Original Article

Clinical and mycological profile of fungal keratitis from North and North-East India

Yamini Tawde, Shreya Singh, Sourav Das, Shivaprakash M Rudramurthy, Harsimran Kaur, Amit Gupta[1], Mounjuri Kataki[2], Pranami Gogoi[2], Anup K Ghosh

Purpose: To study the clinical presentation, mycological profile, and risk factors of fungal keratitis (FK) cases presenting at two tertiary-care centers, one each at North (Chandigarh) and Northeast (Assam) India, and to compare the spectrum of fungi recovered from the clinical and environmental samples at both locations. **Methods:** All patients with suspected FK were enrolled from both the centers between January 2018 and December 2019. Corneal samples were collected and processed as per standard laboratory protocols. Demographic details and clinical and mycological profiles were noted in all patients. Environmental sampling from the soil, air, and the vegetative matter was performed from both locations and neighboring districts. **Results:** Of the 475 suspected cases, 337 (71%) were diagnosed as FK (median age: 50 years; 77.2% males). The presence of diabetes, hypertension, blurred vision, and corneal discoloration was significantly higher in patients with FK compared to those without FK. *Aspergillus* sp. (52.1%) and *Fusarium* sp. (47.61%) were the predominant etiological agents isolated from cases in North and Northeast India, respectively. FK due to melanized fungi was associated with diabetes, trauma with animal tail, and corneal discoloration. A similar spectrum of fungi was seen in environmental and clinical samples in both the regions. **Conclusion:** The difference in etiological agents of FK and environmental fungal isolates in North and Northeast India highlights the need to identify the ecological niche of potential fungal pathogens. Prospective, multicenter studies, systematic environmental sampling, and the evaluation of the differences in causative agents and clinical presentation of FK from different parts of the country can substantially improve our understanding of its region-specific clinico-epidemiological profile.

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Infective keratitis is the inflammation of the cornea due to infections caused by bacteria, viruses, fungi, or parasites.[1] Fungal keratitis (FK) is a very serious, refractory, and potentially sight-threatening corneal infection. Despite the worrisome clinical scenario, cases of FK are often difficult to diagnose due to a broad range of risk factors, nonspecific signs and symptoms, lack of sufficient mycological workup, and also due to the rising numbers of rare and previously unreported fungal agents from such cases.

Both filamentous fungi and yeast can cause FK with variable reports of causative agents from different countries and even between regions within the same country.[2] Since India lies in the tropical belt, favorable environmental conditions for fungal growth are seen throughout the year.[3] The prevalence of FK ranges from 25.6 to 36.7% across India.[4] In North India and Northeast India, the prevalence of FK ranges from 7.3 to 25.6%; in West India, it is 36.3% while a higher prevalence of 36.7% is reported in Southern India.[5] In North and Northeast India, higher susceptibility to FK is associated with agriculture-related activities as it is the most common occupation in these regions, contributing to greater fungal exposure from the environment.

Department of Medical Microbiology, PGIMER, Chandigarh, [1]Advanced Eye Centre, PGIMER, Chandigarh, [2]Department of Medical Microbiology, Dibrugarh Medical College, Assam, India

Correspondence to: Dr. Anup Kumar Ghosh, Research Block-A, 2nd Floor, Sector-12, PGIMER, Chandigarh - 160 012. E-mail: ak_ghosh3@rediffmail.com

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At least 166 genera and 144 species of fungi have been reported to cause human FK including over 100 genera of filamentous fungi, 18 genera of yeasts or yeast-like fungi, and 6 genera of dimorphic fungi.[6,7] *Aspergillus* sp. and *Fusarium* sp. being the predominant causative agents in India.[8] Melanized fungi including *Curvularia* sp., *Alternaria* sp., *Bipolaris* sp., *Cladosporium* sp., and *Scedosporium* sp. are emerging ascausative agents of FK. The filamentous fungi causing FK are most common in tropical regions including India, China, Thailand, Bangladesh, etc; while yeast-like *Candida* sp. is more common in temperate regions or developed nations. Rare fungi like *Alternaria tenuissima*, *Epicoccum nigrum*, *Acrophialophora fusispora*, *Chaetomium globosum*, etc., which are also known plant pathogens are also now being reported to cause FK.[7]

Although, various studies emphasizing the epidemiology and microbiological profile of FK have been reported from India, very few have provided a comprehensive analysis of the clinical and mycological profile.[4,5,8] Additionally, the correlation between environmental fungi with fungi

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isolated in clinical practice has not been investigated to date. A systematic evaluation of the differences in causative agents and clinical presentation of FK from different parts of the country can substantially improve our understanding of its region-specific clinico-epidemiological profile. Studying the spectrum of fungi isolated from cases of FK and its comparison with the profile of environmental fungi in different regions could also provide useful insights regarding disease epidemiology.

Given these concerns, the present study was planned to study the clinical presentation, mycological profile, and risk factors of FK cases from two tertiary-care centers, one each in North and Northeast India. We also aimed to analyze the spectrum of causative agents of FK in both these regions concerning their environment.

Methods

Study population

This prospective study was conducted at the centers, one in North India and the other in Northeast India. Both are tertiary-care centers catering to various parts of North and Northeast India as shown in Fig. 1.

Ethical clearance was taken from the Institutional ethics committees of both participating centers before commencement of the study (ref no. of PGIMER: PGI/IEC/2018/000590 and ref no. AMC: AMC/EC/5856). All patients with suspected FK attending the ophthalmology clinic at both participating centers were prospectively enrolled in this study. The demographic details and clinical history of all patients were recorded using a pre-designed clinical record form. Ocular examination was performed using a slit-lamp biomicroscope. Corneal ulceration defined as loss of epithelium, underlying stromal infiltrate, and suppuration associated with inflammation and with or without the presence of hypopyon. The diagnosis of FK was based on clinical criteria (presence of typical features of FK like corneal ulceration with feathery edges, dry, gray elevated infiltrates, and satellite lesions) along with mycological criteria, presence of septate hyphae or yeast on direct microscopic examination of 10% potassium hydroxide (KOH) + calcofluor white mount prepared from corneal samples.

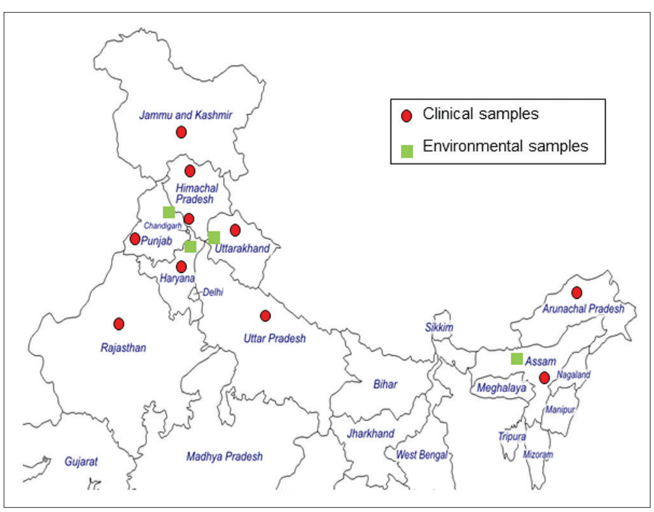

Figure 1: Regional distribution of clinical and environmental samples

Sample collection and processing

After ocular examination, corneal samples (corneal scrapings, corneal button, corneal tissue, and corneal exudates) were obtained by the ophthalmologist under aseptic conditions using a sterile Baird- Parker blade (no. 15) following all safety measures. A part of the sample was placed on a clean glass slide for direct microscopy and the other part was inoculated in a 'C' shape pattern on the surface of Sabouraud dextrose agar (SDA) plates with chloramphenicol (50 mg).

For direct microscopy, a KOH-calcofluor white wet mount was prepared from the sample obtained on glass slides. Inoculated SDA plates were incubated at 25°C for 2 weeks, and examined daily for any visible fungal growth. The ophthalmologist was immediately informed in case any sample was positive on direct microscopy.

Environmental sampling

Environmental samples including vegetative matter, air samples, and soil samples were collected from the three districts surrounding center in North India and five districts surrounding center in Northeast India in January as shown in Fig. 1. All environmental samples from soil and vegetative matter were inoculated in sterile distilled water in a sterile 50 mL falcon and were placed in a shaking incubator at 28°C at 200 rpm for 1 h. Then, 100 µl of this sample was inoculated onto one sterile SDA plate and one Malachite green agar plate[9] by spread plate method and incubated at 28°C for up to 1 week.

During outdoor air sampling, no active intervention to restrict physical activity at the site of sampling was made. An impaction air sampler by BioMérieux®, Sampl'airTM with a suction capacity of 100 L/min was used for sample collection.

Table 1: Comparison of demography, clinical details, and risk factors between patients with and without fungal keratitis

	Fungal keratitis (*n*=337)	Non-fungal keratitis (*n*=138)	*P*
Age			
Median (1-92)	50.0	45.5	
Mean (SD=16)	49.1	47.1	
Gender			
Male	260 (77.2%)	84 (60.9%)	
Underlying condition			
Diabetes Mellitus	46 (13.6%)	0	
Hypertension	29 (8.6%)	0	
Risk factors			
Vegetative matter injury	86 (25.51%)	47 (34.05%)	0.060
Animal tail	3 (0.89%)	0	0.266
Dust	6 (1.78%)	0	0.115
Chemical injury	2 (0.59%)	0	0.364
Iron piece injury	4 (1.18%)	0	0.199
Insect entry	7 (2.6%)	1 (0.7%)	0.298
Foreign particle	6 (1.78%)	0	0.115
Clinical features			
Pain	260 (97.7%)	104 (89.7%)	0.676
Redness	259 (97.4%)	109 (94.8%)	0.614
Watering	254 (95.5%)	97 (84.3%)	0.252
Blurring of vision	213 (81.9%)	12 (12.4%)	0.000
Discoloration	41 (12.1%)	0	0.000

Table 2: Etiology-wise analysis of underlying condition, risk factors, and clinical features in cases of FK

	Aspergillus sp. (*n*=93)	*Fusarium* sp. (*n*=58)	Melanized fungi (*n*=24)	*P*
Underlying conditions				
DM	16 (17.20%)	5 (8.6%)	12 (50.00%)	0.000
HTN	12 (12.90%)	4 (6.89%)	6 (25.00%)	0.79
Risk Factors				
Physical trauma				
Vegetative matter	25 (26.88%)	16 (27.58%)	6 (25.00%)	0.971
Animal tail	0	0	1 (4.16%)	0.042
Dust	1 (1.07%)	2 (3.44%)	1 (4.16%)	0.511
Insect	3 (3.22%)	0	1 (4.16%)	0.349
Chemical injury	0	0	0	--
Iron piece injury	1 (1.07%)	1 (1.72%)	0	0.797
Other foreign particle	3 (9.67%)	1 (1.72%)	0	0.603
Clinical Features				
Pain	86 (92.47%)	53 (91.37%)	23 (95.83%)	0.782
Redness	86 (92.47%)	53 (91.37%)	23 (95.83%)	0.782
Watering	85 (91.39%)	53 (91.37%)	22 (91.66%)	0.999
Blurring of vision	76 (81.72%)	42 (72.41%)	20 (83.33%)	0.335
Discoloration	15 (16.12%)	5 (8.62%)	13 (54.26%)	0.000

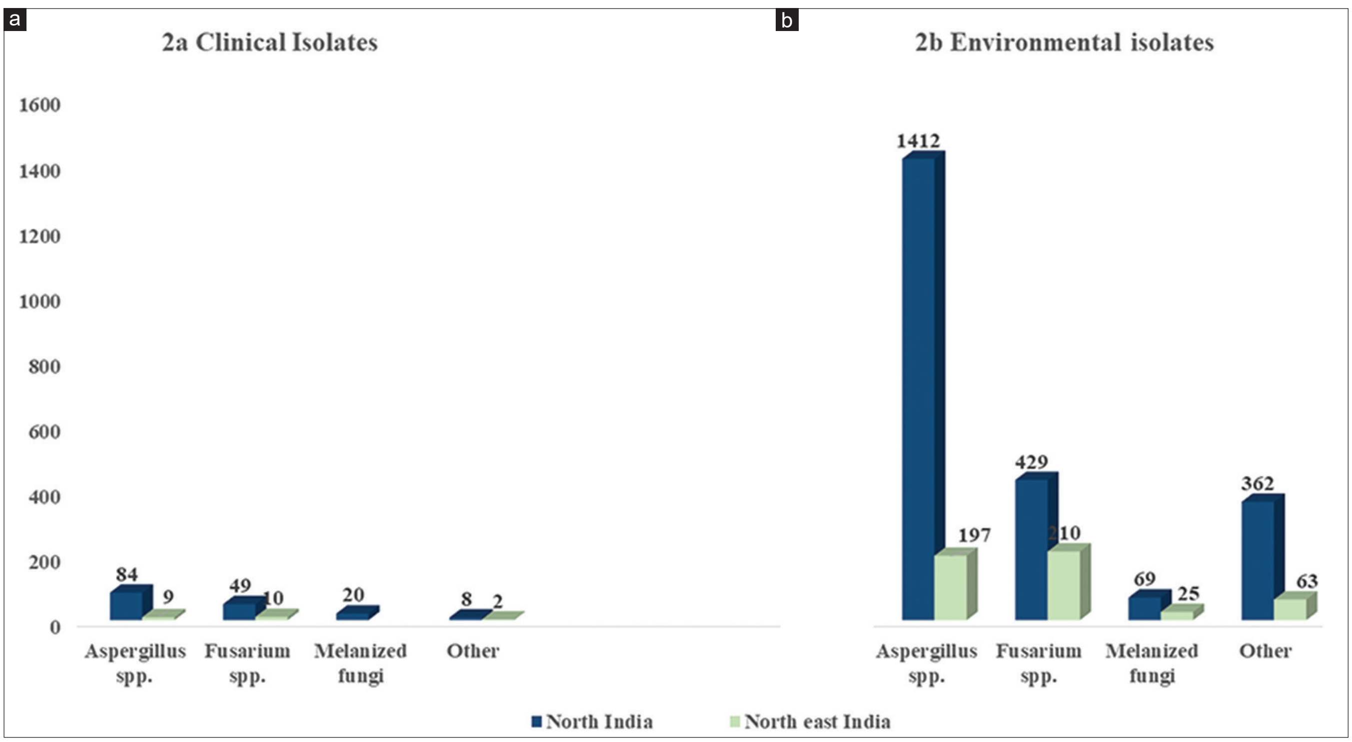

Figure 2: Distribution of fungal agents recovered from (a). clinical and (b). environmental samples at both participating centers

A total of 1,000 L (10 min sampling time per sample) of air was screened. The spores in the suctioned air were allowed to impact on one SDA plate and one Malachite green agar plate and both plates were incubated at 28°C for up to 1 week.

Literature search

A literature search was conducted using the combination of the search terms "Fungal keratitis" and "India" on PubMed from its inception till 30 March 2021. Only studies in English describing the epidemiology of FK in humans were included and review articles, case reports, basic research articles, abstracts, conference proceedings, etc., were excluded. Study details and data including prevalence, risk factors of FK, etiological agent, etc., were extracted.

Statistical analysis

The analysis was carried out using Statistical Package for Social Sciences (SPSS Inc, Chicago, IL, version 23.0 for Windows). Data were represented as mean (standard deviation) and median (range) for quantitative variables and number (frequency/proportions) for qualitative variables. The proportions were compared using the Chi-square test (χ^2) or Fisher's exact test when applicable and a '*PP*'-value of ≤ 0.05 was considered significant.

Results

A total of 475 samples from patients with suspected FK were collected and processed from both the centers between January

Table 3: District-wise distribution of environmental samples in North India and Northeast India

North- East India								
State	District	Village	No of samples	Total Mycelial Count	*Aspergillus* sp.	*Fusarium* sp.	Black fungus	Other mycelial fungus
Assam	Sibsagar	Namtula Hati Camp	5	36	17	9	2	8
Assam	Dhemaji	Silapathar	5	52	23	5	2	22
Assam	Dibrugarh	Thana Chariali	5	84	43	28	2	11
Assam	Dhemaji	Borlung	5	58	32	12	1	13
Assam	Sonitpur	Sonitpur	5	125	42	62	12	9
Assam	Lakhimpur	Sauldhua	5	140	40	94	6	0
Total	6	6	30	495	197	210	25	63
North India								
State	District	Village	No of samples	Total mycelia count	*Aspergillus* sp.	*Fusarium* sp.	Black fungus	Other mycelial fungus
Punjab	Mohali	SAS Nagar	18	248	111	64	2	71
Punjab	Mohali	Jayanti Majri	18	246	84	64	42	56
Punjab	Mohali	Nada	18	225	120	51	2	52
Uttar Pradesh	Saharanpur	Abdullapur	18	543	426	45	5	67
Uttar Pradesh	Saharanpur	Dabkora	18	238	102	85	2	49
Haryana	Yamuna Nagar	Mustafabad	18	347	256	65	12	14
Haryana	Yamuna Nagar	Sarawan	18	435	313	65	4	53
Total	7	7	126	2282	1412	439	69	362

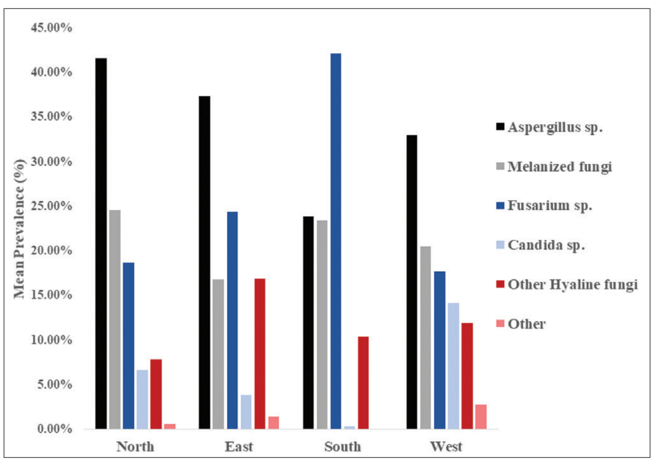

Figure 3: Graphical representation of the mean prevalence (%) of agents causing fungal keratitis reported from 19 studies across different regions of India

2018 and December 2019. Of these, 337 (70.94%) cases fulfilled the diagnostic criteria of FK.

Demographic and clinical findings

The median age of all cases was 50 (range 1–92) and male predominance (77.2%) was seen. The comparison of clinical details between cases with FK and controls without FK is shown in Table 1.

The presence of diabetes mellitus (n = 46, 13.6%) and hypertension (n = 29, 8.6%) was observed only in the FK group. There was significantly higher prevalence of blurring of vision (81.9% vs. 12.4%, P = 0.00) and discolouration (n = 41, 12.1%) in cases with FK.

Mycological findings

Direct microscopy was positive in 318 (66.94%) while 185 (38.94%) were positive by fungal culture. Overall, the most common causative agent of FK in both North and Northeast India was *Aspergillus* sp. (n = 93, 50.27%) which included *A. flavus* (90.32%), *A. fumigatus* (6.45%), *A. niger*, *A. versicolor*, and *Aspergillus* sp. (1.07% each) followed by *Fusarium* sp. (n = 57, 30.81%) including *F. solani* (49.12%), *F. incarnatum* (3.50%), *F. oxysporum* (7.01%), *F. dimerum* (1.75%), and *Fusarium* sp. (38.59%). The melanized group of fungi was recovered in n = 25 (10.81%) and included *Alternaria* sp. (36%), *Curvularia* sp., and *Scedosporium* sp. (20%) each, *Acremonium* sp. (12%), *Cladosporium* sp., and *Bipolaris* sp. (4%) each. Other causative agents of FK were *Candida* sp. (n = 5, 2.7%) including *C. albicans* (40%), *C. guillermondii* (40%), and *C. glabrata* (20%), and unidentified agents (n = 4, 2.1%).

Agent-wise clinical analysis

The agent-wise analysis of clinical parameters is depicted in Table 2. The presence of underlying conditions like diabetes mellitus (50%, P value = 0.000) has been significantly associated with FK due to melanized fungi. Risk factors like trauma due to animal tail (4.16%, P value = 0.042) and clinical features like discoloration of cornea (54.26%, P value = 0.000) had a significant association with FK due to melanized fungi.

Environmental and clinical fungi- regional comparison

Aspergillus sp. was the predominant causative agent found in clinical isolates in North India followed by *Fusarium* sp. and melanized group of fungi while in the case of Northeast India, *Fusarium* sp. was the predominant causative agent followed by *Aspergillus* sp. as shown in Fig. 2a. A total of 126 environmental samples from center in North India and 30 samples from center in Northeast India were

Table 4: Summary of the clinical and mycological details from various epidemiological studies on fungal keratitis from India

Region	Author and Study Period	Microscopy and Culture positive	Gender and Age range	Risk factors
North	Chowdhary *et al*. (2005)[10] Jan 1999- June 2001	119/191 (62.30%) 191/485 (39%)	Male-130/191 (68%) 31-40	Vegetative matter-33/63 (52.3%) Animal mater-12/63 (19%) Dust/stone- 10/63 (15.8%) Iron particle- 5/63 (7.9%)
	Saha *et al*. (2006)[11] Jan 2000-Dec-2004	67/346 (19.36%) 74/346 (21.38%)	Male-60/77 (77.9%) 50-60	NM
	Chander *et al*. (2008)[12] Jan 1999- Dec 2003	52/64 (81.25%) 34/64 (53.12%)	Male-124/154 (80.52%) 21-50	Vegetative matter-24/64 (37.50%) chronic antibiotic usage -16/64 (25%) Use of topical corticosteroids-5/64 (7.81%)
	Ghosh *et al*. (2016)[8] 2005-2011	765/2495 (31%) 393/765 (51%)	Male- 602/765 (78.7%) 21-60	Vegetative matter-50/197 (25.38%) Foreign particle-51/197 (25.88%) Dust/stone- 10/197 (5.07%)
	Roy *et al*. (2017)[13] Jan 2010-Dec 2015	147/400 (36.75%) 94/400 (23.50%)	Males-72/94 (76%) 31-40	Corneal trauma-84/94 (89.36%)
	Satpathy *et al*. (2018)[1] Jan 2001-Dec 2016	NM 4069/18898 (21.5%)	Males-12717/18898 (67.3%) 41-50	NM
East	Basak *et al*. (2005)[2] Jan 2001-Dec 2003	231/1198 (19.3%) 509/1198 (42.5%)	Males-846/1198 (70.6%) 31-40	Vegetative matter-715/1198 (59.68%) Dust/stone- 134/1198 (11.2%) Insect-86/1198 (7.2%) Foreign particle-29/1198 (2.4%)
	Nath *et al*. (2010)[3] Apr 2007- Mar 2009	124/188 (65.2%) 157/310 (50.64%)	Males- 108/157 (68.7%) 41-50	Vegetative matter-103/120 (85.83%) Animal mater-9/120 (7.5%) Dust/stone- 1/120 (0.8%) Insect-1/120 (0.8%) Iron particle- 1/120 (0.8%)
	Rautaraya *et al*. (2011)[5] Jul 2006- Dec 2009	264/997 (26.4%) 215/264 (81.4%)	Male- 185/264 (70.1%) 50-60	Corneal trauma-106/264 (40.15%)
	Paty *et al*. (2018)[4] 3 months	13/50 (26%) 16/50 (32%)	Male- 11/16 (68.75%) 30-45	Vegetative matter-62.5%
South	Srinivasan *et al*. (1997)[14] Jan 1994- March 1994	NM 138/297 (46.46%)	Male-266/434 (61.3%) 51-60	Vegetative matter-168/284 (59.15%) Dust/stone- 51/284 (18%) Animal mater-16/284 (5.6%) Iron particle- 14/284 (4.9%)
	Bharathi *et al*. (2007)[15] Sep 1999- Aug 2002	NM 1090/1171 (92.69%)	Males-1879/3183 (59.03%) 21-50	Vegetative matter-675/1095 (61.28%) Dirt- 163/1095 (14.88%) Dust/stone- 79/1095 (7.21%) Animal mater-62/1095 (5.66%)
	Gopinath *et al*. (2009)[16] Feb 1991- Jun 2001	1511/1598 (94.6%) 1598/3563 (44.8%)	Male- 4087/5897 (69.3%) NM	Ocular Trauma-712/869 (81.9%)
	Chidambaram *et al*. (2018)[17] Feb 2012- Feb 2013	NM 191/252 (77%)	Male- 162/252 (64%) 37-60	Ocular Trauma-181/252 (72%)
West	Baradkar *et al*. (2008)[18] Jan 2005- Jun 2006	14/34 (41.17%) 34/260 (13.07%)	Males 20-50	Ocular Trauma-9/34 (26.47%)
	Deorukhkar *et al*. (2012)[19] Dec 2004- Dec 2009	NM 311/537 (57.91%)	Males- 582/852 (68.31%) 21-30	Ocular Trauma-502/537 (93.48%)
	Binnani *et al*. (2016)[20] Jul 2005- Jun 2012	66/180 (36.67%) 180/480 (58.33%)	Male- 129/180 (71.66%) 21-40	Vegetative matter-77/180 (42.78%) Foreign particle-36/180 (20%)
	Ostwal *et al*. (2016)[21] Apr 2013- Mar 2015	20/100 (20%) 24/100 (24%)	Male- 83/100 (83%) 25-34	Ocular trauma
	Mohod *et al*. (2018)[22] Jan 2015- Feb 2017	NM 52/88 (59.09%)	Male- 54/88 (61.36%) 41-60	Ocular trauma -22/88 (25%)

collected during the post-harvest period in both the centers. The environmental isolates showed a similar pattern of distribution [Fig. 2b]. In North India, a total of 2,282 mycelia was isolated including *Aspergillus* sp. (61.87%), *Fusarium* sp. (19.23%), and melanized fungi (3.02%) while in Northeast India, a total of 495 mycelia were isolated including *Fusarium* sp. (42.42%), *Aspergillus* sp. (39.79%), and melanized fungi (5.05%) as shown in Table 3.

Literature review

A total of 842 results were obtained after the search and after screening the titles and abstracts; 19 studies were found to fulfill the inclusion criteria.[1-5,8-22] The details of all included studies are provided in Table 4.

The mean prevalence of the causative agents analyzed by a comparative study of epidemiological data is shown in Fig. 3. *Aspergillus* sp. is predominant in all three regions, that is, North India, East India, and West India followed by melanized fungi and *Fusarium* sp. in North and West India and *Fusarium* sp. being the second most common in East India. In South India, *Fusarium* sp. is the predominant causative agent followed by *Aspergillus* sp. and melanized fungi. A detailed region wise distribution of causative agents from various region is shown in supplementary Fig. 1.

Discussion

In the present study, we describe the distribution of agents casing FK at one center each from North and Northeast India and also compare it with the environmental isolates. We observed a male predominance (77.2%) in FK cases which concurs with the findings of other studies from this region.[5,8] Higher incidence of FK in males can be attributed to environmental exposure during farming since both regions are known to be involved in agrarian activities.

On comparison of clinical details and various risk factors among patients with and without FK, diabetes (13.6%) and hypertension (8.6%) were significantly associated FK. A study by Bharathi *et al*.[15] showed diabetes to be the predominant systemic disease associated with fungal infections. Diabetes is a major risk factor for various fungal infections.[23] In a study by Dan *et al*.,[24] diabetes was found to increase the severity of keratitis by delaying corneal re-epithelization. Also, a study by Chang *et al*.[25] showed that patients with DM were found to be 1.31 times at higher risk to develop corneal ulcers compared to non-DM patients. The decrease in the trophic effect of the trigeminal sensory nerve in DM leads to a reduction in the secretion and stability of the precorneal tear film.[26] In patients with diabetes, altered commensal flora with changes in the activity of antimicrobial enzymes exaggerates the establishment of pathogenic organisms on the ocular surface. Defective immune response in diabetes along with the easy establishment of pathogens and delayed epithelial healing may contribute to ineffective clearance of pathogenic fungi causing FK in patients with diabetes.

Among all etiological agents, we observed a significant association of diabetes with FK due to melanized fungi. Generally, the melanized group of fungi cause slowly progressive diseases with lower virulence at initial presentation compared to the hyaline group of fungi which shows advanced ulcer in short duration.[27,28] Melanin is known to weaken the host immune response and increases the resistance of pathogens to the host immune response also. It reduces the susceptibility of melanized cells to antifungal drugs.[29] In patients with diabetes, since there is already immune dysregulation, melanized fungi may have an advantage over hyaline molds enabling them to colonize and invade corneal epithelia more readily.

Although trauma due to vegetative matter was more common in cases with FK compared to non-fungal keratitis, there was no statistical significance. Trauma with vegetative matter like sugarcane leaf, husk, tree branches, paddy grains, etc., is likely during the post-monsoon harvest period (September to January) due to which most FK cases are observed during this time.[8] The present study also observed the highest number of FK (40.80%) cases during the winter season in January which is the harvest period (data not shown). A previous study by Ghosh *et al*.[8] from our institute also showed similar results.

A clinical feature like discoloration (55%) of the cornea was observed to be significantly associated with FK caused by a melanized group of fungi. The presence of melanin in melanized fungi may be the cause of discoloration of the cornea. This specific clinical presentation might indicate to the clinician to suspect that FK is caused by a melanized group of fungi. A study by Venkatesh Prajna *et al*.[28] also demonstrated that pigmentation of a corneal ulcer can be a prognostic factor for poor visual outcomes.

We observed that *Aspergillus* sp. (52.1%) was the predominant causative agent of FK in North India while *Fusarium* sp. (47.61%) was most common in Northeast India. On reviewing the literature, we observed an interesting difference in causative etiological agents in different regions with a higher prevalence of FK due to *Fusarium* sp. in South India (42.15%) followed by East (24.37%), North (18.72%), and West (17.69%) India. These regional variations in agent distribution may be due to regional differences in rainfall, humidity, type of soil, cultivated crops, etc., suggesting the role of agroclimatic and geographical situations in influencing etiological patterns of FK across the country.

In North India, the primary crops are wheat, sugarcane, and oilseed whereas in Northeast India, rice is the major cultivation,[30] also the average rainfall in Northeast India is around 2,818 mm which is comparatively high than that of North India, that is, 617 mm.[31] The environmental factors in Northeast India are quite similar to that of South India, hence, this might be the reason for *Fusarium* sp. being the predominant causative agent in both these regions. The differences in the environmental conditions of various regions might provide the necessary niche for the growth of their predominant causative agent and should be investigated.

As the major source of fungi causing FK is vegetative matter from the environment, we performed environmental sampling in the harvest month of January to check for any correlation between clinical and environmental isolates. *Aspergillus* sp. was the predominant organism isolated from environmental samples in North India whereas *Fusarium* sp. was the predominant organism in Northeast India. The use of Malachite green agar (MGA) in the present study ensured the isolation of *Fusarium* sp. which may have been missed or reduced if only SDA was used. A spatiotemporal association was observed between the predominant agents isolated from the clinical cases and the environment. Various studies have checked the association of clinical and environmental isolates in various diseases[32,33] but this is the first study to date to check any correlation between FK clinical and environmental isolates. However, more studies with larger samples size and with molecular typing of clinical and environmental isolates are required to confirm this association. Multicenter studies on the sampling of different types of environmental samples, such as different types of soil, specific vegetation like wheat versus rice, air samples over different seasons, etc., could provide clues regarding the local niches and potential sources of infection in different agents. The limitation of this study includes lack of objective measurement of visual acuity, corneal ulcer size, and follow-up of cases with FK. Although we have provided some preliminary information, future studies with a larger number of environmental samples are warranted. Systematic and detailed environment sampling and correlation of environmental and clinical must also be addressed in future studies.

Conclusion

This study demonstrated the difference in etiological agents in India with a predominance of *Aspergillus* sp. in North India and *Fusarium* sp. in Northeast India. FK due to melanized fungi was significantly associated with diabetes and corneal discoloration. The regional distribution of fungi in environmental sampling was concordant with the agent distribution in clinical samples. A review and comparative analysis of the prevalence of the agents of FK in different regions of India showed a predominance of *Fusarium* sp. in South India and *Aspergillus* sp. in other parts of the country. Prospective, multicenter studies across the country with both clinical and environmental samplings are needed for a better understanding of the factors affecting agent distribution.

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Conflicts of interest

There are no conflicts of interest.

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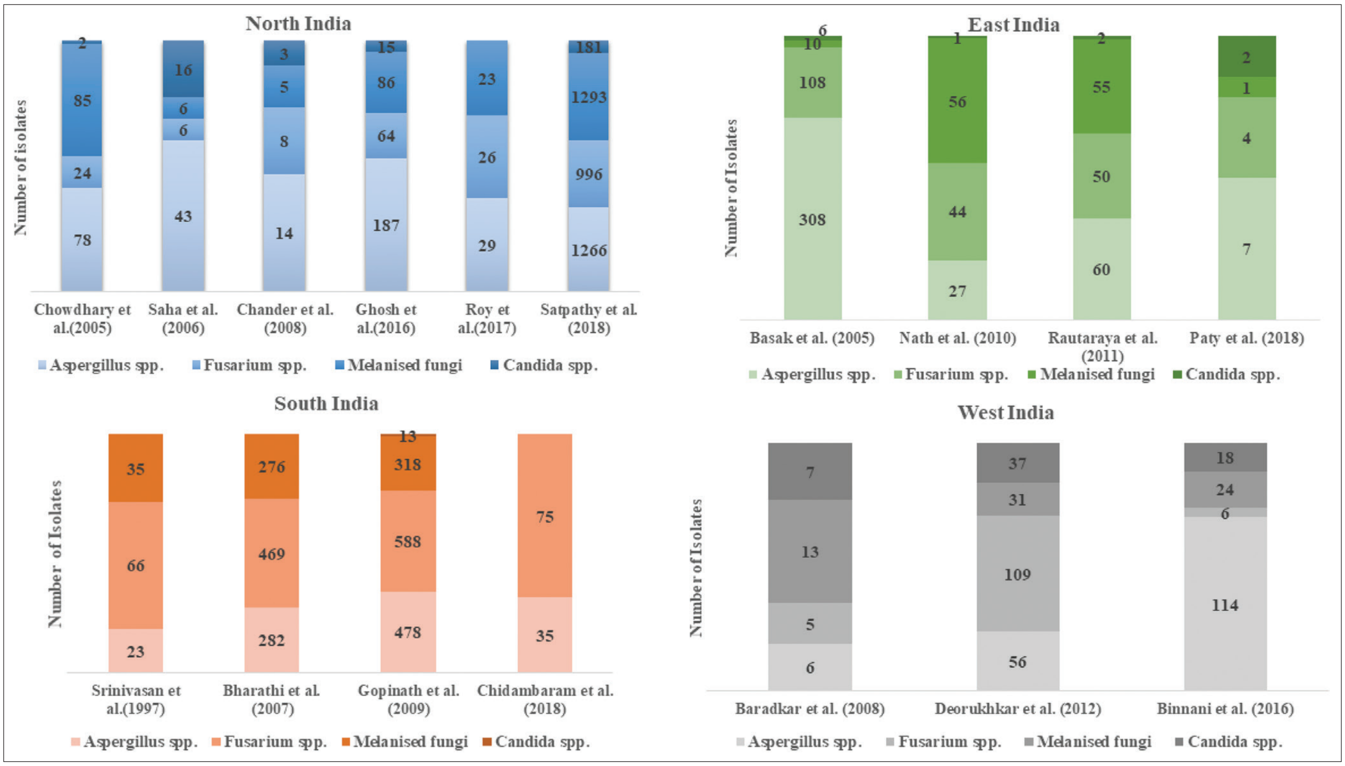

Supplementary Figure 1: Region wise distribution of etiological agents