



Trends of Fungal Keratitis in a Tertiary Care Hospital in Tripura

KEYWORDS

Suppurative keratitis, Mycotic keratitis, Ocular trauma

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ABSTRACT Aim : To study the trends of fungal Keratitis in a tertiary care hospital of Tripura. Materials and Methods: In this study 100 numbers of corneal scrapings were collected from patients presented in the department of ophthalmology since 1/11/2013 to 31/10/2014, with the clinical diagnosis of corneal ulcer with or without hypopyon were subjected for microbial investigation to find out the etiological agents. Result & observation: Out of 100 cases 63% were found to be culture positive, of this 36 (57.1%) patients had bacterial growth, 27(42.9%) had fungal growth and 06(9.5%) cases had mixed bacterial and fungal growth. The Males are the more preponderant sex group accounts 68% . Staphylococcus aureus was the most commonly isolated bacterial organism (25.4 %) followed by Staphylococcus epidermidis(07) (11.1%), Escherichiae coli(05)(7.9%), Pseudomonas spp (04)(6.3%), Klebsiella spp(03)(4.7%) and Acinetobacter spp(01)(1.6%) the most frequently occurring gram-negative organism. . Aspergillus spp (Aspergillus flavus, fumigates & niger) were the major fungal etiological accounts 15.9% of the total 27 (42.7 %) fungal corneal ulcers followed by Candida albicans(07), Fusarium solani(04), Penicillium species(02), Rhodotorula spp(01) & Trichosporon spp(01). Conclusion: Mycotic keratitis will continue to be an important cause for ocular morbidity despite of bacterial agent. Clinical suspicion by the ophthalmologists should be confirmed by microbiological finding prior to initiating corticosteroids and anti-bacterial antibiotics. Precise identification of the causative fungus and institution of appropriate treatment strategy could save the blindness of the eye.

Introduction:

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes for blindness followed by cataract especially in developing countries in comparison to developed countries where 90% people live with this problem (WHO).worldwide 161 million people are visually impaired, 124 millions low vision and 37 millions blind, but the magnitude is much more in India, having one-fourth the world's total blind population¹⁻³. As per recent study Corneal scarring second only to cataract as the major etiology of blindness and visual disability in many of the developing nations in Asia, Africa and the Middle East⁴. A wide spectrum of microbial organisms can produce corneal infections by invading the corneal stroma when defence mechanism of corneal epithelium are compromised⁵. The incidence of mycotic keratitis is more in tropical and subtropical countries due to the climatic environment. Impact of climatic changes by altering local weather or ecological patterns effect human health by resulting bacterial and fungal proliferation^{6,7}. Climatic conditions of North Eastern part of India prevailing with heavy rainfall and humidity throughout the year may result proliferation of microbes in traumatised eye. Most of the people are living under poverty line; hence cultivation, farming, intensive labouring etc are being the only profession for their survival. These may predispose the reason for traumatised of the cornea accidentally or incidentally. However there is always a poor data available in this region which practically emphasised design the present study.

Materials and Methods:

After a thorough clinical evaluation and slit lamp biomicroscopic examination by a dedicated ophthalmologist samples collected from all the 100 no from suspected patient of corneal ulcer who were attending in OPD of ophthalmology department of Tripura Medical College

during last one year. The Ulceration and Suppurative keratitis was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon. Typical viral ulcers and healing ulcers were excluded as were Mooren's ulcers, marginal ulcers, interstitial keratitis, sterile neurotrophic ulcers, and any ulcers associated with autoimmune conditions. All the Clinically suspected patients with infectious keratitis of different of age group except preschool children in different sex were included in our study. Once a clinical diagnosis of corneal ulcer was made, their Socio-demographic data, duration of symptoms, predisposing risk factors, clinical details, prior treatment modalities and associated ocular and systemic diseases etc are also recorded in a standardized patient data sheet pro-forma (Table-1).

Table No.01:

Standard Clinical Examination Form for Corneal Ulcer

Patient Proforma

Patient details Name: _____ Address: _____	Age: _____	Patient number: Sex: M F
Ophthalmic History: Trauma _____ Ocular surface disorder _____ Corneal exposure _____ Diabetes mellitus _____		Eye surgery _____ Contact lens wears _____ Trichiasis _____ Dacryocystitis _____
Does the patient have a history of diabetes? Y/N. _____		
If yes, for how long _____		
If other, give details: _____		
Current topical antibiotic Y / N specify _____		
Current topical antifungal Y / N specify _____		
Current topical steroid Y / N specify _____		
Traditional eye medicine Y / N specify _____		
Presentation Date of primary presentation: / / _____		
Eye RE / LE / Bilateral _____		
Duration of symptoms _____ days		
Visual Acuity (uncorrected) Right _____ Left _____		

BASE - LINE EXAMINATION:

Ulcer size:

Diagram:

Lid Edema: Mild/Moderate/ severe

Depth of Ulcer: Deep/Superficial

Depth of infiltrate: Anterior stroma /Mid stroma/ Posterior stroma

Hypopyon: Absent/ Present

Height _____ mm

REVIEW Date: ____/____/____

MICROBIOLOGY RESULTS

Gram stain _____ date ____/____/____

KOH _____ date ____/____/____

Lactophenol _____ date ____/____/____

Cotton Blue _____ date ____/____/____

Culture (BA) _____ date ____/____/____

Culture (SDA) _____ date ____/____/____

Assessment:

Eye: RE / LE / Bilateral

Visual Acuity Right _____ Left _____

Diagram with ulcer size

Depth of infiltrate: Mid stroma/Anterior stroma/Posterior stroma

Hypopyon: Absent/ Present

Height _____ mm

Healing/Working/No Change/Perforation

After a detailed ocular examination of every patient corneal scrapings were obtained under magnification of slit lamp or operating microscope after instillation of 4% lignocaine (lidocaine) without preservative by using standard techniques, by an ophthalmologist under aseptic conditions from each ulcer using a sterile Bard-Parker blade (No 15). The scraping material were obtained from the leading edge and the base of each ulcer directly onto sterile slides for 10% KOH wet mount, Gram's staining and 100µl saline aliquots for culture in solid media such as Sheep's Blood agar, Mac-Conkey agar, or Sabouraud's dextrose agar with chloramphenicol in a row of C-shaped streaks^{3,8,9}. In addition to above techniques modified AFB staining and parasitological study of wet mount from the saline aliquot. Sabouraud's Dextrose Agar plates were incubated at 28°C and were examined and observed at regular intervals for 1-2 wks for fungal culture.

Sheep's Blood agar, MacConkey agar Plates for bacterial culture were kept at 37°C and were observed for 01-02 days. Fungal growth was macroscopically identified on the basis of colony texture, color, diffusible pigments, growth rate, aerial and submerged hyphae and microscopically the morphological features like mycelium with lactophenol cotton blue, 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 aetiology 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 with tropical antibacterial (Fluoroquinolones) and antifungal eye drop after the microbiological sample was taken and in most of the cases after getting the smear result. For fungal ulcers, 5% natamycin 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:

In this present study corneal scrapings were collected from 100 numbers of patients with the clinical diagnosis of corneal ulcer with or without hypopyon those were presented in the department of Ophthalmology of Tripura Medical College, Hapania, and Tripura since last one year (1/11/2013 to 31/10/2014). A little hike of increase of incidence was observed during the month December to February. All of them were subjected for microbial investigation to find out the etiological agents. Of these 100 cases, 63% were found to be culture positive for bacteria and fungus and all the samples were positive for direct microscopy with gram's stain and KOH mount examination, 30% scrapings were culture sterile and also did not show any evidences of bacterial or fungal elements on direct microscopic examination for bacteria and fungus. Four cases among the 63 cases showed growth on culture though microscopically negative initially. The remaining seven cases did not show growth of any microbes despite of positive microscopic findings. All the samples might have received the treatment with topical antibiotics, antifungal, and corticosteroids time and again before presenting to hospital.

Table No.2: Age and sex distribution of corneal ulcers:

Age(yrs)	Male (%)	Female(%)	Total(%)	Bacteria (%)	Fungus (%)
0-12yrs	01(01)	01(01)	02(02)	01(2.7)	00(00)
13-24yrs	12 (12)	02(02)	14(14)	02(5.5)	01(3.7)
25-40yr	20 (20)	09(09)	29(29)	09(25)	11(40.7)
41-60yrs	18 (18)	14(14)	32(32)	16(44.4)	09(33.3)
61>yrs	17 (17)	06(06)	23(23)	08(22.2)	06(22.2)
Total	68	32	100	36 (57.1%)	27(42.9%)

The Male patients were the most affected sex group accounts 68% in compare to female (32%). Total 63 cases were found to be culture positive with 36 bacterial and 27 fungal. The most commonest affected age group was 41-60 years in both sexes, maximum of 32% patients attended in the hospital from this age group, 29% from 25-40yrs and 23% from 61>yrs age group during the period of one year. More than 75% patients of our study were from different villages of Tripura and majority were farmer, followed by house wife, daily worker (Labor) by profession. Ocular trauma was found to be associated as major causes of corneal injury in 60.3% cases. Beside this, other predisposing factors like Diabetes, Exposure keratitis, Contact lens use, Corneal foreign body, dacryocystitis (NLD), dirty water, injury by nail, metallic substance, injury by flying insect etc. were noticed as being associated with cases with bacterial and fungal ulcers. There was not a single case of post ocular surgery, neither reported nor even any parasitic case of *Acanthamoeba* species in our observations. Vegetable matters with leaf was the commonest agent (44.4%) injuring the cornea. The different predisposing factors and cause of ocular trauma associated with cases with bacterial

and fungal ulcers are shown in Table 3 & 4.

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

Factors	Bacterial corneal ulcers (n=30) %	Mixed bacterial and fungal Ulcer (n=06)%	Fungal corneal ulcer (n=21) %	Total (N=63) (%)
Topical antibiotic use	05 (16.6)	02 (33.3)	05 (23.8)	12 (19.0)
Ocular trauma	22 (73.3)	02 (33.3)	14 (71.4)	38 (60.3)
Diabetes	03 (10)	02 (33.3)	01(4.7)	06(9.5)
Exposure keratitis	01 (3.3)	00	01 (4.7)	02 (3.1)
Contact lens use	01 (3.3)	00	00	01 (1.6)
Corneal foreign body	00	00	01 (4.7)	01 (1.6)
Dacryocystitis (NLD)	08 (26.6)	00	08(30.1)	16 (25.3)
*Others	00	00	00	00

*Others include Post ocular surgery, Viral keratitis etc

Table. 4: Ocular trauma-causing agents associated with microbial keratitis

Cause of Trauma	Pure Bacterial Ulcer (N=30) (%)	Mixed Bacterial and Fungal Ulcer (N=06) (%)	Pure Fungal Ulcer(N=21) (%)	Total (N=63) (100%)
Vegetable matter— Leaf	16 (53.3)	02(33.3)	10 (47.6)	28(44.4)
Vegetable matter— Stem	02 (6.6)	02(33.3)	02 (9.5)	06 (9.5)
Vegetable matter— Hay	04 (13.3)	01(16.6)	04 (19.0)	09 (14.3)
Cow tail injury	02 (6.6)	01(16.6)	02 (9.5)	05 (7.9)
Soil	02 (6.6)	00	01 (4.8)	03 (4.8)
*Others	04 (13.3)	00	02 (9.5)	06 (9.5)
Total	30 (100)	06(100)	21 (100)	57 (84.1)

*Others includes Dirty water, Injury by nail, Metallic substance, Injury by flying insect etc.

Hypopyon was present in 41 (65.0%) cases of pure bacterial 20(31.7%)and fungal 21(33.3%) ulcers cases presented with no perforation. 25 (60.9%)cases out of 41cases responded after three weeks treatment with gradual healing with variable degree of opacity.16 cases did not improved till last follow up. Not a single case developed any sequelae or endophthalmitis .

Cultures were positive in 63 cases of corneal ulcers. 36 (57.1%) patients had bacterial growth and 27(42.9%) fungal growth. In 6 cases there was a growth of bacteria mixed with fungus. Detail growth bacterial and fungal isolates are shown in Table No.05. Of the 36 isolates 23 (63.8%) were gram positive and 13 (36.1%) were gram negative. *Staphylococcus aureus* was the most commonly isolated bacterial organism 16 (25.4 %) and of all positive bacterial cultures.

The other isolated gram-positive organisms were *Staphylococcus epidermidis* 07 (11.1%), *Escherichiae coli* 05 (7.9%), *Pseudomonas spp* 04(6.3%), *Klebsiella spp*(03)(4.7%)and *Acinetobater spp*(01)(1.6%) the most frequently occurring gram-negative organism was isolated from bacteriological growth cultures. (Table 4). There were 27 fungal agents like *Aspergillus spp*-10(15.9%),*Candida albicans*-07(11.1) *Fusarium spp*-04(6.3%), *Curvularia*-02(3.1%), *Penicillium spp*-02(3.1%), *Rhodotorula spp*-01(1.6) and *Trichosporon spp*-01(1.6) isolated from 63 patients which also shown in Table 4. In mixed cases *Aspergillus spp*-03,*Candida albicans*-02 and *Curvularia*-01 etc were. isolated along with bacteria.

Table. 5: Microbial agents isolated from corneal ulcers.

Name of bacteria isolated	No. of bacteria isolated, n=36 (57.2%)	Name of fungus isolated	No. of fungus isolated, n=27 (42.7%)	Total
<i>Staphylococcus aureus</i> (16)	16(25.4)	<i>Aspergillus flavus</i> (04) <i>Aspergillus fumigates</i> (04) <i>Aspergillus niger</i> (02)	10 (15.9)	26(41.3)
<i>Staphylococcus epidermidis</i> (CNS)(07)	07(11.1)	<i>Candida albicans</i> (07))	07(11.1)	14(22.2)
<i>Escherichiae coli</i> (05)	05(7.9)	<i>Fusarium solani</i> (04)	04(6.3)	09(14.3)
<i>Pseudomonas spp</i> (04)	04(6.3)	<i>Curvularia lunata</i> (02)	02(3.1)	06(9.5)
<i>Klebsiella spp</i> (03)	03(4.7)	<i>Penicillium species</i> (02)	02(3.1)	05(7.9)
<i>Acinetobater spp</i> (01)	01(1.6)	<i>Rhodotorula spp</i> (01)	01(1.6)	02(3.1)
		<i>Trichosporon spp</i> (01)	01(1.6)	01(1.6)
Total	36(57.2)		27(42.7)	63(100)

Discussion:

Ocular fungal infections, or ophthalmic mycoses, are being increasingly recognized as an important cause of morbidity and Blindness. Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes incidence of which has increased worldwide in the last few years due to the improvement in microbiologic diagnostic techniques and because of introduction of new therapeutic measures such as wide-spread use of broad-spectrum antibiotics, immunosuppressive drugs and corticosteroids. In India, the prevalence of mycotic keratitis varies between 6-46.8 per cent in various regions¹⁰. In this study, Male (68%) age group is the most affective predominant group in comparison to female(38.1%), this may be due to over exposure to environment, similar findings are present with most of the Indian study like Bharathi MJ et al¹¹, Jadhav S.V¹², Gopinathan U¹³ and many others. Chander J et al¹⁷ in his study, shown most frequently affected age group is between 21yr-50yrs which almost correlates with our findings, 41-60yrs age group is most affected group followed by 26-40 and 61>yrs age group in both sexes. But in contrast findings of Jadhav S.Vet al and Basak SK, does not match, their study shows 21-40yrs age group is affected group ^{12, 15}

A significant increase of incidence of suppurative keratitis was observed during harvesting seasons of November-December than the wet, humid months of the year but few say during the hot and humid months^{14,15}. In this study we found the incidence increasing trend during the period from December-February as because this period coincided with the harvesting season in this region. In this present study we observed that many predisposing factors are responsible for ocular trauma of 60.3% which lead to corneal ulcer in majority of the cases. Vegetable matter mostly with leaf, paddy plants, paddy or paddy stalks responsible for causing supportive as well mycotic corneal ulcers in 40-50%cases alone. Besides Vegetable Stem, hay, Cow tail injury, Soil, muds, dirty water, Injury by nail, Metallic substance, Injury by flying insect etc. reported as frequent cause that responsible for formation of ulcer superficial to deep. Underlying disease like, Diabetes mellitus, Dacryocystitis or frequent injudicious use of tropical medication are underlying predisposing factors.. In our observation, villagers with their different profession like farmer, house wife, daily workers, agriculture workers etc are the commonest group of people are worst sufferer. Similar results are compared with the studies conducted like Madurai, Srinivasan M et al³, West Bengal, Basak SK et al¹⁵, Assam Reema Nath et al¹⁴

Cultures were positive in 63 cases of corneal ulcers where 30 (47.6%) patients had bacterial growth, 27(42.9.3%) had fungal growth and 06 cases had mixed bacterial and fungal growth. Our study results are almost correlated with the studies conducted in Madurai, Tamil Nadu 1997³, Ghana study in 1985¹⁹, Jadhav s.v study in Pune¹² Anil Kumar et al, in Gujarat, Western India¹⁹ etc. But it does not approach the 80% or 81% isolation rate reported in Nepal 1991¹⁷ or Bangladesh 1994 study.¹⁸ Mono microbial infection was seen in majority (80.9%) of the cases the most common being bacterial (47.6%) followed by 33.3% fungal growth. Of these 63 cases of corneal ulcers 36 bacterial isolates were isolated from 36 bacterial growths. *Staphylococcus aureus* (25.4%) is being the most common bacterial agent isolated in our study followed by *Staphylococcus epidermidis*(11.1%), *Escherichiae coli* (7.9%), *Pseudomonas spp* (6.3%) and *Acinetobater spp*(1.6%) respectively. Similar figures have been reported from Madurai(Srinivasan M et al)³, Andhra Pradesh(Kothapalli Sreelakshmi et al)¹⁶, in Gujarat(Anil Kumar et al)²⁰ and Ghana(Maria Hagan et al)¹⁹. As per study of Reema Nath et al¹⁴ and Basak SK et al¹⁵ 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% cases¹⁵. We too got few cases with mixed infection.

In our study there is a gradual trends of mycotic keratitis which is the major concern nowadays. *Aspergillus spp* (*Aspergillus flavus*, *fumigates* & *niger*) were the major fungal etiological accounts 15.9% of the total 27 (42.7 %) fungal corneal ulcers followed by *Candida albicans*(11.1%), *Fusarium solani*(6.3%), *Penicillium species*(3.1%), *Rhodotulra spp*(1.6%)& *Trichosporon spp*(1.6).Though our finding do not correlates the study Bharathi MJ et al¹¹ and Reema Nath et al¹⁴ with very high incidence (60.6%), but our results are within the Indian national range 6-46.8% which was shown in many Indian studies^{10,12,15,17,21}. Moreover this is our first experience, to see its gradual trends of mycotic keratitis in our centre which is already the major concern in world literature. 30% remained culture sterile, 07% culture negative despite strong clinical suspicion and positive microscopic result and 04% were culture positive but with negative microscopy in this present study. There may be five underlying causes (a)The reasons cultures to be sterile even when microscopy was positive could be that of patients were using tropical antibacterials, antifungals and corticosteroids before collection of corneal scraping. (b) Collection of inappropriate and inadequate quantity¹⁷.(c) Several limitations have been reported when such smears or mounts are used for corneal scrapes, including low sensitivity, frequent misinterpretation, presence of artifacts, and lack of detection of *Candida* and other yeasts²⁴. (d) KOH preparation tends to absorb carbon dioxide from air and form carbonate crystals thus reducing the effective hydroxide may lead to error in examination. (e) Moreover some medically important fungus produce metabolic end products which may results inhibition of growth²⁵.On direct microscopic examination with Gram's stain and 10% KOH mount examination techniques are still good, less expensive, users friendly and will remain gold standard test despite of its detection error.

Conclusion:

In summary, the supurative keratitis is major public health concern. Bacteria may be the one of the major causes but this trends are reducing gradually because of peoples are mostly inhabiting rural areas, poverty, ignorance, involved in outdoor and agriculture activity as a result of which mycotic keratitis will continue to be an important cause for ocular morbidity. Present study provides regional trend of fungal etiology in fungal keratitis. In conclusion, clinical suspicion by the ophthalmologists should be confirmed by microbiological finding prior to initiating corticosteroids and anti-bacterial antibiotics. Precise identification of the causative fungus and institution of appropriate treatment strategy could save the blindness of the eye.

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