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Original Research Paper

Fungal Etiology and Outcome of Ocular Diseases in Patients Attending A Tertiary Care Hospital in Eastern Rajasthan

Medical Science

KEYWORDS : Ocular mycosis, fungal keratitis, early antifungal treatment

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ABSTRACT

Context: Corneal disease is the second most common cause of blindness after cataract and infectious keratitis is one of the predominant cause. The incidence of fungal keratitis is more than 50% in tropical and sub tropical countries. However, distribution may vary considerably depending upon ethnogeographic and individual patient factors. It is essential to determine the local etiological profile of ocular mycosis to enable appropriate choice of therapeutics and management.

Aims: To know the fungal etiology and clinical outcome of ocular mycosis in patients attending a tertiary care hospital in Eastern Rajasthan.

Materials and Methods: Ocular samples from 90 clinically suspected patients over a period of two years were processed for mycological diagnosis. Fungal identification was done by standard conventional methods. Antifungal susceptibility testing with fluconazole (25µg) and voriconazole (1µg) was done for yeast isolates by disk diffusion and automated broth dilution method using Vitek 2C (Biomerieux, France).

Results: Fungal etiology was established in 43.33%cases. Positivity rate was highest in cases of fungal keratitis. Filamentous fungi were the commonest with predominant Aspergillus spp. (37.04%) and Fusarium spp.(18.52%). Trauma was seen to be the most common predisposing factor. In follow up 71.42% of the fungi positive cases responded well to fungal eye drops (natamycin 5% / fluconazole 0.2% / voriconazole 1%) with or without oral fluconazole(150 mg) / itraconazole(100 mg) BD for 15 days.

Conclusions: This study reveals fungal keratitis as the most prevalent ocular infection in our region. In order to enable better management of disease relating to eye , increased clinical suspicion, better sampling rate and availability of rapid methods for fungal diagnosis is imperative.

Introduction:

Infection of eye leads to conjunctivitis, keratitis, endopthalmitis and other infections which are responsible for increased incidence of morbidity and blindness worldwide [1,2]. Corneal diseases is the second most common cause of blindness, after cataract in developing countries .The World Health Organization estimates that in every year, about 1.5-2.0 million new cases of monocular blindness in developing countries is secondary to corneal ulceration[3]. Among infectious corneal ulcers, fungal keratitis is the most challenging and ironically most prevalent in developing countries[4]. In some cases when medical treatment fails early surgical debridement is imperative[5].

Prevalence of fungal keratitis is not just affected geographically but also varies with time ,type of weather and individual patient factors which tend to favour specific organism[6]. In the best of our knowledge studies to know the fungal etiology of ocular mycosis in Rajasthan has not been reported in literature till date. Hence this study was conducted to know the prevalence of ocular mycosis and its clinical outcome in our region.

Materials and Methods:

The study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College , Ajmer (Rajasthan) from December 2012 to November 2014.The total number of patients admitted in ophthalmology department during this period was 6010. Clinically suspected cases of infection were 292(4.86%) [corneal ulcer and abscess 245(83.90%), endopthalmitis 47(16.10%)].Patients who were already on antifungal / antibacterial treatment/ not willing to take the treatment/ move to still more higher centres for treatment were not included in this study. Hence, only samples from 90(30.82%) clinically suspected cases of ocular mycosis were processed for mycological diagnosis. A detailed study of cases was done to know the demographic features, possible predisposing factors, duration of symptoms, microbiology results, therapy received and outcome at completion of treatment. All the patients were examined in the eye OPD by an ophthalmologist. Clinical diagnosis of fungal corneal ulcer was based on slit lamp biomicroscopic examination. Feathery margins[Figure1] or satellite lesion [Figure 2] around the lesion and presence / absence of hypopyon was recorded. Corneal scrapping was taken from the edge and base of the ulcer using sterile Bard Parker blade no.15 following the instillation of local anaesthesia eye drops (4% xylocaine) under aseptic condition and inoculated onto culture plates bedside. Scrapping were also sent between two slides for KOH examination. Vitreous aspirate and abscess discharge was taken in cases of endophthalmitis and corneal abscess respectively.

Direct microscopy was done using 10% potassium hydroxide, Gram staining(if adequate material available). Ocular material was inoculated bedside onto Sabouraud's dextrose agar with chloramphenicol in two plates in the form of 'C' streaks was incubated at 250C and 370C respectively for maximum four weeks before declaring negative. Only growth occurring on the 'C' streaks was considered to be significant. Blood agar was also inoculated. Growth on the medium was identified by standard mycological methods. Filamentous fungi were identified on the basis of growth rate, colony morphology, microscopic appearance in lactophenol cotton blue mount and slide culture. Yeast was identified on the basis of colony morphology, germ tube test, urease production, corn meal agar morphology and CHROM agar morphology. Samples yielded bacterial growth on blood agar (n=5) were excluded from the study.

Antifungal susceptibility testing with fluconazole (25µg) and voriconazole (1µg) by disk diffusion method and automated broth dilution method with Vitek 2 compact (Biomerieux ,France) was done for yeast isolates. All materials and culture media were obtained from Hi media Laboratories, Mumbai, India

Results:
Out of 90 patients presenting with ocular infection, fungal etiology was identified in 39(43.33%) cases and bacterial in 5(5.55%). Of the 39 fungus positive cases, KOH wet mount was positive for fungus in 30(76.92%) cases and fungal culture was positive in 27(69.23%) cases [Table A]. Demographic data of the patients are given in [Table B]. Clinical and mycological results are given in [Table C,D] respectively. Only two yeasts were isolated, viz.Candida albicans and Cryptococcus neoformans. Candida albicans was found to be sensitive to both the drugs tested (voriconazole and fluconazole) while Cryptococcus neoformans was found to be resistant to both. Out of 39 positive cases, 18 were lost during follow up. Out of 21 cases followed up, 15(71.43%) responded well to local antifungal eye drop (natamycin 5% / fluconazole 0.2% / voriconazole 1%) with or without oral fluconazole (150 mg) / itraconazole (100 mg) BD for 15 days. While 6 (28.57%) showed poor response to antifungal treatment with azoles at the end of 15 days. These patients were in the age group of 50-70 years and had no history of diabetes, hypertension or immunocompromised condition. Out of these, one patient whose sample tested positive for Cryptococcus neoformans improved clinically with amphotericin-B treatment. Enucleation had to be done in one case of Aspergillus keratitis complicated by painful blind eye and perforation with secondary glaucoma. Occupationally, 23 of the 39 cases were farmer-labourers and most of them were males. All 39 of them belonged to low socio-economic groups.

Discussion:
Mycotic keratitis has emerged as a major ophthalmic problem since its recognition in 1879 by Leber. He described Aspergillus invasion of cornea in hypopyon corneal ulcer. Etiology of fungal keratitis is influenced by ethnogeographic factors. It is relatively common in agrarian tropical countries compared to Western countries. Several studies from India and elsewhere show filamentous fungi as the main causative agent of fungal keratitis. A comparative analysis of different studies by various authors is shown in [Table E].The incidence of fungal keratitis in this study was 43.33%. The incidence of fungal etiology in the cases of endophthalmitis and abscess was very low . This is probably due to lesser sample size and negligible sample volumes. Vitreous aspirate and abscess discharge were seen to be poor samples for culture diagnosis primarily due to inadequate volume. Corneal scrapping was found to be the most appropriate sample for mycological diagnosis.

Tanure et al (2000) reported chronic surface diseases, contact lenses usage, use of topical corticosteroids and trauma as the common risk factors (in decreasing frequency) for development of fungal keratitis. However, in this study injury mainly due to vegetative matter /surgical trauma was the most predominant risk factor associated with fungal keratitis. Two third of fungal keratitis cases were males, probably reflecting significant occupational exposure of males to fungal etiological agents in outdoor working environment. Most of them were either agricultural or outdoor labourers. Elderly people in the age group of 51-71 years were found to be the most susceptible to fungal keratitis.

In this study, the majority of mycotic keratitis was due to filamentous fungi. Among these hyalohyphomycetes were predominant , mainly Aspergillus spp.(37.04%) followed by Fusarium spp.(18.52%) similar results were reported from other studies in India [6-11].While most studies from India and South East Asia report Fusarium as the predominant etiological agent for fungal keratitis[4,12-15], those from United kingdom report Candida spp. as the predominant corneal pathogen [16]. This variation in fungal etiology probably reflects the difference in ethnogeographic factor of the Eastern (developing/ agrarian /tropical) and Western (developed/urban/temperate) countries. It can also be due to difference in socioeconomic condition of the patients in these two settings. However studies from USA report changing trends in fungal etiology[17,18].

In this study isolation of Aspergillus spp. was seen to show predominance during the rainy season and immediately thereafter. However, isolation of Fusarium did not show any seasonal predominance which probably indicates its indigenous and ubiquitous presence in our environment. Epicoccum, Stemphilum and Scytalidium were also isolated in this study which was ubiquitously found in air, soil, and food stuff and decaying vegetation. Till date, Epicoccum has not been reported as a causative agent of corneal infection in literature. In this study this species was isolated post operatively from a patient who has undergone cataract surgery.

In follow up, 71.43% of cases responded well to the standard treatment, while 28.57% showed poor response. In cases poorly responding to standard therapy, treatment with amphotericin -B based on culture diagnosis was found to be effective. In recalcitrant cases, repeated epithelial debridement had to be done to enhance the drug penetration. Hence amphotericin-B seems to be the empirical drug of choice in our region. However, the need for injection and associated toxicity leads to its restricted use. Local eye drops natamycin and voriconazole can be an alternative in such cases [19,20]. However, a combination of medical and surgical treatment is usually most effective for the management of fungal keratitis.

Conclusion: This study reveals fungal keratitis as the one of the most prevalent ocular infection in our region. Agricultural workers and outdoor labourers prone to trauma were the most affected population. This regional information on fungal etiology and clinical outcome shall enable initiation of most appropriate empirical treatment. On a wider perspective, this information will also guide us in formulating recommendation for preferred practice pattern and initiation of preventive measures in the population at risk. In order to enable better management of disease relating to this precious organ, increased clinical suspicion, better sampling rate and availability of rapid methods for fungal diagnosis is warranted.

Table A : Fungal etiology

KOH / Culture	Fungal etiology %
KOH positive culture positive	18(46.15)
KOH positive culture negative	12(30.77)
KOH negative culture positive	9(23.08)
Total	39

Table B : Demographic data ,age group and predominant predisposing factor

					Category of trauma				
Age group	Male	Female	Total	History of trauma	Sand / Dust	Vegetative matter	Foreign body	Burn	Surgery
0 - 10	-	-	-	-	-	-	-	-	-
11 -20	-	2	2	0	-	-	-	-	-
21 -30	2	-	2	0	-	-	-	-	-
31 - 40	1	3	4	3	1	-	2	-	-
41 -50	3	1	4	3	1	2	-	-	-
51 - 60	6	4	10	9	2	3			4
61 - 70	14	2	16	10	-	5	1	1	3
71 - 80	1	-	1	1	-	-	-	-	1
Total	27	12	39	26	4	10	3	1	8

Table C : Incidence of fungal infection in various ocular infections

Clinical manifestation	Clinical suspected cases of infection	Sample received in mycology department %	Fungal etiology (KOH / Culture)%
Keratitis without abscess	222	75(33.78)	34(45.33)
Endophthalmitis	47	9(19.15)	1(11.11)
Keratitiswith abscess	23	6(26.09)	4 (66.67)
Total	292	90(30.82)	39(43.33)

Table D : Mycological profile of ocular infection in the study

Fungi (n = 27)		Isolates	No. of isolates %
		Aspergillus spp.	10 (37.04)
Filamentousfungi=25 (92.59)	Hyaline =19 (70.37)	*Aspergillus niger*	5(1)* (18.52)
		Aspergillus flavus	3 (11.11)
		Others	2 (1)* (7.41)
		Fusarium spp.	5 (18.52)
		Fusarium solani	1 (3.70)
		Fusarium oxysporum	1 (3.07)
		Others	3 (11.11)
		Acremonium strictum	1 (3.70)
		Trichothecium sp.	1 (3.70)
		Sterile hyphae	2 (7.41)
	Pheoid = 6 (22.22)	*Bipolaris spicifera*	1 (3.70)
		Epicoccum sp.	1 (3.70)
		Scytalidium sp.	1 (3.70)
		Stemphylum sp.	1 (3.70)
		Non sporulating Phaeohyphomycetes	2 (7.41)
Yeast = 2 (7.41)		Candida albicans	1 (3.70)
		Cryptococcus neoformans	1 (3.70)

*** Isolated from corneal abscess**

Note : All other isolates are from culture of corneal scrapping (keratitis)

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Table E : Studies on fungal etiology of keratitis

Place	Authors Reference no	Years	Incidence of fungal infection %	Predominant fungal isolates %
Philadelphia	Tanure *et al* [17]	2000	-	*Candida albicans* (45.8) *Fusarium* (25)
Ghana	Leck *et al* [23]	2002	36.2	Aspergillus Fusarium
Tamil nadu	Barathi *et al* [12]	2003	34.44	*Fusarium* (42)
Florida	Alfonso *et al* [26]	2006	20.8	*Fusarium* (54.1) *Curvularia* (26.4) *Aspergillus* spp.(17.3) *Candida albicans* (91)
Delhi	Saha *et al* [7]	2006	22.25	*Aspergillus flavus* (31.16) *Aspergillus fumigatus* (16.88) *Fusarium* (7.79) *Candida albicans* (7.79)
Melbourne	Bhartiya *et al* [22]	2007	62.5	*Candida albicans* (37.2) *Aspergillus fumigatus* (17.1) *Fusarium* (14.3)
Queensland	Thew *et al* [25]	2008	-	*Fusarium* (50)
Delhi	Sherwal *et al* [8]	2008	32.50	*Aspergillus* spp. (56.42) *Curvularia* (17.95)
Sari	Shokoi *et al* [21]	2006	31.81	Aspergillus fumigatus Fusarium
London	Tuft *et al* [16]	2009	-	*Candida* (57.5) *Filamentous fungi* (42.5)
Bangladesh	Akter *et al* [15]	2009	58.92	*Aspergillus* spp.(45.45) *Fusarium* (24.24)
Philadelphia	Yildiz *et al* [18]	2010	85.90	*Fusarium* (43.28) *Candida albicans* (25.37)
Varanasi	Tilak *et al* [9]	2010	45.56	*Aspergillus* spp.(47.22) *Fusarium* (19.44)
Kerela	Geethakumari *et al* [13]	2011	69.78	*Fusarium* (37.05) *Aspergillus*(26.07) *Penicillium* (20.09) *Candida* (1.79)
Amritsar	Gill *et al* [10]	2011	43.6	*Aspergillus* (50) *Candida* (20) *Fusarium* (15)
Pune	Jadhav *et al* [6]	2012	26.83	*Aspergillus* spp.(48.71) *Candida* spp.(25.64) *Fusarium* (7.79)
Malayasia	Fadzillah *et al* [4]	2012	25.27	*Fusarium* (46) *Candida* (12.20) *Aspergillus* (9.75)
Vietnam	Nhung *et al* [14]	2012	52.84	*Fusarium* (40.7) *Aspergillus* (25.9)
Egypt	Shabrawy *et al* [24]	2013	58	*Penicillium* (24.2) *Aspergillus fumigatus* (21.2)
Chandigarh	Punia *et al* [11]	2014	-	*Aspergillus flavus* (59.09) *Fusarium* (15.91)
Ajmer	Rastogi *et al* [present study]	2014	43.33	*Aspergillus* (37.04) *Fusarium* (18.52)

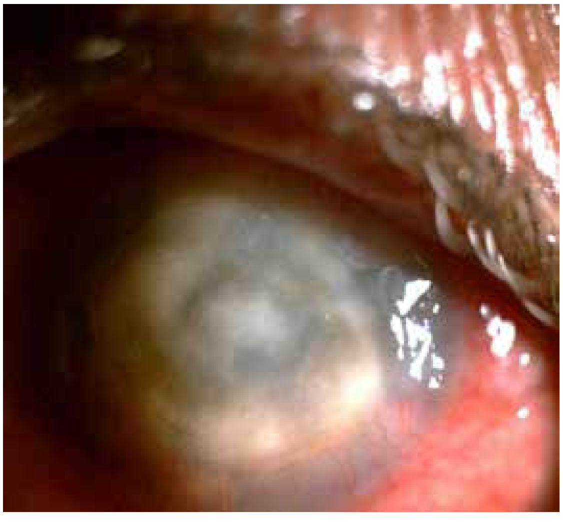

Figure 1 - slit lamp examination showing fungal corneal ulcer with feathery margin

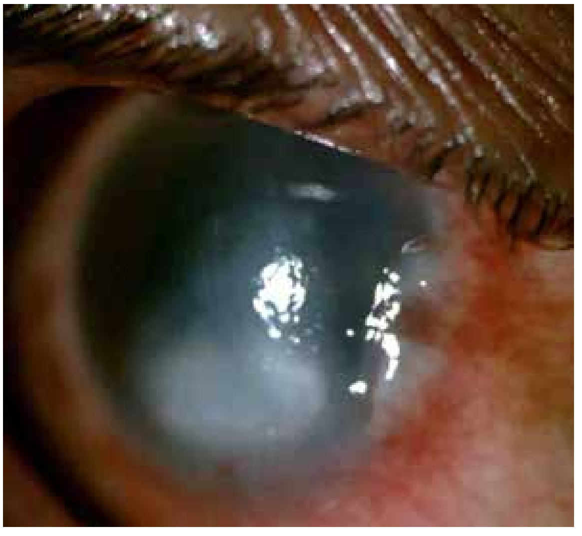

Figure 2 - slit lamp examination showing fungal corneal ulcer with sattellite lesion and indolent vascularization

References:

1. Chirambo MC, Tielsch JM, West KP, Katz J. Blindness and visual impairment in Southern Malawi. Bulletin of the World Health Organization 1986; 64: 567-72.
2. Juarez-Verdayes MA, Reyes-Lopez MA, Cancino-Diaz ME, et al.Isolation, vancomycin resistance and biofilm production of Staphylococcus epidermidis from patientswith conjunctivites, corneal ulcers and endophthalmitis.Rev Latinoam Microbiol 2006; 48 (3-4): 238-46.
3. Whitcher JP, Srinivasan M, Upadhyay MP.Corneal blindness: a global perspective," Bulletin of the World Health Organization,2001;79:214–221.
4. Fadzillah MT, A Norhayati, Siti-Raihan I, M. Ibrahim. A5-Year Retrospective Review of Fungal Keratitis at Hospital Universiti Sains Malaysia. Interdisciplinary Perspectives on Infect Dis2012,ArticleID851563,6pages
5. Thomas PA. Current prespectives on ophthalmic micosis. Clin Microb Rev 2003; 16 :730-97.
6. Jadhav SV, Gandham NR, Mishra RN,Ujagare MT, Sharma M, Sardar M. Prevalence of fungal keratitis from tertiary care hospital from western part of India. Int J.Microbiol Res 2012; 4:211-214.
7. Saha R, Das S. Mycological profile of infectious Keratitis from Delh. Indian J Med Res 2006; 123: 159-164.
8. Sherwal BL,Verma AK. Epidemiology of ocular infection due to bacteria and fungus - A prospective study. J Med Edu Rec 2008, 127-131
9. Tilak R, Singh A, Maurya OPS, Chandra A, Tilak V, Gulati AK. Mycotic keratitis in India: a five-year retrospective study. J Infect Dev Ctries 2010; 4(3):171-174
10. Gill PK , Devi P. Keratomycosis - A retrospective study from a North Indian tertiary care institute JIACM Journal, Indian Acad Clin Medicine 2011; 12(4): 271-3.
11. Punia RS, Kundu R, Chander J, Arya SK, Handa U, Mohan H. Spectrum of fungal keratitis: clinicopathologic study of 44 cases.Int J Ophthalmol 2014;7(1):114-117
12. Bharathi MJ, Ramakrishnan R, Vasu S, et al. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. Indian J Ophthalmol 2003; 51:315-321.
13. Geethakumari PV, Remya R, et al. Bacterial Keratitis and Fungal Keratitis in South Kerala: A Comparative Study. Kerala. J Ophthalmol 2011; 23: 43-46.
14. Nhung PH, Thu TA, Ngoc LH, Ohkusu K, Ezaki T (2012) Epidemiology of Fungal Keratitis in North Vietnam. J Clin Exp Ophthalmol 2012; 3:238.
15. Akter L, Salam MA, Hasan B, Begum N, Ahmed I. Etiological agents of suppurative corneal ulcer: study of 56 cases. Bangladesh J Med Microbiol 2009;3(2):33-36
16. Tuft SJ, Tullo AB. Fungal keratitis in the United Kingdom 2003–2005. Eye 2009 ; 23 : 1308-1313.
17. Tanure MA, Cohen EJ et al. Spectrum of keratitis at Wills Eye Hospital. Philadelphia, Pennsylvania. Cornea 2000; 19: 307-12.
18. Yildiz EH, Abdalla YF, Elsahn AF, Rapuano CJ, Hammersmith KM, Laibson PR, Cohen EJ. Update on fungal keratitis from 1999 to 2008.Cornea 2010;29(12):1406-1411
19. Tuli SS. Fungal keratitis.Clin Ophthalmol. 2011;5:275-279
20. Anutarapongpan O, O'Brien TP. Update on Management of Fungal Keratitis. Clin Microbial 2014; 3: 5
21. Shokohi T, Nowroozpoor-Dailami K, Moaddel-Haghighi T. Fungal keratitis in patients with corneal ulcer in Sari, Northern Iran.Arch Iran Med 2006;9(3):222-227
22. Bhartiya P, Daniell M, Constantinou M, Islam FMA, Taylor HR. Fungal keratitis in Melbourne. Clin Experiment Ophthalmol 2007;35(2):124-130
23. Leck AK, Thomas PA, Hagan M, KaliamurthyJ, Ackuaku E, John M et al.: Etiology of suppurative corneal ulcers in Ghana and South India, and epidemiology of fungal keratitis. Br J Ophthalmol 2002, 86:1211–1215.
24. Shabrawy RME, Badawy NESE, Ashraf, Harb AW. The incidence of fungal keratitis in Zagazig University Hospitals, Egypt and the value of direct microscopy and PCR technique in rapid diagnosis. J Microbiol Infect Dis 2013; 3 (4): 186-191.
25. Thew MR , Todd B.Fungal keratitis in far North Queensland, Australia. Clin Experiment Ophthalmol 2002; 36(8) : 721-4.
26. Alfonso EC, Miller D, Cantu-Dibildox J, O'Brien TP,Schein OD. Fungal Keratitis Associated With Non-therapeutic Soft Contact Lenses. Am J Ophthalmol 2006;142: 154 –155.