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Original Research Article

A prospective study to determine epidemiology, predisposing factors and microbiology of keratitis in north India

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ABSTRACT

Background: Corneal opacification due to keratitis is a leading cause of blindness, with fungal pathogens being important causative agents. Thus, a prospective study was designed to identify the etiological agents; and assess the epidemiological features and risk factors for infective keratitis in India with particular reference to fungal keratitis.
Methods: Corneal scrapings were collected from 151 patients and subjected to direct microscopic examination by gram's stain, KOH wet mount, followed by fungal culture in sabouraud's dextrose agar and bacterial culture in MacConkey's agar and blood agar. Identification of fungal growth was done by colony morphology, slide culture and lacto phenol cotton blue wet mount preparation.
Results: Out of the 151 cases of keratitis, 65 (43.0%) showed fungal elements on KOH smear. By culture methods, microbial etiology (fungal/ bacterial) was established in 68 (45.0%) patients. *Aspergillus spp.* (57.7%) was the most frequently isolated fungus, followed by *Fusarium* (15.4%), *Penicillium* (7.7%), *Curvularia* (9.6%), *Alternaria* (5.8%) *and Candida albicans* (1.9%). History of trauma with vegetative and non-vegetative material and prior ophthalmologic intervention were the major identifiable risk factors.
Conclusions: Thus, *aspergillus spp*. is the most common cause for fungal keratitis in India, with *A. fumigatus* and *A. flavus* being the most familiar species, followed by Fusarium. Bacterial isolates were responsible for considerably fewer cases of keratitis.

Keywords: Fungi, Keratitis, India, Aspergillus

INTRODUCTION

Corneal opacification due to keratitis is a major cause of blindness and visual disability, in fact, second only to cataract in developing countries like Asia, Africa and the Middle East. A breach in common defense mechanism like lids, tear film and corneal epithelium leads to corneal invasion due to any microorganisms.[1] Fungal keratitis represents approximately 6% to 53% of all cases of culture-positive infectious keratitis.[2] Reports from different parts of the world suggest a paradigm shift, with an increasing incidence of fungal keratitis during the last four decades, possibly due to increased awareness and availability of fungal culture methods and identification. *Mycotic keratitis* is often associated with unfavorable outcomes due to the slower onset, long course and the diversity of clinical presentations, presenting the greatest challenge to the ophthalmologists.

Minor injury to the eye is a critical predisposing factor. Indiscriminate use of steroids and antibiotics, use of contact lenses, immuno-compromised state and ocular surgery are other causes of increasing incidence of corneal infection.[3] Agricultural workers and laborers are the biggest occupational group as the highest incidence coinciding with the period of maximal activity. In India, a favorable tropical environment, coupled with a primarily agricultural population having a constant risk of plant exposure and low-income status are the predisposing factors for fungal infections. *Aspergillus* is one of the most common fungi in many parts of India, others being *Fusarium*, *Penicillium*, *Candida*, etc. Early diagnosis of fungal etiology is challenging due to mimicking symptoms with bacterial etiology in early stages. At the same time, culture for fungal pathogens takes a long time. So, it is necessary to comprehend the microbiological and clinical characteristics to start proper treatment. Knowledge of regional epidemiology and risk factors are essential to guide appropriate anti-fungal therapy.

Thus, this study was designed to identify the etiological agents and assess the epidemiological features of infective keratitis in the North region, particularly fungal keratitis.

METHODS

The study was a prospective, cross-sectional observational study, conducted at the Department of Microbiology, Santosh Medical College, Ghaziabad, Uttar Pradesh, a tertiary care hospital in North India for two years, from December 2009 to December 2011. The study was ethically approved. After taking informed consent, a total of 151 cases of keratitis attending the OPD/IPD of Ophthalmology were included in the study. Samples were taken on the basis of patient availability in hospital. The only exclusion criteria were - patients other than keratitis.

Several clinical variables were recorded: demographic data, medical history, risk factors (history of ocular trauma with vegetative or non-vegetative matter, use of contact lenses, prior ocular surgery, etc.) and clinical presentation.

After clinical evaluation by the ophthalmologist, a slit-lamp bio-microscopic examination was done by an experienced ophthalmologist. Subsequently, corneal scrapings were collected under strict aseptic conditions after the instillation of topical anesthesia (4% lignocaine) using a sterile no. 15 bard-parker blade. Scrapings were taken from the edges and base of the ulcer. Lids were held widely apart to reduce inadvertent contamination by lid margins or eyelashes. Scraping was taken from both central and peripheral areas using firm but gentle strokes.

The material obtained by scraping was subjected to standard microbiology evaluation. Microscopic examination of ocular specimens was performed by staining heat-fixed smear with Gram's stain and preparing a wet mount with 10% KOH. Samples were immediately inoculated on the surface of sabouraud's dextrose agar (SDA) without delay and incubated at 25℃ and 37℃. Inoculated media was examined daily for 8 weeks and discarded thereafter if no growth was observed. Fungal isolates were identified by colony morphology seen on SDA. Lactophenol cotton blue (LPCB) mount was prepared for identification of filamentous fungi by their microscopic morphology, such as characteristics of hyphae, septation, conidial shape, size, color and arrangement of spores. Slide cultures were set up whenever required. Yeasts isolated were identified by gram's staining and Germ tube test.

Fungal etiology was considered when fungal growth in culture was supported by positive direct microscopy and growth of the same fungus in at least two media or repeated isolation of the fungus on more than one occasion.

Culture for bacterial isolates was done on MacConkey's agar medium and blood agar medium and incubated in ambient air at 37ºc for 24 hours. Bacterial colonies were subsequently identified by colony characteristics, gram's staining and biochemical reactions.

Statistical analysis: Data were expressed in proportion and percentage.

RESULTS

A total of 151 patients of keratitis presenting to the ophthalmology OPD/IPD were included in the study. All the patients had involvement of a single eye; therefore, 151 eyes of 151 patients were evaluated. The epidemiological characteristics of the study subjects are presented in the (Table 1).

Table 1: Clinico-epidemiological profile of patients.

Variables	Keratitis patients (n=151)	Mycotic keratitis patients (n=50)
Sex		
Male	95 (62.9)	34 (68)
Female	56 (37.1)	16 (32)
Age (in years)		
≤21 years	22 (14.6)	8 (16)
21-40	61 (40.4)	18 (36)
41-60	63 (41.7)	22 (44)
≥61	5 (3.3)	2 (4)
History of trauma	81 (53.6)	36 (72)
With vegetative matter	45 (29.8)	21 (42)
With non-vegetative matter	36 (23.8)	15 (30)
No history of trauma	70 (46.4)	14 (28)
Ocular Surgery	3 (2.0)	3 (6)

Out of the 151 cases almost two-third of patients (n=95, 62.9%) were males, while females constituted only about one-third of the sample size (n=56, 37.1%). Age of the patients ranged from 12 years to 75 years (median=34 years). Twenty-two (14.6%) patients were below 20 years, 61 (40.4%) patients were between 20 to 40 years of age, 63 (41.7%) were between 41 and 60 years, while only five (3.3%) patients were above 60 years of age.

Table 2: Etiological agents of mycotic keratitis.

S. no.	Fungus	Pure isolates	Mixed with other fungi	Mixed with bacteria	Total (%)
1.	*Aspergillus fumigatus*	11	2	1	14 (26.9)
2.	*Aspergilus flavus*	11	-	-	11 (21.2)
3.	*Aspergillus niger*	5	-	1	6 (11.5)
4.	*Fusarium spp.*	8	-	-	8 (15.4)
5.	*Penicillium spp.*	3	1	-	4 (7.7)
6.	*Curvularia spp.*	4	1	-	5 (9.6)
7.	*Alternaria spp.*	3	-	-	3 (5.8)
8.	*Candida albicans*	1	-	-	1 (1.9)
	Total no. of isolates (%)	46 (88.5)	4 (7.7)	2 (3.8)	52 (100)

Table 3: Etiological agents of bacterial keratitis.

S. no.	Bacteria	Pure isolates	Mixed with other fungi	Mixed with bacteria	Total (%)
1.	*Staphylococcus aureus*	5	-	2	7 (31.8)
2.	*Pseudomonas aeruginosa*	-	1	2	3 (13.6)
3.	*Klebsiella spp.*	2	-	-	2 (9.1)
4.	*Escherichia coli*	2	-	-	2 (9.1)
5.	*Staphylococcus epidermidis*	7	1	-	8 (36.4)
	Total no. of isolates (%)	16 (72.7)	2 (9.1)	4 (18.2)	22 (100)

History of trauma was seen as a significant risk factor, being present in 81 (53.6%) patients, including 45 (29.8%) patients who had trauma with vegetative matter such as leaves, stems, branches of trees, or other wooden/ paper material. Thirty-six (23.8%) patients complained of injury with non-vegetative material like stones, metal parts/chips, etc. Three patients had prior ophthalmologic intervention (Table 1). However, none of the patients gave a history of use of contact lenses. History of trauma was much higher in culture-proven fungal keratitis cases (72% vs. 53.6%).

Of the 151 cases of keratitis, 65 (43.0%) showed fungal elements on KOH mount, while 86 (57.0%) were negative. In almost half of the 151 cases, fungal or bacterial growth was obtained by culture methods. Single filamentous fungal species were isolated from 45 patients, *Candida albicans* in one isolate and single bacterial isolate in 16 patients. In comparison, six patients had mixed fungal-fungal/ bacterial-fungal/ bacterial-bacterial infection (two cases of each). Thus, a total of 52 fungal pathogens were isolated from 50 cases of culture-positive *mycotic keratitis* (Table 2). Among them, *Aspergillus spp.* (30 out of 52 fungal pathogens, 57.7%) was the most frequently isolated fungus, with A. fumigatus being the most common species. *Alternaria, Curvuaria, Fusarium, and Penicillium* were other common fungi (Figure 1). One case of keratitis due to *Aspergillus fumigatus* was co-infected with *Penicillium*, one with *Curvularia*, and one with *Pseudomonas aeruginosa*. One case of *Aspergillus niger keratitis* was co-infected with *Staphylococcus epidermidis.*

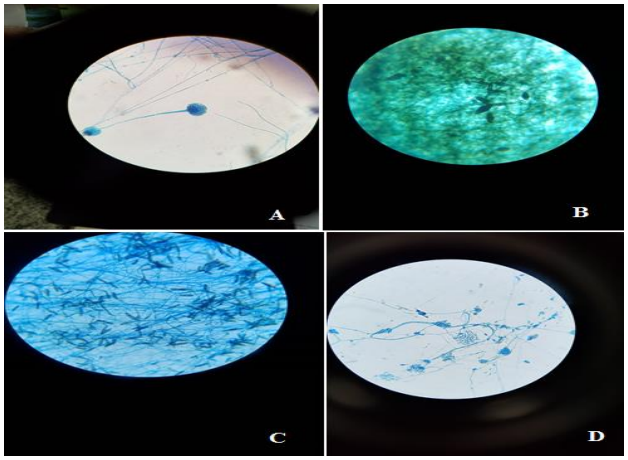

Figure 1: Different fungi seen under microscopy with LPCB preparation (A) *A fumigatus* (B) *Alternaria* (C) *Curvularia* (D) *Fusarium.*

Out of the 151 specimens, 22 bacterial pathogens were found in 20 cases, with *Staphylococcus epidermidis* being the most common isolate (Table 3). *Pseudomonas*

aeruginosa was isolated as mixed infection in three cases, along with *Staphylococcus aureus* (two cases) and *Aspergillus fumigatus* (one case).

DISCUSSION

More than 105 species of different fungi such as *Aspergillus spp., Fusarium spp., Candida spp., Rhizopus, Mucor,* and other fungi have been identified as the etiological agents of fungal keratitis. The importance of fungus in the etiology of keratitis can never be over-emphasized. While other infectious agents like virus, bacteria and parasites are fundamental causes of keratitis, fungal etiology is the one that raises the greatest concern due to its intractable course. Despite advances in diagnosis and medical treatment of fungal infections of the cornea, patients may require surgical intervention. Apart from nonspecific measures, medical therapy consists of nonspecific measures and the use of specific antifungal agents, such as topical natamycin (5%) or amphotericin B (0.15%).

In our study, males outnumbered the females with a ratio of 1.69:1, perhaps due to greater involvement in outdoor work, with more chances of ocular injury. This has also been observed by several other authors.[3-8] Majority of the patients belonged to the middle age group (21 to 60 years), possibly due to the same reason. History of trauma, especially with vegetative matter, maybe an essential risk factor for mycotic keratitis as shown by other authors.[9-11] This is further emphasized by our study, as more than half of the patients with keratitis and more than two-thirds of the fungal keratitis patients had history of prior trauma with either vegetative matter or non-vegetative matter. Although, ocular trauma is the main culprit in developing countries, several studies suggest that contact lens users have more prone to develop infections in developed countries.[12,13]

In KOH wet mount preparation, hyaline, septate and branching hyphal filaments were commonly observed, while phaeoid, septate hyphae were relatively less frequent. Fruiting bodies were not seen in any direct KOH mount; except in one sample in which Aspergillus type conidiation was seen, though speciation was not possible. In our study cases showed presence of fungal hyphae in KOH mount but did not yield any fungal growth on culture.[15] This is similar to the observations of and Nath R et al and Tilak R et al where KOH mount had higher positivity.[14] However, Tahereh et al and Jose et al reported only 71.4% and 90.2% positive KOH smear compared to culture-positive fungal keratitis cases, respectively.[15,16]

As per our criteria, fungal etiology was considered when fungal growth in culture was supported by positive direct microscopy or growth of the same fungus in at least two media or repeated isolation of the fungus on more than one occasion. Therefore, specimens that showed fungal hyphae on KOH wet mount, but were culture-negative; or were culture-positive but KOH negative were not included in the study. This was done to rule out the possibility of contamination, especially during manipulation in the laboratory. Infectious etiology was established in 68 patients by culture, with single filamentous fungi being responsible in 45 cases, Candida albicans in one case, pure bacterial growth in 16 cases and mixed fungal and/or bacterial infection in six cases. Therefore, fungal etiology was established in 50 cases (33.1%) out of 151 cases.

In our study, *Aspergillus spp*. was the most common fungus, with *A. fumigatus* being the most common species, followed by *A. flavus* and *A. niger*. *Fusarium* was the second most frequent fungal pathogen. Dematiceaceous fungi like Curvularia and Alternaria were isolated less frequently (in 4 and 3 cases, respectively). Here, (Table 4) shows the common etiological agents of mycotic keratitis as reported by various Indian authors in the last ten years. Close analysis of these studies also reveals that in India, *Aspergillus spp.* is the most common pathogen responsible for fungal keratitis followed by Fusarium. But some studies reported different types of results. Jampala S et al reported Candida spp as the most frequent isolate.[17] Nath R et al from Assam and Meena et al from Uttarakhand reported *Fusarium* as the most prevalent species followed by *Aspergillus spp*. and *Curvularia spp.*[18] Some studies found Aspergillus spp as common isolate in the north and western India and *Fusarium spp.* in South India.[19] Among the *Aspergillus species, A. fumigatus* was the most prevalent species in our study, as also seen by Sanjeev H et al from Mangalore; at the same time, Punia RS et al and Tilak R et al found A. flavus to be the commonest.[20] These findings suggest that although Aspergillus spp is the most common isolate, some variation may be possible.

Bacterial isolates, either singularly or in combination with other bacteria or fungi, were identified in 20 cases (13.24%) of the 151 keratitis cases. These were considerably fewer as compared to cases of mycotic keratitis, thus emphasizing the importance of fungi in cases of infective keratitis. But variation can't be ignored as suggested by Mudhol R et al.[21] Among bacterial isolates, Staphylococcus epidermidis (36.4%) was most common followed by Staphylococcus aureus (31.8%) and Pseudomonas aeruginosa (13.6%). Several studies like Mudhol R et al, Sedhu PA, Dalmon C, et al, Mohanty et al and Jampala S et al found Gram-positive organisms as common bacterial isolates.[22] In contrast, others like Lin T et al reported Gram-negative bacteria as common bacterial isolates.[23,24]

Table 4: Different fungal isolates in different studies.

Author	Present study	Tewari et al[5]	Punia et al[4]	Srinivas	Sedhu et al[23]	Paty et al[19]	Satpathy et al
Total number of fungal isolates	52	31	44	9	18	16	4069
Aspergillus fumigatus	14 (26.9)	11 (35.4)	5 (11.4)				8.2%
Aspergillus flavus	11 (21.2)		26 (59.1)	20%	66.6%	43.8%	13.1%
Aspergillus niger	6 (11.5)		-				9.8%
Other Aspergillus species	-		-				-
Fusarium spp.	8 (15.4)	7 (22.5)	7 (15.9)	40%		25%	24.5%
Penicillium spp.	4 (7.7)	-	1 (2.3)	-	12.%	12.5%	4.5%
Curvularia spp.	5 (9.6)	6 (16.1)	2 (4.5)	-	-	6.3%	10.2%
Alternaria spp.	3 (5.8)	-	1 (2.3)	-	-	-	10.5%
Candida albicans	1 (1.9)	4 (12.9)	-		22.2%	12.5%	
Other *Candida* species	-	-	-	44.5%	-	-	4.4%
Rhizopus spp.	-	-	1 (2.3)	-	-	-	1%
Mucor spp.	-	-	-	-	-	-	0.2%
Pheoacremonium parasiticum	-	-	1 (2.3)	-	-	-	-
Cladosporium spp.	-	-	-	-	-	-	-
Bipolaris spp.	-	-	-	-	-	-	5.4%
Paeciliomyces lilacinus	-	-	-	-	-	-	0.8%
Scedosporium apiospermum	-	-	-	-	-	-	-
Acremonium spps.	-	-	-	20%	-	-	1.2%
Helminthosporium	-	-	-	-	-	-	5.7%
Trichosporon spp.	-	-	-	-	-	-	-
Cladophialophora carrion	-	-	-	-	-	-	-
Other dematiceous fungi	-	-	-	-	-	-	-
Unidentified	-	4 (12.9)	-	-	-	-	-
Most common fungal pathogen	*Aspergillus spp.*	*Aspergillus spp.*	*Aspergillus spp.*	*Candida spp*	*Aspergillus spp.*	*Aspergillus spp.*	*Aspergillus spp.*

Limitations

Follow up of patients was not done to know effect of treatment. We didn't perform antifungal susceptibility due to unavailability of facility.

CONCLUSION

Thus, the present study concludes that Aspergillus species are the most common cause for fungal keratitis in our region, with A. fumigatus and *A. flavus* being the prevalent species, followed by *Fusarium.* Bacterial isolates are responsible for considerably fewer cases of keratitis. Routine surveillance of fungal keratitis is necessary to know the existing and emerging pattern of pathogens. Trauma with vegetative matter was identified as the most common risk factor.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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