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

Yamini Tawde, Shreya Singh, Sourav Das, Shivaparaksh M Rudramurthy, Harsimran Kaur, Amit Gupta, 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.

Key words: Aspergillus, clinical isolates, environment, fungal keratitis, Fusarium, India

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. Aspergillus sp. and Fusarium sp. being the predominant causative agents in India. Melanized fungi including Curvularia sp., Alternaria sp., Bipolaris sp., Cladosporium sp., and Scedosporium sp. are emerging as causative 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.

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. Additionally, the correlation between environmental fungi with fungi...
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.

**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, 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 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®, SampFairTM 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 |


**Figure 1: Regional distribution of clinical and environmental samples**
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 ($\chi^2$) or Fisher’s exact test when applicable and a ‘$P$’-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

| State   | District  | Village                  | No of samples | Total Mycelial Count | Aspergillus sp. | Fusarium sp. | Black fungus | Other mycelial fungus |
|---------|-----------|--------------------------|---------------|----------------------|----------------|--------------|--------------|-----------------------|
| North- East India | | | | | | | | |
| 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             | 30                   | 495            | 197          | 210          | 25                    | 63                    |

| North India | | | | | | | | |
| 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 | Abdullahpur        | 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             | 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 discoloration (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 Aspergillus fumigatus (90.42%), 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. guillermontii (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 = 0.00) 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]     | 119/191 (62.30%) Male-130/191 | Male-130/191 (68%)    | Vegetative matter-33/63 (52.3%) Animal mater-12/63 (19%) Dust/stone-10/63  |
|          | Jan 1999- June 2001             | 191/485 (39%)                  | 31-40                | (15.6%) Iron particle- 5/63 (7.9%)                                            |
|          | Saha et al. (2006)[11]          | 67/346 (19.36%) Male-60/77    | Male-60/77 (77.9%)    | Vegetative matter-24/64 (37.5%)                                              |
|          | Jan 2000-Dec-2004               | 74/346 (21.38%)                | 50-60                | Chronic antibiotic usage -16/64 (25%)                                         |
|          | Chander et al. (2008)[12]       | 52/64 (81.25%) Male-124/154   | Male-124/154 (80.52%) | Use of topical corticosteroids-5/64 (7.81%)                                   |
|          | Jan 1999- Dec 2003              | 34/64 (53.12%)                 | 21-50                |                                                                                |
|          | Ghosh et al. (2016)[9]          | 765/2495 (31%) Male-602/765   | Male-602/765 (78.7%)  | Vegetative matter-50/197 (25.38%)                                            |
|          | 2005-2011                        | 393/765 (51%)                  | 21-60                | Foreign particle-51/197 (25.88%)                                             |
|          | Roy et al. (2017)[13]           | 147/400 (36.75%) Males-72/94  | Males-72/94 (76%)     | Dust/stone- 10/197 (5.07%)                                                    |
| East     | Satpathy et al. (2018)[17]      | 4069/18898 (21.5%) NM         | 41-50                | Corneal trauma-84/94 (89.36%)                                                |
|          | Jan 2016- Dec 2016              |                                |                      |                                                                                |
|          | Basak et al. (2005)[2]          | 231/1198 (19.3%) Males-846/1198| Males-846/1198 (70.6%)| Vegetative matter-715/1198 (59.68%)                                           |
|          | Jan 2001-Dec 2003               | 509/1198 (42.5%)               | 31-40                | Dust/stone- 134/1198 (11.2%)                                                 |
|          | Nath et al. (2010)[23]          | 124/188 (65.2%) Males-108/157 | Males-108/157 (68.7%) | Insect-86/1198 (7.2%)                                                         |
|          | Apr 2007- Mar 2009              | 157/310 (50.64%)               | 41-50                | Foreign particle-29/1198 (2.4%)                                              |
|          | Rautaraya et al. (2011)[26]     | 264/997 (26.4%) Male-185/264  | Male-185/264 (70.1%)  | Corneal traumas-106/264 (40.15%)                                             |
|          | Jul 2006- Dec 2009              | 215/264 (81.4%)                | 50-60                |                                                                                |
|          | Paty et al. (2018)[24]          | 13/50 (26%) Male-11/16        | Male-11/16 (68.75%)   |                                                                                |
|          | 3 months                         | 16/50 (32%)                    | 30-45                |                                                                                |
| South    | Srinivasan et al. (1997)[14]    | NM                              | Male-266/434 (61.3%)  | Vegetative matter-168/284 (59.15%)                                           |
|          | Jan 1994- March 1994            | 138/297 (46.46%)               | 51-60                | Dust/stone- 51/284 (18%)                                                     |
|          | Bharathi et al. (2007)[15]      | NM                              | Male-1879/3183 (59.03%)| Animal mater-16/284 (5.6%)                                                   |
|          | Sep 1999- Aug 2002              | 1090/1171 (92.69%)             | 21-50                | Iron particle- 14/284 (4.9%)                                                 |
|          | Gopinath et al. (2009)[16]      | 1511/1598 (94.6%) Male-4087/5897| Male-4087/5897 (69.3%)| Vegetative matter-675/1095 (61.28%)                                         |
|          | Feb 1991- Jun 2001              | 1598/3563 (44.8%)              | NM                   | Dirit- 163/1095 (14.88%)                                                     |
|          | Chidambaram et al. (2018)[17]   | NM                              | Male-162/252 (64%)   | Dust/stone- 79/1095 (7.21%)                                                  |
|          | Feb 2012- Feb 2013              | 191/252 (77%)                  | 37-60                | Animal mater-62/1095 (5.66%)                                                 |
|          | Baradkar et al. (2008)[18]      | 14/34 (41.17%) Males           | Male-14/34 (31.07%)   |                                                                                |
|          | Jan 2005- June 2006             | 34/260 (13.07%)                | 20-50                |                                                                                |
|          | Deorukkar et al. (2012)[19]     | NM                              | Male-582/852 (68.31%) | Ocular Trauma-502/537 (93.48%)                                               |
|          | Dec 2004- Dec 2009              | 311/537 (57.91%)               | 21-30                |                                                                                |
|          | Binnani et al. (2016)[20]       | 66/180 (36.67%) Male-129/180  | Male-129/180 (71.66%) | Vegetative matter-77/180 (42.78%)                                            |
|          | Jul 2005- Jun 2012              | 180/480 (58.33%)               | 21-40                | Foreign particle-36/180 (20%)                                                |
|          | Ostwal et al. (2016)[21]        | 20/100 (20%) Male-83/100       | Male-83/100 (83%)     | Ocular trauma                                                                 |
|          | Apr 2013- Mar 2015              | 24/100 (24%)                   | 25-34                |                                                                                |
|          | Mohod et al. (2018)[22]         | NM                              | Male-54/88 (61.36%)   | Ocular trauma -22/88 (25%)                                                   |
|          | Jan 2015- Feb 2017              | 52/88 (59.09%)                 | 41-60                |                                                                                |

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 (5.03%) 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.[10,5,8,22] 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.[18] 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.[30] 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.[31] 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.[32] 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,[33] 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.[34] 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.

**Financial support and sponsorship**

The study was funded by Department of Biotechnology under twinning project between Northeast India and North India.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Satpathy G, Ahmed NH, Nayak N, Tandon R, Sharma N, Agarwal T, et al. Spectrum of mycotic keratitis in north India: Sixteen years study from a tertiary-care ophthalmic centre. J Infect Public Health 2019;12:367-71.
2. Basak SK, Basak S, Mohanta A, Bhowmick A. Epidemiological and microbiological diagnosis of suppurative keratitis in Gangentic West Bengal, eastern India. Indian J Ophthalmol 2005;53:17-22.
3. Nath R, Baruah S, Saikia L, Devi B, Borthakur A, Mahanta J. Mycotic corneal ulcers in upper Assam. Indian J Ophthalmol 2011;59:367-71.
4. Pady BP, Dash P, Mohapatra D, Chayani N. Epidemiological profile of mycotic keratitis in a tertiary care center of eastern Odisha. J Dr NTR Univ Health Sci 2018;7:23-5.
5. Rautaraya B, Sharma S, Kar S, Das S, Sahu SK. Diagnosis and treatment outcome of mycotic keratitis at a tertiary eye care center in eastern India. BMC Ophthalmol 2011;11:1-8.
6. Mills B, Radhakrishnan N, Karthikeyan Rajapandian SG, Rameshkumar G, Lalitha P, Prajna NV. The role of fungi in fungal keratitis. Exp Eye Res 2021;202:108372.
7. Ghosh A, Kaur H, Gupta A, Singh S, Rudramurthy SM, Gupta S, et al. Emerging dematiaceous and hyaline fungi causing keratitis in a tertiary care centre from North India. Cornea 2020;39:868-76.
8. Ghosh AK, Gupta A, Rudramurthy SM, Paul S, Hallur VK, Chakrabarti A. Fungal keratitis in North India: Spectrum of agents, risk factors and treatment. Mycopathologia 2016;181:843-50.
9. Alborch L, Brugalt MR, Cabafies FJ. Comparison of two selective culture media for the detection of *Fusarium* infection in conventional and transgenic maize kernels. Lett Appl Microbiol 2010;50:270-5.
10. Chowdhary A, Singh K. Spectrum of fungal keratitis in North India. Cornea 2005;24:8-15.
11. Saha R, Das S. Mycological profile of infectious Keratitis from Delhi. Indian J Med Res 2006;123:159-64.
12. Chander J, Singla N, Agnihotri N, Arya SK, Deep A. Keratomycosis in and around Chandigarh: A five-year study from a north Indian tertiary hospital. Indian J Pathol Microbiol 2008;51:304-6.
13. Roy P, Das S, Singh NP, Saha R, Kajla G, Snehaa K, et al. ScienceDirect changing trends in fungal and bacterial profile of infectious keratitis at a tertiary care hospital: A six-year study. Clin Epidemiol Glob Heal 2016;5:40-5.
14. Srinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, et al. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalmol 1997;81:965-71.
15. Bharathi MJ, Ramakrishnan R, Meenakshi R, Padmavathy S, Shivakumar C, Srinivasan M. Microbial keratitis in South India: Influence of risk factors, climate, and geographical variation. Ophthalmic Epidemiol 2007;14:61-9.
16. Gopinathan U, Sharma S, Garg P, Rao GN. Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: Experience of over a decade. Indian J Ophthalmol 2009;57:273-9.
17. Devi J, Venkatesh N, Srikantti P, Lanjewar S. Epidemiology, risk factors, and clinical outcomes in severe microbial keratitis in India. Ophthalmic Epidemiol 2018;25:297-305.
18. Baradkar VP, De A, Mathur M, Lanjewar M, Kumar S. Mycotic keratitis from Mumbai. Bombay Hosp J 2008;50:200-4.
19. Deorukhkar S, Katiyar R, Saini S. Epidemiological features and laboratory results of bacterial and fungal keratitis. A five-year study at a rural tertiary-care hospital in western Maharashtra, India. Singapore Med J 2012;53:264-7.
20. Binnani A, Gupta PS, Gupta A. Epidemiology-clinico-microbiological study of mycotic keratitis in North-West Region of Rajasthan. Mycopathologia 2018;183:717-22.
21. Ostwal KI. Mycotic keratitis in Solapur (A two years study). Natl J Lab Med 2016;5:30-3.
22. Mohod P, Nikose A, Laddha P, Bharti SB. Incidence of various causes of infectious keratitis in the part of rural central India and its visual morbidity: Prospective hospital-based observational study. J Clin Ophthalmol Res 2019;7:31.
23. Hoffman JJ, Burton MJ, Leck A. Mycotic keratitis — A global threat from the filamentous fungi. J Fungi (Basel) 2021;7:1-36.
24. Dan J, Zhou Q, Zhai H, Cheng J, Wan L, Ge C, et al. Clinical analysis of fungal keratitis in patients with and without diabetes. PLoS One 2018;13:e0196741.
25. Chang YS, Tai MC, Ho CH, Chu CC, Wang JJ, Tseng SH, et al. Risk of corneal ulcer in patients with diabetes mellitus: A retrospective large-scale cohort study. Sci Rep 2020;10:1-6.
26. Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in non-insulin-dependent diabetes mellitus. Ophthalmology 2001;108:566-92.
27. Garg P, Gopinathan U, Choudhary K, Rao GN. Keratomycosis: Clinical and microbiological experience with dematiaceous fungi. Ophthalmology 2000;107:574-80.
28. Venkatesh Prajna N, Krishna T, Mascarenhas J, Srinivasan M, Agarwal T. Predictors of outcome in fungal keratitis. Eye 2012;26:1226-31.
29. Nosanchuk JD, Casadevall A. Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Antimicrob Agents Chemother 2006;50:3519-28.
30. Geology India-Agriculture-Tutorialspoint. Available from: https://www.tutorialspoint.com/geography/geography_india_agriculture.htm. [Last accessed on 2021 May 18].
31. Average Rainfall of States in India. Available from: http://www.rainwaterharvesting.org/urban/rainfall.htm. [Last accessed on 2021 May 18].
32. Rahkhya R, Singh G, Urhekar AD. Virulence factors detection in *Aspergillus* isolates from clinical and environmental samples. J Clin Diagn Res 2017;11:DC13-8.
33. Cho SY, Lee DG, Kim WB, Chun HS, Park C, Myong JP, et al. Epidemiology and antifungal susceptibility profile of *aspergillus* species: Comparison between environmental and clinical isolates from patients with hematologic malignancies. J Clin Microbiol 2019;57:1-13.
Supplementary Figure 1: Region wise distribution of etiological agents