

## A Prospective Cross-Sectional Assessment of the Clinico Microbiological Profile of Patients with Fungal Corneal Ulcer

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### Abstract

**Aim:** To evaluate the clinical features, laboratory diagnosis and microbiological profile of fungal keratitis.

**Method:** A prospective cross-sectional study was conducted on 50 patients with features of corneal ulcer presenting to the Department of Ophthalmology Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India. from March 2018 to February 2019. Corneal ulcer with or without intact epithelium were included in the study. Clinical diagnosis of fungal keratitis was made on the basis of following features: dry raised slough, stromal infiltrate with feathery edges, satellite lesions and thick endothelial exudate. A standardized proforma was filled up for each patient documenting socio-demographic features duration of symptoms, history of trauma, associated ocular and systemic conditions, prior therapy received, and all other clinical findings including visual activity.

**Results:** Out of the 50 patients of corneal ulcers included, mycotic etiology was established in 27 cases [54%]. Out of 30 fungal corneal ulcer cases males (19, 70.4%) were affected more than females (8, 29.6%). Majority of the patients were more than 40 years of age (11, 40.7%) followed by 31-40 years of age group (7, 25.9%). Most patients were from rural areas (17, 62.9%). Corneal trauma (22, 81.5%) was the most common predisposing factor. 4 patients (14.8%) had co-existing ocular diseases responsible for development of fungal keratitis. Use of topical steroids predisposing to fungal keratitis accounted for 3 cases (11.1%) and associated systemic diseases were seen in 7 cases (25.9%).

**Conclusion:** A good clinical evaluation aided by microbiological support will help in better diagnosis and treating the corneal ulcer. In cases of fungal corneal ulcer, *Fusarium* spp is the most common organism responsible followed by *Aspergillus* spp.

**Keywords:** Corneal Ulcer, Fungal Keratitis, Microbiological.

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## Introduction

Corneal ulcer is an inflammatory or, more seriously, infective condition of the cornea involving disruption of its epithelial layer with involvement of the corneal stroma. In ophthalmology, a corneal ulcer usually refers to having an infectious cause while the term corneal abrasion refers more to physical abrasions [1]. Fungal corneal ulcer or fungal keratitis or keratomycosis refers to an infective process of the cornea caused by any of the multiple pathologic fungi capable of invading the ocular surface. It is most typically a slow, relentless disease that must be differentiated from other types of corneal conditions with similar presentation; especially its bacterial counterpart, which accounts for the majority of the microbial corneal infections. It can cause rapid visual loss and pain.

Risk factors that can predispose to fungal keratitis include contact lens wear, eye trauma with vegetative matter, previous ocular surgery, topical steroid use, and immunosuppression. The infection probably starts when the epithelial integrity is broken either due to trauma or ocular surface disease and the organism gains access into the tissue and proliferates. Proteolytic enzymes, fungal antigens and toxins are liberated into the cornea with the resulting necrosis and damage to its architecture thus compromising the eye integrity and function.

Corneal ulceration results in 1.5-2 million new cases of corneal blindness annually, posing a major public health problem according to the World Health organization (WHO) reports [2]. Fungi are the most common etiological agents which account for 30–40% whereas bacteria account for 13–48% of all cases of suppurative keratitis; this varies by geographical area [3]. The burden of corneal disease in our country is reflected by the fact that 90% of the global cases of

ocular trauma and corneal ulceration leading to corneal blindness occur in developing countries [4].

Corneal ulcer is an ophthalmic condition requiring prompt medical attention. Thus, precise knowledge of the causative agents and their susceptibility patterns is important for deciding the proper course of treatment [5]. Infectious corneal ulcers need to be treated as soon as possible to preserve vision. If left untreated, a fungal infection can lead to perforation of the cornea, loss of vision, and even loss of the eye.

### Methodology:

A prospective cross-sectional study was conducted on 50 patients with features of corneal ulcer presenting to the Department of Ophthalmology Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India from March 2018 to February 2019

### Inclusion criteria:

Corneal ulcer with or without intact epithelium were included in the study. Clinical diagnosis of fungal keratitis was made on the basis of following features: dry raised slough, stromal infiltrate with feathery edges, satellite lesions and thick endothelial exudate.

### Exclusion criteria:

Complicated corneal ulcers like those associated with endophthalmitis and perforation, typical viral ulcers, mooren's ulcers, interstitial keratitis, sterile neurotrophic ulcers, and any ulcers associated with autoimmune diseases were excluded from the study.

### Methodology

A standardized proforma was filled up for each patient documenting socio-demographic features duration of symptoms, history of trauma, associated ocular and systemic conditions, prior

therapy received, and all other clinical findings including visual activity.

**Clinical examination:** Each patient was subjected to a detailed slit-lamp examination. The ulcer was stained with 2% sodium fluorescein dye and its size was measured with variable slit on the slit lamp, the longest vertical and the widest horizontal dimensions were recorded in millimeter (mm). The size and depth of the infiltrate was also recorded. Hypopyon when present was recorded, and its height measured in mm. Other features of the ulcer like margins, floor, and presence of satellite lesions, retained foreign body and pigmentation over the ulcer were noted. Use of contact lenses and topical corticosteroids/ antibiotics were also recorded. Under aseptic precautions corneal scrapings were taken by an experienced ophthalmologist. The procedure was performed after instillation of 4% topical lignocaine under slit-lamp magnification, using a sterile no.15 bard-parker blade. The material was first obtained from the leading edge and then one more scraping from the base of the ulcer was inoculated into the surface of solid media such as sheep's blood agar, chocolate agar and Sabouraud's Dextrose Agar (SDA) in a row of C-shaped streaks. The material was also smeared on 2 separate glass slides one for gram stain and the other for 10% KOH preparation [6]

**Microbiological examination:** Aerobic incubations were done for all inoculated media [7]. Sabouraud's dextrose agar (SDA) were incubated at 27°C and after daily examination they were discarded

after 3 weeks, if there was no growth. The blood agar, chocolate agar was incubated at 37°C examined at 24 hrs and 48 hrs and then discarded if there was no growth [7]. All laboratory methods were performed following standard protocols [6, 7]. Microbial cultures were considered positive if at least one of the following criteria were met; Growth of the same organism was demonstrated on 2 or more solid media or confluent growth at the site of inoculation on one solid medium or Growth on one medium consistent with direct microscopic observations as in KOH preparation or Gram stain [6, 7]. Fungi were identified by their colony characteristics on SDA and by their microscopic appearance in lactophenol cotton blue (LPCB).

#### Results:

Out of the 50 patients of corneal ulcers included, mycotic etiology was established in 27 cases [54%]. Out of 30 fungal corneal ulcer cases males (19, 70.4%) were affected more than females (8, 29.6%). Majority of the patients were more than 40 years of age (11, 40.7%) followed by 31-40 years of age group (7, 25.9%). Most patients were from rural areas (17, 62.9%). Corneal trauma (22, 81.5%) was the most common predisposing factor. 4 patients (14.8%) had co-existing ocular diseases responsible for development of fungal keratitis. Use of topical steroids predisposing to fungal keratitis accounted for 3 cases (11.1%) and associated systemic diseases were seen in 7 cases (25.9%).

**Table 1: Demographic details, predisposing factors and traumatic agents in all the cases**

Variables:		Number	%
Gender	Male	19	70.4
	Female	8	29.6
Age	<21	3	11.1
	21-30	6	22.2
	31-40	7	25.9
	>40	11	40.7

<b>Lifestyle</b>	<b>Rural</b>	17	62.9
	<b>Urban</b>	10	37.1
<b>Predisposing factors</b>	<b>Corneal trauma</b>	22	81.5
	<b>Co-existing ocular disease</b>	4	14.8
	<b>Topical steroids use</b>	3	11.1
	<b>Systemic diseases</b>	7	25.9
<b>Traumatic agent</b>	<b>Vegetative matter</b>	14	51.8
	<b>Foreign body</b>	7	25.9
	<b>Insects</b>	3	11.1
	<b>Others</b>	3	11.1

Out of 50 patients, pure mycotic growth was detected in 27 cases (54%), 3 cases (6%) showed bacteria mixed with fungus, and Pure bacterial etiology was established in 9 cases (18%). 11 cases were culture negative (22%). 20 out of 27 (74.1%) positive fungal culture were hyaline (non-pigmented) group and 7 cases (25.9%)

were dematiaceous (pigmented) group. The most commonly isolated fungal species out of non-pigmented group was *Fusarium* species (11 cases, 40.7%) followed by *Aspergillus* species (6 cases, 22.2%) and in pigmented group, most common isolated species was *Cladosporium* species (4 cases, 14.8%).

**Table 2: Fungal pathogens isolated from microbiological examination**

Type of fungi	Species name	Number	%
<b>Hyaline fungi</b>	<b><i>Fusarium</i> spp.</b>	11	40.7
	<b><i>Aspergillus</i> spp.</b>	6	22.2
	<b><i>Mucor</i> spp.</b>	2	7.4
	<b><i>Rhizopus</i> spp.</b>	1	3.7
<b>Dematiaceous fungi</b>	<b><i>Cladosporium</i> spp.</b>	4	14.8
	<b><i>Curvularia</i> spp.</b>	2	7.4
	<b><i>Biopolaris</i> spp.</b>	1	3.7

**Table 3: Correlation between clinical diagnosis and microbiological diagnosis**

Results	Detection of fungal elements in 10% KOH smear	Detection of fungal elements in gram-stained smear	Clinical suspicion of fungal keratitis on slip lamp examination
<b>Positive</b>	27	25	31
<b>Negative</b>	23	25	19
<b>Total</b>	50	50	50

### Discussion:

Fungal keratitis is a fungal infection of the cornea, which can lead to blindness [9]. It generally presents with a red, painful eye and blurred vision. There is also increased sensitivity to light, and excessive tears or discharge. The symptoms of fungal keratitis typically emerge over 5-10 days and present with a painful eye, blurred vision, and redness of eye. There is

increased sensitivity to light, and excessive tears or discharge. The symptoms are markedly less as compared to a similar bacterial ulcer [8]. The eyelids and adnexa involved shows edema and redness, conjunctiva is chemosed. Ulcer may be present. It is a dry looking corneal ulcer with satellite lesions in the surrounding cornea. Usually associated with fungal ulcer is hypopyon, which is mostly white

fluffy in appearance. Rarely, it may extend to the posterior segment to cause endophthalmitis in later stages, leading to the destruction of the eye.

The precipitating event for fungal keratitis is trauma with a vegetable / organic matter. A thorn injury, or in agriculture workers, trauma with a wheat plant while cutting the harvest is typical. This implants the fungus directly in the cornea. The fungus grows slowly in the cornea and proliferates to involve the anterior and posterior stromal layers. The fungus can break through the descemet's membrane and pass into the anterior chamber. The patient presents a few days or weeks later with fungal keratitis. In our study also, in 51.8% of cases, vegetative matter was the traumatic agent out of 81.5% cases accounting for corneal trauma. Similarly, corneal trauma has been identified as major cause of fungal keratitis in many studies [10-12].

Out of 30 fungal corneal ulcer cases males (19, 70.4%) were affected more than females (8, 29.6%) in this study. Similarly according to study conducted by Titiyaletal[13] and Shubhra Mehta [14], males were more affected compared to females. But in a study conducted by Upadhyay et al in Nepal [15], they have found almost equal distribution among both the sexes. In our study, 40.7% patients belonged to age group of more than 41 years. A similar study by Li et al. also noted that the age group which is highly presented as corneal ulcer belongs to age group 50-59 years, accounting for 83.21% [16] and SharmilaSuwal et al also reported 40% of their corneal ulcer belongs to age group of 51 to 60 years of age.[17] In contrast to this, Rumpa Saha from new Delhi Showed most affected age group was 31-40 years [18].

Among the fungal isolates in our study Fusarium spp 11(40.7%) was the most

isolated species followed by Aspergillus spp. 6(22.2%). In contrast to Alkatan et al [19] study who had reported aspergillus spp 27.6% as higher incidence than Fusarium spp 17.2%. Comparable results were obtained in studies done by Idiculla et al revealing Fusarium spp (50%) and Aspergillus spp (34.4%) predominate fungal isolates.[20] The difference in the isolation rates of these fungal pathogens can be explained by the difference in the climate and the natural environment of individual regions. Studies in the bihar region have shown a higher incidence of Fusarium as compared to studies in the bihar region [21].

### Conclusion:

A good clinical evaluation aided by microbiological support will help in better diagnosis and treating the corneal ulcer. In cases of fungal corneal ulcer, Fusarium spp is the most common organism responsible followed by Aspergillus spp. KOH wet mount preparation have more sensitivity than gram stain smear and helps in the early detection of species responsible for fungal infection.

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