

## Mycotic corneal ulcers in upper Assam

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**Purpose:** To study the association of various risk factors and epidemiological variables of mycotic keratitis treated at a tertiary referral hospital of upper Assam. **Materials and Methods:** In this hospital-based prospective study a total of 310 consecutive corneal ulcer cases attending the ophthalmology outpatient department of Assam Medical College were enrolled between April 2007 and March 2009. After clinical and slit-lamp biomicroscopic examination in all suspected cases, smears and culture examination for fungus was done to establish the etiology. Demographic information and associated probable risk factors of individual cases were noted in a predesigned questionnaire. **Results:** In 188 (60.6%) cases fungal etiology could be established. Out of them 67.6% were males. The most commonly affected age group was 41-50 years (25.5%). The maximum (23.4%) cases were reported during the paddy harvesting season in Assam (January and February). Fungal element could be demonstrated in 65.2% cases in direct potassium hydroxide (KOH) mount. The commonest predisposing factor was corneal injury (74.5%). While diabetes was a significant systemic predisposing factor in mixed bacterial and fungal infections in 11.1% cases, blocked naso-lacrimal duct was the local predisposing factor in 11.1% of cases. *Fusarium solani* (25%) was the commonest isolate followed by *Aspergillus* species (19%), *Curvularia* species (18.5%) and *Penicillium* species (15.2%). Yeasts were isolated in 2.7% (n=5) cases. **Conclusions:** Ocular trauma was the commonest cause of fungal corneal ulcer in Assam and *Fusarium solani* was the commonest species responsible for it. Most of the mycotic ulcer cases come from rural areas including the tea gardens.

**Key words:** Assam, corneal ulcer, fusarium, mycotic keratitis

Corneal ulceration is a major cause of blindness in developing countries. Most microorganisms can invade the corneal stroma if the normal corneal defense mechanisms are compromised.<sup>[1]</sup> Being in the subtropical region, the northeastern part of India has conducive environmental conditions like high rainfall, longer rainy season, and high humidity throughout the year for fungal growth. Moreover, the majority of the people are engaged in agriculture including the labor-intensive tea industry. These make them more vulnerable for corneal ulceration and fungal infection. However, systemic studies on fungal corneal ulcer from this region are scanty. In the above context, the present study was undertaken to know the risk factors and other epidemiological variables of fungal corneal ulcer cases reporting to a tertiary referral hospital of Assam.

## Materials and Methods

In this hospital-based prospective study, 310 consecutive patients of corneal ulcers attending the outpatient department of the ophthalmology department of the Assam Medical College and hospital were included. Except infants below one year of age, cases of all ages and either sex were included in the study. After clinical evaluation by the ophthalmologist, slit-lamp biomicroscopic examination was done by an experienced ophthalmologist. Cases with suppurative corneal infiltrate

and overlying epithelial defect, with or without history of trauma by plant matter were clinically suspected as mycotic keratitis. Once a clinical diagnosis of corneal ulcer was made, a trained interviewer filled up the proforma [Table 1]. Though all efforts were made for hospitalization to treat and follow up the cases, some patients opted for domiciliary treatment with regular follow-up for various reasons. Demonstration of fungal elements on corneal scraping and/or culture was regarded as confirmatory criterion for mycotic ulcer. Corneal scrapes were taken by applying topical anesthesia (4% lignocaine) with a sterile Bard parker blade (no. 15). Materials obtained were processed for direct 10% potassium hydroxide (KOH) mount examination, Gram stain and for culture in blood agar, chocolate agar, Sabouraud's dextrose agar with chloramphenicol (50 mg/ml), brain heart infusion broth and non-nutrient agar with an overlay of *Escherichia coli*. Sabouraud's dextrose agar plates were incubated at 28°C and were examined and observed at regular intervals for 15 days. Plates for bacterial culture were kept at 37°C and were observed for seven days. Fungi (when grown) were identified on the basis of colony texture, color, diffusible pigments, growth rate, aerial and submerged hyphae; microscopic features like mycelium, conidium, relationship between hyphae and fruiting bodies were noted from the culture. Slide cultures in potato dextrose agar and corn meal agar were used for observation of conidiogenesis of filamentous fungi for identification. Identification of yeast-like fungi was done by Dalmau plate morphology, urease production, assimilation and fermentation of sugar. Fungal etiology was considered when fungal growth was supported by positive direct microscopy or when growth of the same fungus was observed in more than two media or repeated isolation of the fungus on more than one occasion. Treatment was started after the microbiological sample was taken and in most of the cases after getting the smear result. For fungal ulcers, 5% natamycin

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**Table 1: Proforma**

Assam Medical College and Hospital, Dibrugarh  
 "Microbial agents of corneal ulcers in Upper Assam" - A study on patients attending Ophthalmology OPD, Assam Medical College and Hospital, Dibrugarh.

Serial No:

Hospital Registration No:

M.R.D. No. (if indoor patient):

Professor/Associate Professor/Assistant Professor of OPD:

Name of the patient:

Age:

Sex:

Occupation:

Consent:

Chief Complaints:

History of present illness:

\*Any history of trauma:

\*If any history of trauma, causative agent:

Sketch of  
the Ulcer

Treatment history of present illness (if any):

\*Duration of treatment

\*Any history of steroid medication

\*History of any surgical procedure on the eye

Past history if significant:

Examination of the patient: (1) General Examination (2) Systemic (Ocular) Examination

Ocular Examination (of both eyes)

1. VISUAL ACUITY (a) Distance (b) Near

2. EXTERNAL OCULAR EXAMINATION

(a) Inspection in diffuse light

(b) Focal (Oblique) illumination examination by slit-lamp

Examination sequence:

1. Head posture
2. Forehead
3. Eyebrows
4. Eyelids
5. Lacrimal apparatus
6. Eyeball
7. Conjunctiva
8. Sclera
9. Cornea
  - a) Size
  - b) Shape
  - c) Surface
  - d) Sheen
  - e) Transparency
  - f) Vascularization
  - g) Sensation
  - h) Bio-microscopic examination by staining with:
    - (i) Sodium-Fluorescein (ii) Rose Bengal

Detailed Examination of the Corneal Ulcer:

- a) Site
- b) Size
- c) Shape
- d) Depth

e) Floor

f) Margins

10. Anterior Chamber:

a) Depth

b) Hypopyon

c) Abnormal contents

11. Iris

12. Pupil

13. Lens

14. Any other significant findings

3. FUNDUS EXAMINATION (if possible):

Treatment provided in Assam Medical College and Hospital:

Follow-up findings:

Final Outcome:

eye drop was started hourly. Periodical debridement was done unless corneal thinning precluded it till the ulcer bed was clean. All the cases which failed to improve within seven days were reviewed for other local or systemic causes. Cases with deep ulcers involving the posterior stroma, non-resolving hypopyon, hypopyon more than one-third of the anterior chamber, impending or actual corneal perforation or in cases where endophthalmitis was suspected, oral fluoroquinolone and/or oral fluconazole were given. Depending upon the fungal species and response to therapy natamycin eye drop was substituted with itraconazole (1%) or voriconazole (1%) eye drop and oral antifungal therapy.

## Results

Out of 310 cases of corneal ulcers mycotic etiology was established in 188 (60.6%). Pure mycotic growth was detected in 157 and 27 showed bacteria mixed with fungus. Four cases were smear-positive for fungal hyphae but fungus could not be isolated in culture. These four cases were included based on clinical features and history of trauma by plant matter as well as direct smear result.

Pure bacterial etiology was established in 98 (31.6%) cases and in three (0.96%) cases *Acanthamoeba species* infection could be established.

In cases of pure mycotic ulcers, males were affected more than females (108 males and 49 females) [Table 2]; 87.9% patients were from villages and tea-garden areas and only 11.5% were from urban areas; 56.7% patients were tea-garden workers and 43.3% patients were rice-field workers. The most common affected age group was 41-50 years in both sexes, maximum (21.7%) patients attended the hospital during January-February (this period coincided with the harvesting season in this region). History of topical antibiotic use (85.3%) was found to be the most common. Ocular trauma was found to be associated with 76.4% cases. Diabetes was an associated factor in four (2.5%) cases, two such cases had infection with yeast. Other risk factors are shown in Table 3.

Hypopyon was present in 58 (36.9%) cases of pure fungal ulcers and 12 (7.6%) cases presented with perforation. Sixty-three (40.1%) cases of fungal ulcers improved clinically within

**Table 2: Age and sex distribution of corneal ulcers**

Age (Years)	Male (%)	Female (%)	Total (%)	Bacterial (%)	Fungal (%)
1-10	18 (5.8)	10 (3.2)	28(9.0)	7 (7.1)	19 (10.1)
11-20	22 (7.1)	6 (1.9)	28(9.0)	16 (16.3)	9 (4.8)
21-30	32 (10.3)	14 (4.5)	46 (14.8)	9 (9.2)	37 (19.7)
31-40	29 (9.4)	16 (5.1)	45(14.5)	13 (13.3)	27 (14.4)
41-50	42(13.5)	19 (6.1)	61(19.7)	17 (17.3)	48 (25.5)
51-60	39 (12.6)	18 (5.8)	57(18.4)	22 (22.4)	24 (12.8)
61-70	21 (6.8)	8 (2.6)	29 (9.4)	10 (10.2)	12 (6.4)
71-80	12 (3.9)	4 (1.3)	16 (5.1)	4 (4.1)	7 (3.7)

**Table 3: The different factors associated with cases with fungal and bacterial ulcers**

Factors	Fungal corneal ulcer (n=157)	Mixed bacterial and fungal ulcer (n=27)	Bacterial corneal ulcers (n=98)
Topical antibiotic use	134 (85.3)	27 (100)	22 (22.4)
Ocular trauma	120 (76.4)	(59.3)	43 (43.8)
Diabetes	4 (2.5)	3 (11.1)	3 (3.1)
Exposure keratitis	2 (1.3)	1 (3.7)	5 (5.1)
NLD block	3 (1.9)	3 (11.1)	12 (12.2)
Contact lens use	-	-	-
Corneal foreign body	-	-	5 (5.1)
Vitamin A deficiency	-	-	3 (3.1)
Viral keratitis	-	-	2 (2.04)
Post ocular surgery	-	-	2 (2.04)

NLD: Naso-lacrimal duct, Figures in parentheses are given in percentage.

seven days of starting treatment, whereas 45 (28.7%) cases took two weeks or more for improvement after starting antifungal treatment. Nine (5.7%) cases did not show any improvement up to 15 days of treatment or till the last follow-up. Sixty-nine (43.9%) cases healed with variable degrees of opacity, 19 (12.1%) cases healed after perforation leading to other sequels, two (1.3%) cases developed endophthalmitis.

Vegetative matter was the commonest agent (65.6%) injuring the eye [Table 4].

In cases of bacterial ulcers trauma was found to be predisposing factor in 43.8% cases against 76.4% cases of pure mycotic ulcers. Other risk factors are shown in Table 3.

In mixed (bacteria and fungus) infections, 63% (n=17/27) presented with hypopyon and 40.7% (n=11/27) presented with perforated ulcer. Bacterial isolates were coagulase-negative *Staphylococcus* (n=6), *Corynebacterium* species (n=6), *Staphylococcus aureus* (n=4), *Streptococcus* species (n=4), *Streptococcus pneumoniae* (n=3), *Escherichia coli* (n=2), *Acinetobacter lwoffii* (n=1) and non-fermenting Gram-negative bacilli (n=1). The commonest fungus isolated from 29.6% cases was *Aspergillus* spp [Table 5]. The different predisposing and associated factors found in this group are shown in Table 3.

Direct microscopic examination with 10% KOH showed

**Table 4: Corneal trauma-causing agents associated with fungal keratitis**

Cause of trauma	Pure fungal ulcers (%) n=120	Mixed bacterial and fungal ulcers (%) n=16
Vegetable matter—Leaf	60 (50)	6 (37.5)
Vegetable matter—Stem	9 (7.5)	3 (18.8)
Vegetable matter— Hay	34 (28.3)	5 (31.2)
Cow tail injury	9 (7.5)	-
Injury by nail	5(4.2)	1 (6.3)
Injury by flying insect	1(0.8)	-
Metallic substance	1 (0.8)	-
Soil and dirty water	1 (0.8)	1 (6.3)

presence of fungal element in 124 cases (65.2%). Hyaline fungus was found to be the commonest cause. The fungal species isolated are shown in Table 5 [Figs. 1 and 2].

Although all culture plates were examined daily for 15 days, no new growth could be detected after the fifth day.

## Discussion

In an earlier report from Assam, Dutta *et al.*, reported the incidence of fungal keratitis to be 32%.<sup>[2]</sup> However, our study showed a very high incidence (60.6%). Bharathi *et al.*, from South India reported almost equal incidence of bacterial and fungal infections.<sup>[1]</sup> Of course, from India, as high as a 63% incidence of fungal keratitis has been reported from West Bengal.<sup>[3]</sup> One apparent reason for the high incidence of mycotic keratitis in our study might be due to the inclusion of referred cases from peripheral hospitals which were treated empirically with antibacterials and failed to respond to treatment initially. This fact has also been supported by the highest (85.6%) association of mycotic keratitis cases with antibacterial treatment in our study. Out of 121 patients reported within the first week of development of symptoms, 47.1% were fungal and 28.9% were bacterial; which also indicates a higher prevalence of fungal keratitis in this part of India.

Mixed infection with bacteria is another problem in the diagnosis and treatment of fungal keratitis. Studies elsewhere from India reported mixed infection in 9.5-12% cases.<sup>[3,4]</sup> In our study 8.7% cases with mixed infection were detected. Agricultural activity and occupational ocular injury were the

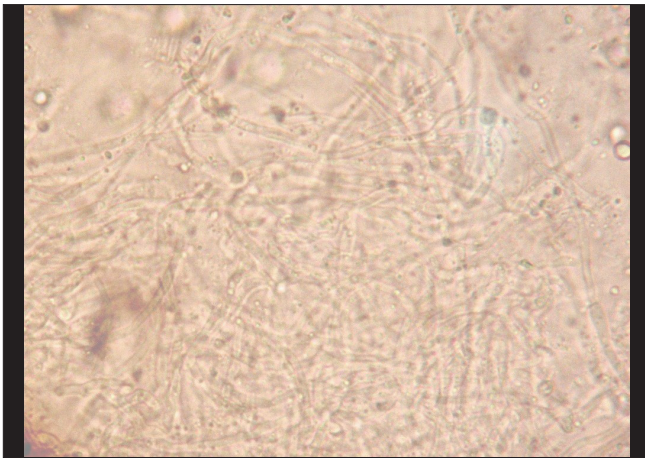


Figure 1: Abundant hyaline septate hyphae in 10% KOH



Figure 2: Corneal ulcer caused by *Fusarium solani*

Table 5: Fungal species isolated

Fungus isolated	Pure isolates N=157	Mixed with Bacteria n=27	Percentage of Culture- positive cases (n=184)
<i>Fusarium solani</i>	44	2	25
<i>Curvularia lunata</i>	25	3	15.2
<i>Penicillium</i> species	24	4	15.2
<i>Cladosporium</i> species	15	0	8.2
<i>Aspergillus fumigatus</i>	12	3	8.2
<i>Curvularia</i> species	6	0	3.2
<i>Bipolaris spicifera</i>	6	1	3.8
<i>Aspergillus flavus</i>	5	3	4.3
<i>Aspergillus</i> species	5	0	2.7
<i>Aspergillus niger</i>	5	2	3.8
Sterile hyphae	5	2	3.8
<i>Paecilomyces lilacinus</i>	3	0	1.6
<i>Scedosporium apiospermum</i>	2	0	1.1
<i>Acremonium killiense</i>	2	0	1.1
<i>Trichosporon</i> species	1	1	1.1
<i>Candida parapsilosis</i>	1	0	0.5
<i>Candida albicans</i>	0	2	1.1

principal factors associated with fungal corneal infection in this study. Male preponderance among them was similar with other studies from India.<sup>[4,5]</sup> Though a maximum number of 23.4% patients attended the hospital during January-February, the flow of patients was seen throughout the year. This period corresponds to the harvesting season in Assam leading to injury by vegetative matter as also indicated in studies elsewhere.<sup>[6]</sup> The percentage of trauma varies in different reports. In 37 (19.7%) cases of mycotic ulcers in our study no obvious history of trauma could be elicited. Of course, 16 (8.5%) of them had other risk factors like diabetes, exposure keratitis following hyperthyroidism and blocked naso-lacrimal duct.

Standard treatment protocols were followed in treating the



Figure 3: Colony of *Fusarium solani* in C streaks in blood agar medium

cases,<sup>[7,8]</sup> including oral antifungals.<sup>[9]</sup> Most of the cases (132 cases) showed good response to treatment within 15 days. These cases were either admitted to the hospital or were in regular follow-up for monitoring. Some patients were lost to follow-up. Mainly patients from the lower socioeconomic backgrounds, many of whom were the sole bread-earners of the family; in spite of motivation opted for domiciliary treatment. Some (38.2%, n=72) of the patients came to the hospital after two weeks of injury or development of symptoms and 23 of them had perforation of cornea. Therapeutic penetrating keratoplasty could not be done in many cases because of non-availability of donor corneas during this period. It could be done in three (13%) cases. The best corrected visual acuity of these cases improved to 20/200. Before keratoplasty the visual acuity was less than counting fingers at 5 meters.

Percentage of agreement between direct KOH mount and culture was 65.2%, and specificity was 96.8% which is similar to the experience elsewhere.<sup>[4,10-12]</sup>

Four samples, which were KOH mount-positive for fungal elements did not yield any growth in culture. These samples showed more than 10 hyphal filaments per high-power field. Others have also reported similar findings.<sup>[10-12]</sup> Two of these

cases responded to antifungal therapy and two other cases did not turn up for follow-up. Sharma *et al.*, opined that KOH-positive cases could be considered as fungal ulcers irrespective of the fungal culture positivity.<sup>[12]</sup> The probable cause of this may be due to presence of non-viable fungal elements at the time of culture due to prior administration of antifungal drug, but the exact cause in our cases could not be ascertained.

The most common fungus found in this study was [Fig. 3] *Fusarium solani* (25%), which is at a variance with the other studies from India. Studies elsewhere reported *Aspergillus* species (40-59%) to be commoner than others.<sup>[3-5,10]</sup> But Bharathi *et al.*, reported *Fusarium* species to be the commonest fungus in their study.<sup>[1,13]</sup> *Curvularia* spp was implicated as the causative agent in 18.5% cases in the present study which is in agreement with others (10-29%).<sup>[4,5,10,14,15]</sup> In 15.2% cases *Penicillium* species was isolated as against 10.1% of Basak *et al.* Yeast isolation from corneal ulcers is generally low in India.<sup>[3,4]</sup> But Saha *et al.*, reported 21.6% cases.<sup>[10]</sup> Diabetes was an associated factor in seven fungal keratitis cases in our study, three such cases had infection with yeast. But there was history of diabetes in only 2.2% cases with mold isolation. Because of less number of cases, the associated predisposing factors for yeast infection could not be ascertained. But diabetes is certainly an important predisposing factor in these cases.

In our study, ocular trauma was the most common predisposing factor in 74.5% cases; most common trauma causing agent was plant matter including 47.9% by leaf, 9.3% by stem and 29.3% by hay. Ocular trauma caused by vegetable matter has been reported in 61.28% cases in one study from India.<sup>[16]</sup> Though contact lens wear is a common predisposing factor in developed countries,<sup>[17]</sup> no such case could be detected in this study probably because only a limited number of people in our group used contact lenses. Bharathi *et al.*, reported this factor in less than 2% cases.<sup>[18]</sup> In our study, none of the cases gave history of using any topical corticosteroid.

Fungal infections were found in 60.6% cases, which is higher than many of the studies worldwide. *Fusarium solani*, *Aspergillus* spp, *Curvularia* spp and *Penicillium* spp are the most common species isolated from fungal keratitis. Rural agricultural workers were more vulnerable to fungal keratitis.

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