

Spectrum of Mycotic corneal ulcers in Mid Western peripheral region of Terrain belt of Nepal and Indo-Nepal Border

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ABSTRACT

Background: The study was set in two different tertiary eye care hospitals of Nepal. The study area covered a large population of mid western, far western peripheral terrain regions of Nepal as well as the corresponding Indo-Nepal border. Looking at the high prevalence of mycotic corneal ulcers in the region, the study aimed to find out the pattern of culture proven fungal corneal isolates in diagnosed cases of mycotic corneal ulcers.

Methods: A prospective hospital based study was carried out on 2768 consecutive patients presenting with corneal ulcer in the outpatient department of Ophthalmology in Nepalgunj Medical College and Nepalgunj Eye Hospital, Nepalgunj Nepal from July, 2011 to July, 2012. Patient particulars with regard to the socio demographic data, predisposing risk factors, prior treatment modalities, laboratory results and the distribution pattern of culture proven fungal isolates were analyzed.

Results: Diagnosis of various types of pure fungal keratitis was established post investigations in 760 (27.45%) of all patients. Men 444 (58.42%) were more commonly affected than women. The young adults age group of 31-40 years was most commonly involved 220 (28.94%) in number. Trauma or injury to cornea 431 (56.71%) was the commonest predisposing risk factor. Fusarium species (Fusarium spp.) were the commonest fungal isolates accounting for 287 (37.76%) of diagnosed fungal ulcer cases, followed by curvularia 136 (17.89%).

Conclusion: Fusarium species are the commonest fungal isolates in cases of fungal corneal ulcer in mid western peripheral terrain belt of Nepal followed by curvularia and unidentified dematiaceous fungi.

Keywords: Corneal ulcer; fungal isolates; fusarium species; cornea; mycotic fungal corneal ulcers

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Background:

Corneal ulcer is a disease that warrants special attention and intervention as it may lead to irreversible damage to the cornea in short span of time and consequently blinding eye disease. Suppurative (bacterial and mycotic) corneal ulcer is still one of the leading causes of blindness. In Nepal annual

incidence is 799 cases per 1,000,000 population, of which nearly 22 % are of fungal origin.¹

Corneal ulcer, trauma to the eyes and sequale of corneal ulcer especially corneal scarring/corneal opacity stands only second to cataract worldwide, as the commonest cause of blindness in any age group. Various studies have concluded;

it is very common in the developing and underdeveloped nations. Hereby showing the importance of prevention, early diagnosis and treatment of suppurative corneal ulcers cases.¹⁻⁶

Previously, it was thought various ocular pathologies like trachoma, neonatal Ophthalmia, xerophthalmia and onchocerciasis (in Africa) play big role in developing corneal scarring or corneal opacity added by harmful eye practices by patients, medications by village healers and lack of specialist medical personnel. However, all recent studies and conclusions have made it clear that primary corneal ulceration is the major cause of corneal scarring or opacity.^{3,7,8,9}

Actual data on the annual incidence of infective ulceration are difficult to obtain in most countries. Available data indicate that, in the United States there are 11 corneal ulcers per 100,000 population annually.¹⁰ In southern part of India the number is 10 times higher with 113 per 100,000 population per year.¹¹ Corneal injury with vegetative matters highly increases the risk of developing fungal corneal ulcers. However, the culture proven fungal isolates can differ in different geographical areas.^{12,13}

As the recent studies or survey done in Nepal have been pointing towards changing pattern of ocular morbidity.¹⁴ This study was an attempt to find out and gather valuable information related to common types of pure fungal isolates in cases of diagnosed fungal corneal ulcers in mid western, far western peripheral terrain region of Nepal and corresponding areas of Indo-Nepal border. So that it helps in providing concrete evidence, in diagnosing and treating the cases more accurately.

Methods:

A hospital based, prospective, observational study was carried out with a primary objective to find out the distribution pattern of the common fungi in cases of fungal corneal ulcer from July, 2011 to July, 2012. All corneal ulcer cases attending the outpatient department and cornea clinic of two of the tertiary eye hospitals of mid western peripheral terrain belt of Nepal, (Nepalgunj Eye Hospital and Nepalgunj Medical College and Teaching Hospital, Nepalgunj) were included in the study. An informed consent was taken from all the study subjects. The study strongly adhered to the Tenets of the Declaration of Helsinki and also was approved by the ethical research committee of Nepalgunj Eye Hospital, Nepalgunj. Both the tertiary eye hospitals are well equipped with modern laboratory investigations.

The cases which were clinically diagnosed as a typical viral corneal ulcer, healing corneal ulcers, cases with mixed infections, non suppurative corneal ulcers, cases who have been already undergone investigations and were on treatment for corneal ulcer on presentation and cases not willing to be the part of study were excluded from the study.

A total of 2768 cases, after exclusion based on the above specified criteria, were evaluated in the outpatient department and cornea clinic over a period of one year. Corneal ulcer was defined as loss of corneal epithelium with variable corneal stromal infiltration, with clinical evidence of infection with or without hypopyon. A detailed history taking including systemic illnesses, clinical examination including ocular/slit-lamp bio-microscopic examination of the anterior segment of the eye with more focus to the corneal ulcer was carried out; when needed and when visible fundus evaluation was carried out in affected eye while routine detailed ocular examination was carried out in normal eye. In the affected eye after staining the ulcer with sodium fluorescein the size of the ulcer, stromal infiltrate and depth was measured using the variable slit on the slit lamp and recorded in millimeter. The hypopyon if present was noted and measured similarly in millimeters. The ulcer margin, thinning of the floor, satellite lesions, any retained foreign body and pigmentation over the ulcer surface was recorded. A diagram of each ulcer was drawn on the standardized form by performing frontal and cross sectional sketches. Associated ocular conditions like blepharitis, conjunctivitis, dacryocystitis, corneal anesthesia, dry eyes, lid abnormalities, lagophthalmos, past surgery in the cornea, use of contact lens and corticosteroids were noted.

One corneal swab and three corneal scrapings were collected from each patient by an Ophthalmologist with all aseptic precautions to detect or to exclude possible bacterial corneal ulcer or mixed growth. Corneal swab was taken by rubbing the ulcerated area of the cornea with sterile cotton swab soaked with sterile normal saline before instillation of local anesthetic and was used for Gram, Giemsa staining purpose.¹⁵ For taking corneal scrapings; two drops of preservative free local anesthetic (1% Proparacaine) were put in the affected eye. Five minutes after instillation of local anesthetic, three corneal scrapings were taken by sterile Bard Parker No. 15 scalpel blade or a flame sterilized platinum Kimura spatula under a biomicroscope. Great care was taken for not to touch the lashes or lids and to obtain material from the base and the peripheral margins of ulcer. The swab was inoculated onto Blood agar, MacConkey's agar, and Chocolate agar media and incubated aerobically at 37 degrees Celsius for

maximum up to 48 hours. To ensure 5-10% CO₂, incubated Chocolate agar plates were put under candle extinction jar. All the bacterial isolates were identified by their colony morphology, gram staining, motility testing by hanging drop preparation, pigment production and relevant biochemical tests.^{16,17}

To detect fungal corneal ulcers, corneal scrapings were used for wet preparation in 10% KOH, Gram stain and Giemsa stain. For fungus culture; materials obtained by scraping were spot inoculated on plain Saboraud's dextrose agar medium (SDA) and in to Brain Heart Infusion Broth (BHI).

The inoculation technique consisted of "C" streaks on the culture plate, with the idea to localize the site of implantation of the corneal scraping on the agar media. Inoculated SDA media was incubated at 25 degree Celsius. Only growth occurring on the "C" streaks was considered as significant and out growth away from the "C" streak was discarded as contaminants.¹⁸

The plates which did not show any evidence of growth after 14 days were discarded. For identification of fungal species that grown in SDA, microscopical examination in wet preparation was done besides subculturing onto SDA media.

Any growth observed was identified using standard bacteriological and mycological techniques.¹⁹ Microbial cultures were considered significant if any/all of the following criteria were satisfied: (1) growth of the same organism was demonstrated on more than one solid medium; (2) confluent growth was seen at the site of inoculation on one solid medium; (3) growth of one medium was consistent with direct microscopy findings (appropriate staining and morphology with Gram stain); (4) the same organism was grown from repeated scraping material.²⁰

Cultures were considered negative if no growth was found after even 96 hours. Identification of fungi in SDA medium was done by a trained microbiologist and a group of ophthalmologists. For all cases, treatment was commenced empirically with broad spectrum topical antibiotics immediately after the diagnosis was made, most of the cases were admitted, while the cases refusing admission were followed up daily with same medications. Subsequent treatment was tailored according to the microbiological diagnosis and sensitivity results. The final visual acuity was defined as the visual acuity on discharge from the ward. The patients were asked to follow-up every 7 days for 3 weeks and then 2 weekly for 2 months, after which follow-up was individualized as per patient's requirements. A careful examination of the staining pattern and culture

growth with the help from the microbiologist made sure correct data and information were entered in the proforma specifically designed for the study. Positive findings were recorded and data was entered in the statistical package for social sciences (SPSS) data base version 19.0 and the results were interpreted.

Results:

A total of 2768 consecutive cases meeting inclusion criteria were included in the study. Out of the total study population 760 (27.45%) cases were pure fungal isolates. Men 444 (58.42%) were more commonly affected than women 316 (41.48%). The commonest age group affected was young adults in the prime working age groups. Though the number of cases with proven fungal isolates was in decreasing trend towards the elderly age group and younger age groups; accounting for around 14% in patients above 60 years of age and approximately 12% in patients below 20 years of age (Table 1).

Table 1: Distribution of fungal corneal ulcers in different gender and age groups

Age group (Years)	Male	Female	Total	Percentage (%)
0 - 10	18	12	30	3.94
11 - 20	35	25	60	7.89
21 - 30	76	65	141	18.55
31 - 40	135	85	220	28.94
41 - 50	70	48	118	15.52
51 - 60	49	36	85	11.18
61 - 70	40	31	71	9.34
71 - 80	18	12	30	3.94
81 - 90	3	2	5	0.65
Total	444 (58.42%)	316 (41.58%)	760	100 (99.55)

By occupation; more than half patients 51.18% of total fungal isolates were actively involved in agricultural work and farming followed by housewives 15.79%, students 10.72% and other occupations (Table 2).

Table 2: Distribution of fungal isolates according to occupation of patients

Occupation	Number of pure fungal isolates in patients	Percentage (%)
Agriculture (Farmers)	389	51.18
Housewives	120	15.79
Students	80	10.52
Office holders	56	7.36
Children	35	4.60
Labourers	30	3.94
Others	50	6.57
Total	760	100

Trauma or injury to cornea 431 (56.71%), prior topical steroids use 89 (11.71%), chronic ocular surface disorder 76 (10.00%) were the most common predisposing risk factors noted which were followed by systemic diseases, contact lens wear and others (Table 3).

Table 3: Distribution of risk factors amongst the patients

Risk factors	Number of cases	Percentage (%)
Trauma	431	56.71
Topical steroid use	89	11.71
Chronic ocular surface disorder	76	10.00
Systemic diseases	54	7.10
Contact lens wear	52	6.84
Others	58	7.63
Total	760	100

Fusarium species (*Fusarium* spp.) were the commonest fungal isolates accounting for 287 (37.76%) of diagnosed fungal ulcer cases, followed by *Curvularia* 136 (17.89%), unidentified dematiaceous fungi 112 (14.73%), *Aspergillus* species 95 (12.50%) and unidentified hyaline in 47 cases (6.18%). While *Candida* species, *Bipolaris* species, *Penicillium*, *Alternaria*, *Mucor*, *Rhizopus* and unidentified were the other fungal isolates diagnosed (Table 4).

Table 4: Pure fungal isolates identified from patients with corneal ulcers

Isolate	Total number (n = 760)	Percentage (%)
<i>Fusarium</i> species	287	37.76
<i>Curvularia</i>	136	17.89
<i>Aspergillus fumigatus</i>	47	6.18
<i>Aspergillus flavus</i>	45	5.92
<i>Aspergillus niger</i>	3	0.39
Unidentified dematiaceous fungi	112	14.73
Unidentified hyaline	47	6.18
<i>Candida albicans</i>	21	2.76
<i>Candida tropicalis</i>	11	1.44
<i>Candida parapsilosis</i>	3	0.39
<i>Alternaria</i> species	9	1.18
<i>Bipolaris</i> species	8	1.05
<i>Penicillium</i> species	8	1.05
<i>Mucor</i>	4	0.52
<i>Rhizopus</i>	3	0.39
Unidentified	6	0.78
Others	10	1.31
Total	760	100 (99.92)

Discussion:

A variety of factors determine clinical outcome in microbial (fungal and bacterial) keratitis. Epidemiological patterns may vary from country to country and between different geographical regions within a country. Corneal trauma and ulceration is the second leading cause of unilateral blindness. Corneal ulceration is seen in all ages.¹

In this study, fungal corneal ulceration was more common in men (58.42%), although earlier studies done in various parts and regions of India have reported a comparable gender distribution.^{13,21,22} Interestingly, in a study done in Kingdom of Bahrain²³ the study has reported a higher incidence of corneal ulcer in women.²³ The higher incidence rates seen in men in this study may be due to the higher risk of exposure to injury during fieldwork/agricultural work and farming compared to women therefore developing more fungal corneal ulcers. Ulceration was more frequent among

both men and women during the middle decades of life. Possibly, due to these age groups being maximally active outdoors. In this study too, the commonest occupation of the patients having proven fungal isolates were farmers which was followed by housewives, which correlates well with the occupational background.

The most common predisposing factor found in our study was corneal injury, accounting for 56.71 % of total fungal isolates. This figure has been echoed by another study from south India.¹³ The agents responsible for this trauma were primarily agricultural matter such as rice stalks, thorns, tree branches and leaves and paddy grain. Animal products commonly implicated in corneal injury were the tails of cows and cow dung which consequently leads to develop a variable sized corneal injury leading to fungal corneal ulcers. Unlike in developed countries where causes such as the use of contact lens are reported to be common.^{21,23} This is explained by the fact that most fungi isolated from the cultures of patients with corneal ulcer in this study are also commonly present as atmospheric saprophytes and can initiate an ulcer even with a small abrasion. Fungal corneal ulcer in contact lens users in our study accounted for a total of 6.84% of total fungal isolates which is quite less and correlates well with previous reports.^{21,23} Present study showed prevalence of chronic ocular surface disorder to be 10.00%, prior topical steroid use 11.71% and underlying systemic diseases 7.10%. Squamous blepharitis, meibomian gland dysfunction, chronic dacryosistitis were the reasons for ocular surface disorder becoming one of the very important risk factors to develop fungal corneal ulcer.

Of the systemic diseases Diabetes mellitus, immuno suppressive chronic systemic diseases, Hensen's disease, Rheumatoid arthritis, Tuberculosis contributed for 7.10% of risk factor for developing fungal corneal ulcers. Which did correlated well with a previous report from Nepal.²⁴

The commonest fungus isolated in this study was fusarium species accounting for 37.76% of total fungal isolates, followed by curvularia species in 17.89% of cases, unidentified dematiaceous species in 14.73% of cases and aspergillus species in 12.50% of cases. Our study correlates well with a similar study done in Nepal earlier in the western peripheral terrain belt of Nepal and Indo-Nepal border²⁴ Ghana and southern India.²⁵ Where 32% and 39.9 % of cases were due to fusarium species respectively.^{24,25} The fusarium species are common plant pathogens particularly of cereal crops and saprophytes of plant debris and are found in soil. The higher incidence of fusarium species may be due to the widespread cereal-crop cultivation in this region.²⁴

Curvularia species were found to be more prevalent in our study (17.89 %) than in the study done in Ghana and Southern India in which the prevalence was 9.6 %.²² In a similar study from Nepal²⁴ curvularia species were found to be more prevalent, which correlated well with this study.²⁴ In another study done in northern part of India, aspergillus species were found to be the most common fungal isolate accounting for 41 % of cases. Though, in our study aspergillus species accounted for 12.50% of all pure fungal isolates.²⁶ However few studies have shown aspergillus species to be the commonest fungal isolate isolated from patients with fungal corneal ulcer.^{21,22,27} This may be due to differences in the climate and natural environment of the areas studied.

This study showed high prevalence of unidentified dematiaceous and hyaline species. Dematiaceous species were diagnosed on the basis of the brown pigmentation similarly the hyaline species growth were identified as whites colonies.

Though, there has been already a report on culture positive fungal microbial keratitis from western terrain belt of Nepal,²⁴ we believe the difference in geographical location and economic status plays a sufficiently important role to warrant the setup of our present study and helps in finding out the variation in the fungal isolates. Importantly, this study had more study subjects and more number of pure fungal isolates.

Conclusion:

In contrast to the other studies done in Nepal, this study found fusarium species to be the most common fungal isolate causing corneal ulcer followed by curvularia, unidentified dematiaceous fungi and aspergillus species. The higher incidence of fusarium species is contributed by the widespread cereal-crop cultivation in the study region. The study differed from various other studies primarily due to geographical location of the study population followed by occupation and socio economic status.

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Conflict of interest: None

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