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A cross sectional study on clinico microbiological profile of patients with fungal corneal ulcer attending a tertiary care hospital of Shimoga district, Karnataka

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Abstract

Introduction: Corneal ulceration is the most common cause of preventable blindness, second only to cataract in developing countries. Mycotic keratitis accounts of 30-62% of all culture positive infectious keratitis in several Indian studies. Mycotic keratitis is a significant problem in a country like India with a predominantly agrarian population, who encounter ocular trauma with vegetative material.

Aim: To study the clinical profile of patients with fungal corneal ulcers in relation to age, sex, risk factors, clinical presentation and laboratory diagnosis in the outpatient department of a tertiary care hospital of Shimoga district.

Materials and Methods: A prospective cross sectional study was carried out on all consecutive patients presenting with corneal ulcer attending the out-patient department of a tertiary care hospital of Shimoga district, Karnataka, between August 2013 to July – 2014.

Results: Out of the total 90 cases of corneal ulcer, 30 were fungal culture positive. Out of these 30 cases, males [21, 70%] were affected more than females [P < 0.0001]. The age group most commonly affected was 31-40 years [8, 26.67%], patients [24, 80%] residing in rural areas were more affected and most patients were agricultural workers [19, 63.6%]. Corneal trauma [27, 90%] was the most common risk factor [P < 0.0001] and 17 patients [62.96%] had history of trauma with vegetative matter [P < 0.0001]. Incidence of fungal corneal ulcer was highest during the paddy harvesting season. The sensitivity of 10% KOH wet mount preparation was higher [96.96%] than gram – stained smear [87.88%S] [P < 0.0001].

Fungal culture was positive in 30 cases [33.33%]. The most commonly isolated fungal species was Fusarium species [12 cases, 40%] followed by Aspergillus species [8 cases, 26.66%].

Conclusion: Ocular trauma with vegetative material especially in agriculture workers was an important cause of mycotic keratitis. Direct microscopic examination of KOH wet mount preparation is a rapid, sensitive, technique of laboratory diagnosis in mycotic keratitis. It is particularly important for the early initiation of antifungal treatment.

Keywords: Clinico-microbiological profile, Culture, Fungal keratitis, microscopy, Risk factors.

Introduction

Corneal ulcerations is the most common cause of preventable blindness, second only to cataract in developing countries.[1,2,3] Prevalence of corneal blindness is expected to increase from 0.66% [2001] to 0.84% in 2020.[14] The factors that have been held responsible for this increasing incidence of fungal keratitis include widespread use of broad spectrum antibiotics and steroids, prolonged use of contact lens and the growing number of corneal surgeries being performed. Mycotic keratitis accounts for 30-62% of all culture positive infectious keratitis in several Indian studies.[5,6,7,8]

In our country with a predominantly agrarian population ocular trauma with vegetative material is very common. The tropical climate favors fungal growth. Therefore, with all these risk factors mycotic keratitis is a significant problem. The aim of this study was to evaluate the clinical features and laboratory diagnosis of Fungal keratitis, so as to assist in the early initiation of antifungal treatment.

Materials and Methods

A prospective cross sectional study was conducted on all patients with features of corneal ulcer presenting to the out-patient department of Shimoga Institute of Medical Sciences (tertiary care hospital) between August-2013 to July-2014.

Sample size was calculated by the formula $4pq/d^{(2)}$ where p is prevalence, q is (1-p) and d is precision. Considering the prevalence of culture positive fungal keratitis to be 30% and precision of 10%, sample size calculated was 84 and it was rounded off to 90.

Corneal ulceration was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.[5] Corneal ulcer with intact epithelium were also 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.

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.

The Institutional Ethical Committee approval was taken for the study. A written informed consent was taken from all patients.

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.

Data was analysed by using excel spread sheet for data entry and SPSS for data analysis. The study variables were defined into continuous and categorical part and then accordingly the data was expressed in terms of percentages and then the normally distributed data was analyzed and compared by using Pearson's chi square test to each set of variables.

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.

Associated ocular conditions like Blepharitis, Dacrocystitis, spheroidal corneal degeneration, Bullous Keratopathy, Bell's palsy, lagopthalmos, Trichiasis, ocular anesthesia, ocular leprosy were noted.[5,7] 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.[9]

Laboratory procedure: Aerobic incubations were done for all inoculated media.[5] 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 were incubated at 37°C examined at 24 hrs and 48 hrs and then discarded if there was no growth.[5]

All laboratory methods were performed following standard protocols.[5,9] Microbial cultures were considered positive if atleast one of the following criteria were met; Growth of the same organism was demonstrated on 2 or more solid medial 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.[5,9]

Fungi were identified by their colony characteristics on SDA and by their microscopic appearance in lactophenol cotton blue (LPCB).

Results

Out of the 90 cases of corneal ulcers included, mycotic etiology was established in 30 cases [33.33%]. Out of 30 fungal corneal ulcer cases males (21, 70%) were affected more than females (9, 30%). Majority of the patients were in the 31-40 years of age group (8.26, 67%). Most patients were from rural areas (24, 80%) with agricultural work (19, 63.67%) as their occupation.

Corneal trauma (27, 90%) was the most common predisposing factor (P <0.0001). Seventeen patients (62.96%) had corneal injury with vegetative matter (P < 0.0001). six patients had co-existing ocular diseases responsible for development of fungal keratitis. Use of topical steroids predisposing to fungal keratitis accounted for 2 cases (6.67%). Association with systemic diseases was seen in 5 cases (16.66%) (Table 1).

Seven patients (22%) reported within first week after onset of symptoms, six patients (20%) in 2nd week, eight patients (26.66%) in 3rd week, five patients (16.66%) in 4th week and 4 patients after 4th week of their illness.

Maximum cases were seen during the paddy harvesting season i.e. October to December (14 cases, 46.66%).

Clinical features of the 30 fungal corneal ulcers on slit lamp examination include:- dry, thick and raised corneal surface in 22 (73.33%) cases, stromal infiltration with feathery margins in 20 (66.66%) cases, immune ring in 2 (6.66%) cases, satellite lesions in 5 (16.66%) cases, hypopyon in 17 cases (56.66%) corneal abscess in 1 case (3.33%).

Pure mycotic growth (fungal culture positive) was detected in 30 cases and 5 cases (5.55%) showed bacteria mixed with fungus. Pure bacterial etiology was established in 28 cases (31.11%). 1 case showed Acanthamoeba species growth (1.11%). 26 cases were culture negative (28.88%). 22/30 positive fungal culture were hyaline (non-pigmented) group and 8 cases were dematiceous (pigmented) group. No yeast or yeast-like organisms were isolated. The most commonly isolated fungal species was Fusarium species (12 cases, 40%), followed by Aspergillus species (8 cases, 26.66%). (Table 2)

Table 1: Demographic characteristics of 30 cases of fungal keratitis

Demographics	Particulars	n(%)
Gender	Male	21(70%)
	Female	9(30%)
Age in Years	<21 Years	4(13.33%)
	21-30 Years	6(20%)
	31-40 Years	8(26.67%)
	41-50 Years	7(23.33%)
	>50 Years	5(16.67%)
Residence	Rural	24(80%)
	Urban	6(20%)
Occupation	Agricultural Worder	19(63.6%)
	Labourer	4(13.33%)
	Students	2(6.7%)
	Tradesman/ Professional	2(6.7%)
	Household	2(6.7%)
	Unemployed	1(3.33%)
Predisposing Factors	A. Corneal Trauma	27(90%)
	B. Co-existing Ocular disease	6(20%)
	Spheroidal degeneration	1(3.33%)
	Dry Eye	1(3.33%)
	Lagophthalmos	1(3.33%)
	Chronic dacrocystitis	3(10%)
	C. Use of topical steroids	2(6.67%)
	D. Systemic diseases	5(16.66)
	Diabetes Mellitus	4(13.33%)
	Leprosy	1(3.33%)
Traumatic Agents	1. Vegetative matter	17 Cases (62.96%)
	a. Paddy/ hay	8 Cases (29.62%)
	b. Tree Branch/ Thorn	4 Cases (14.81%)
	c. Other Vegetative Matter*	5 Cases (18.51%)
	*Grass, Corn, Stalks, Wood, Leaf	
	2. Soil/ Sand/ Stone	3 Cases (11.11%)
	3. Insects	2 Cases (7.4%)
	4. Cow Tail	2 Cases (7.4%)
	5. Metallic Foreign Body	2 Cases (7.4%)
	6. Finger Nail	1 Cases (3.7%)
	7. Unknown	0

Table 2: Fungal pathogens isolated from 30 culture positive cases of fungal keratitis

Fungal isolates	Total No of isolates No. (%)
1} Hyaline fungi	22(73.33%)
Fusarium species	12 (40%)
Aspergillus species	8 (26.66%)
Mucor species	1 (3.33%)
Rhizopus species	1 (3.33%)
2}Dematiaceous fungi	8(26.66%)
Cladosporium species	5 (16.66%)
Curvularia species	1 (3.33%)
Biopolaris species	1 (3.33%)
Unidentified Dematiaceous fungal species	1 (3.33%)

Table 3: Correlation between direct microscopic (10% KOH and gram-stained smear) diagnosis and culture based diagnosis and also clinical diagnosis and culture based diagnosis of 90 cases of corneal ulcers

S. No.	Name of Investigation	Results	No.	Presence of fungal growth in culture		Senstivity (%)	Specificity (%)
				Positive	Negative		
1.	Detection of fungal elements in 10% KOH smear	Positive	33	32*	1	96.96	98.24
		Negative	57	1	56		
		Total	90	33	57		
2.	Detection of fungal elements in gram stained smear	Positive	29	29	0	87.87	100.00%
		Negative	61	4	57		
		Total	90	33	57		

3.	Clinical suspicion of fungal keratitis on slit lamp examination	Positive	34	31	3	93.93	94.73%
		Negative	56	2	54		
		Total	90	33	57		

*Out of 32 cases pure fungal growth was defected in 30 cases, remaining 2 were mixed with bacteria.

Discussion

Fungi are ubiquitous eukaryotic microorganism. Fungi take the form of either yeasts or molds. Yeasts are unicellular and grow by budding. Molds also called filamentous fungi, are composed of hyphae and are multicellular. Molds grow by extension and branching of hyphae. Molds may be classified as septate or non-septate. Fungi reproduce sexually by the formation of spores and asexually by forming conidia or sporangiospores. The disease causing fungi in the cornea usually are in an asexual phase of their life cycle when they are cultured from infected cornea.[10,11,12,13]

Young male individuals from rural areas and those engaged in agricultural activity were more commonly affected than others. Most of the patients were in 31-40 years of age group. Male preponderance of corneal ulcers has been described in many studies.[5,6,7,20,21]

Increased incidence of fungal keratitis in agricultural workers has been reported.[1,5,6,7,21] Corneal injury particularly with vegetative matter was the most common predisposing factor. Paddy / hay was the most common traumatic agents, paddy being the major crop in this part of Karnataka. Corneal trauma has been identified as cause of fungal keratitis.[1,5,7,22]

Pre-existing ocular diseases accounted for 20% of cases. Association between pre-existing ocular diseases and fungal keratitis is shown in many studies.[5,7,22] Indiscriminate use of over the counter topical corticosteroids accounted for (6.67%) of cases. Systemic diseases like diabetes was seen in 4(13.33%) of cases.

Clinical examination with a slit lamp was helpful in picking up classical clinical features of fungal keratitis. In this study, on slit lamp examination 22(73.33%) of cases showed dry, thick and raised corneal surface, 20 [66.66%] cases showed stromal infiltration with feathery margins, dendritic pattern in 2 (6.66%) cases, immune ring in 2(6.66%) cases, satellite lesions in 5 (16.66%) cases. The sensitivity and specificity of clinical diagnosis of fungal keratitis made by an ophthalmologist using slit lamp bio-microscope was 93.93% and 94.73% respectively. Similar observation was seen in other studies also.[7,22]

Out of the 90 cases, of corneal ulcer, 30 cases (33.33%) were fungal culture positive. So, the prevalence of fungal keratitis in our study was 33.33%. Incidence of fungal keratitis in some other regions is as follows 7.3% in North India,[14] 32% in East India,[15] 34.4%,[7] 51.9%[5] in various South Indian studies. This regional variation is because fungal keratitis is more common in hot and humid climate than in temperate regions.

The most common fungal isolate in this study was Fusarium 40% followed by Aspergillus species 26.66%. Similarly Fusarium species was the predominant species in many studies done in India[5,7,18] as well as other countries like South Florida[19] and Ghana.[20] But some other Indian studies reported Aspergillus species [40-59%] as the commonest fungal isolate.[8,14,16,17] No yeast or yeast like organisms were isolated in this study, but a few studies in India have reported yeast isolates.[8,16]

The sensitivity of KOH wet mount preparation (96.96%) was higher than that of gram-stained smear (87.88%) in the detection of fungal filaments. This has been proved in several studies.[7,22]

In this study, the incidence of fungal keratitis was highest during the paddy harvesting season i.e. October to January. Similar results were seen in the study by Bharathi et al.[7] In coastal Karnataka, higher incidence is reported in October, June and January[23] and in Hyderabad, higher incidence is reported during winter (October to January) and monsoon (June to September) seasons.[6]

Fungal corneal ulcers is an important cause of ocular morbidity. Farmers are at high risk. KOH wet mount preparation helps in the early detection and initiation of appropriate antifungal therapy.

References

1. Upadhyay M P, Karmacharya P C, Koirala S, Tuladhar N.R., Bryan L E, Smolin G et al (1991).
2. Epidemiological characteristics, predisposing factors and etiologic diagnosis of corneal ulceration in Nepal, Am J Opthalmol;111:92-99.
3. Whitcher JP, Srinivasan M, Upadhyay MP (2001). Corneal blindness: a global perspective Bull world Health Organ; 79:214-21.
4. Gilbert CE, Wood M, Wadel K, Foster A (1995) Causes of childhood blindness in East Africa, results in 491 pupils attending 17 School for the blind in Malawi, Kenya and Uganda. Opthalmic Epidemiol;2:77-84.
5. Dandona R, Dandona L, Childhood blindness in Indian: a population based perspective. Br. J Opthal 2003;87:263-265.
6. Srinivasan M, Gonzales C A, George C, Cevallos V, Mascarenhas JM, Asokan B, et al. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai South India. Br J Opthalmol 1997,81:965-71.
7. Gopinathan U, Garg P, Fernandes M, Sharma S, Atmanathan S, Rao GN. The epidemiological features and laboratory results of fungal keratitis. A 10 year review at the referral eye care center in South India. Cornea 2002;21:555-59.
8. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Planiappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three – year study. Indian J Opthalmol 2003;51:315-21.

9. Basak SK, Basak S, Mohanta A, Bhowmick A. Epidemiological and microbiological diagnosis of suppurative keratitis in Gangetic West Bengal, Eastern India. Indian J Opthalmol 2005,53:17-22.
10. Sharma S, Athmanathan, Diagnostic procedures in infectious keratitis. In: Nema HV, Nema N, editors. Diagnostic procedures in opthalmology.
11. New Delhi: Jaypee Brothers Medical Publishers; 2009,pg 316-331.
12. Durand M, Fungal keratitis, conjunctivitis and canaliculitis: Mycology. In: Foster CS, Azar DT, Dohlman CH. The Cornea, scientific Foundations and clinical practice, fourth edition: Lippincott Williams and Wilkins, 2005 pg 397-402.
13. O'Day DM. Fungal keratitis. In: Pepose JS, Holland GN, Wilhemus KR editors. Ocular infections and immunity. St. Louis: Mosby: 1997.Pg 263-64.
14. Ananthanarayanan R, Paniker CKJ, editors. Textbook of Microbiology, 9th ed, Chennai: Universities Press [India] Ltd, 2013 Pg589-594.
15. Cooper BH. Taxomomy, Classification and Nomenclature of fungi. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, editors. Manual of clinical microbiology. 4th ed Washington D.C: American society for microbiology; 1985,Pg 495-499.
16. Chander J, Sharma A. Prevalence of fungal corneal ulcers in Northern India Infection 1994;22:207-9.
17. Dutta LC, Dutta D, Mohanty P, Sharma J. Study of fungal keratitis. Indian J Opthalmol 1981;29:Pg407-40.
18. Chowdhary A, Singh K, spectrum of fungal keratitis in North India, Cornea 2005;24:8-15.
19. Saha R, Das S, Mycological profile of infectious keratitis from Delhi. Indian J Med Res 2006;123:159-64.
20. Nath R, Beruah S, Saikia L, Devi B, Borthakur AK, Mahantha J, Mycotic corneal ulcers in upper Assam. Indian J Opthalmol 2011;59:367-71.
21. Liesegang TJ, Foster RK. Spectrum of microbial keratitis in South Florida. Am J Opthalmol 1980;90:38-47.
22. Hagan M, Wright E, Newman M, Dolin P, Johnson G. Causes of suppurative keratitis in Ghana. Br J Opthalmol 1995;79:1024-28.
23. Agarwal PK, Roy P, Das A, Banerjee A, Maity PK, Banerjee AR. Efficacy of topical and systemic intraconazole as a broad – spectrum antifungal agents in mycotic corneal ulcer: A preliminary study. Indian J Opthalmol 2001;49:173-76.
24. Bandyopadhyay S, Das D, Mondal KK, Ghanta AK, Purkrit SK, Bhasrar R. Epidemiology and laboratory diagnosis of fungal corneal ulcer in the sundarban region of West Bengal, Eastern India. Nepal J Opthalmol 2012;4(7):29-36.
25. Kotigadde S, Ballal M, Jyothirlatha, Kumar A, Rao SPN, Shivananda PG. Mycotic keratitis. A study is coastal Karnataka Indian J Opthalmol 1992;40:31-33.