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Mycological profile of infectious Keratitis from Delhi

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***Background & objectives*: Corneal blindness is a major health problem worldwide and infectious keratitis is one of the predominant causes. The incidence of fungal keratitis has increased over the last few years. Though a few studies have been carried out on mycotic keratitis from north and other parts of India, there are none from Delhi. Keeping this in mind, this study was conducted to evaluate the frequency of positive fungal cultures in infectious keratitis and of the various fungal species identified as aetiologic agents in patients attending a tertiary care hospital in East Delhi.**

***Methods*: Corneal scrapings from 346 patients of corneal ulcer with suspected fungal aetiology were subjected to direct examination by 10 per cent KOH mount, Gram stain and culture. The results were examined retrospectively and analyzed.**

***Results*: Of the 346 patients of corneal ulcer investigated, in 77 (22.25%) cases fungal aetiology was identified. Males were more commonly affected and were mostly in the age group of 31-40 yr. It was seen that trauma was the most common predisposing factor especially in the agriculturists and the farmers. *Aspergillus flavus* was the most common fungus isolated in 31.16 per cent cases, followed by *A. fumigatus* (16.88%) and *Fusarium* spp. (7.79%). Yeasts were also isolated in 21.62 per cent cases. Both yeasts and mycelial fungi were isolated in 6.5 per cent of cases.**

***Interpretation & conclusion*: Because of serious consequences of infectious keratitis, it is important to know the exact aetiology of corneal ulcer to institute appropriate therapy in time. Laboratory confirmation should be undertaken and fungal infection should be ruled out before prescribing corticosteroids and antibacterial antibiotics.**

Key words *Aspergillus* - corneal ulcer - fungal keratitis

Mycotic keratitis is an important ophthalmologic problem especially in outdoor workers in tropics. It is an important cause of corneal blindness and usually carries an unfavourable prognosis due to its protracted course and requirement of specific therapy. A report on the causes of blindness worldwide consistently lists corneal ulceration second only to cataract as the major aetiology of blindness and visual disability in many of the developing nations in Asia, Africa, and the Middle East[1]. The diversity of clinical presentation observed in each case and also new emerging cases each year pose a diagnostic and

therapeutic challenge to the ophthalmologists. More than 70 genera of filamentous fungi and yeasts have been identified as the aetiological agents of fungal keratitis[2]. The prevalence of individual pathogens largely depends on geographical and climatic factors. Keratomycosis occurs mainly in the warm climates and coincides with seasonal increase in temperature and humidity. Trauma, particularly by vegetative or soil matter, seems to be the most common predisposing factor for keratomycosis[3]. Apart from that, injudicious use of topical corticosteriods and antibacterial agents for external ocular disease enhances the risk further[4]. It may also occur though rarely among the contact lens wearers[5] or by retained intracorneal hair[2].

Though studies on mycotic keratitis have been carried out in various parts of India, no report is available from eastern part of Delhi. This study was thus undertaken to evaluate the frequency of positive fungal cultures in infectious keratitis and of the various fungal species identified as aetiologic agents in the patients attending a tertiary care hospital in East Delhi.

Material & Methods

This retrospective study comprised a total of 346 patients of corneal ulcer treated and investigated in the Departments of Ophthalmology and Microbiology of University College of Medical Sciences (UCMS) and Guru Teg Bahadur Hospital (GTBH), Delhi from January 2000 to December 2004. Thirty patients suffering from bacterial, Herpes simplex and Acanthamoeba keratitis were excluded from this study. Data collected included age, sex, information on the history of the infectious process as well as the use of corticosteriods, topical antibiotics or herbal medicine, previous eye surgeries or any condition of immunosuppression, or use of cosmetic or therapeutic contact lens.

In all cases, collection of corneal scrapings for analysis was carried out directly from the base and margin of ulcers aseptically using Kimura's spatula under direct vision through slit lamp after instillation of anaesthetic eye drops (4% xylocaine). Direct microscopy was performed in 10 per cent KOH wet mount and smears were prepared from each sample for Gram stain also for the demonstration of hyphae, pseudohyphae and yeasts cells. For fungal cultures samples were seeded on the following media: two sets of Sabouraud's dextrose agar (SDA) with antibiotics chloramphenicol (50 mg/ml) and gentamicin (20 mg/ml) and maintained at 25 and 37^{0}C separately over a period of four weeks; one set of brain heart infusion agar (BHIA) containing chloramphenicol (50 mg/ml) and cycloheximide (actidione) (500 mg/ml) were maintained at 25^{0}C for 4 wk and observed daily. All culture media and antibiotics were obtained from Hi-media Laboratories, Mumbai, India. The characteristics considered for fungus identification were macroscopic aspects of texture, colour, growth rate and microscopic aspects such as mycelium and conidium types, relationship between hyphae and fructification organs by lactophenol cotton blue mount. Micro culture on slides was the technique used for observation of filamentous fungi[6]. The yeast isolates were identified by standard tests like germ tube, different spore production on corn meal agar (CMA) or rice starch agar (RCA), urease production and sugar fermentation and assimilation tests[6].

The results were considered positive when smear results were consistent with culture or growth of the organism was demonstrated on two or more media with negative smear results or repeated appearance in smear with negative culture results.

Statistical analysis: Statistical analysis was done by Modules Exact test of SPSS.

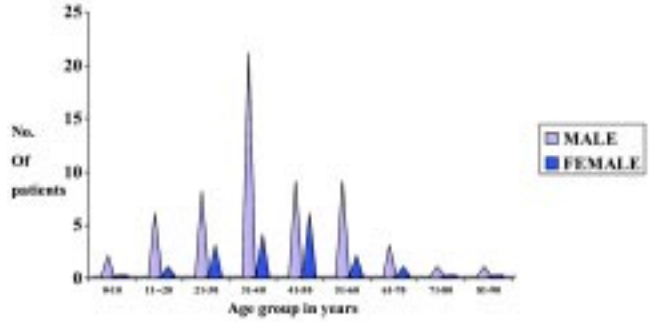

Fig. Age and sex distribution of fungal corneal ulcer patients.

Results

Out of the 346 cases of corneal ulcer investigated, in 77 (22.25%) a fungal aetiology was identified. Of these, 60 (77.9%) were male and 17 (20.07%) female, with a male : female ratio of 3.5:1. The most common age group affected was between 31 to 40 yr in both sexes (Fig.).

Unilateral mycotic infection was observed in 73 patients and bilateral in 4. In cases with bilateral affection of the eye, the same fungus was isolated from both the eyes. Of the 346 cases studied, direct microscopic examination was positive in 67 (19.36%) and in 74 (21.38%) cases, fungi were isolated by culture. Three cases revealed sterile culture inspite of positive direct microscopic findings and in seven cases only cultures were positive. Mycelial fungi were isolated in 58 (78.37%) cases and yeasts in 16 (21.62%) cases. In 5 (6.5%) patients both yeasts and mycelial fungi were isolated. There was an 87.93 per cent (51/58) agreement between the direct examination and culture for filamentous fungi, whereas the same for yeasts was 100 per cent (16/16).

The annual frequency of corneal ulcer and those with a positive culture is shown in Table I.

Among the identified filamentous fungi, most were hyaline. Of the 77 positive specimens, the most frequent agent isolated was *Aspergillus flavus* in 24 (31.16%), followed by *Aspergillus fumigatus* in 13 (16.88%), *Fusarium* species and *Candida albicans*

Table I. Annual frequency of corneal ulcer and fungal keratitis (2000-2004)

Year	Total no. of corneal ulcer samples	Fungal keratitis with positive cultures no. (%)
2000	66	7 (10.6)
2001	73	8 (10.95)
2002	62	15 (24.19)
2003	80	19 (23.75)
2004	65	25 (38.46)
Total	346	74 (21.38)

Table II. Annual frequency of hyaline, dematiaceous fungi and yeasts in 77 patients with fungal aetiology (2000-2004)

Filamentous fungi	2000	2001	2002	2003	2004	Total (%)
Hyaline:						
Aspergillus flavus	2	3	5	6	8	24 (31.16)
A. fumigatus	2	1	2	4	4	13 (16.88)
A. terreus	0	0	0	1	1	2 (2.59)
A. nidulans	0	0	0	1	0	1 (1.29)
Aspergillus spp.	0	0	2	0	1	3 (3.89)
Fusarium spp.	1	1	0	2	2	6 (7.79)
Acremonium spp.	0	0	1	0	1	2 (2.59)
Penicillium spp.	0	0	0	0	1	1 (1.29)
Total hyaline fungi	5	5	10	14	18	52 (67.53)
Dematiaceous:						
Alternaria spp.	0	1	0	0	0	1(1.29)
Dreshlera spp.	0	0	1	0	0	1 (1.29)
Cladosporium spp.	0	0	0	0	1	1 (1.29)
Curvularia spp.	1	0	0	0	0	1 (1.29)
Bipolaris spicefera	0	0	0	0	1	1 (1.29)
Fonsecaea pedrosoi	0	0	0	1	0	1 (1.29)
Total dematiaceous fungi	1	1	1	1	2	6 (7.79)
Total filamentous fungi	6	6	11	15	20	58 (75.32)
Yeasts:						
Candida albicans	1	1	1	1	2	6 (7.79)
Candida tropicalis	0	0	1	1	1	3 (3.89)
Candida parapsilosis	0	0	1	1	0	2 (2.59)
Other *Candida* spp.	0	1	1	1	2	5 (6.49)
Total *Candida* spp.	1	2	4	4	5	16 (20.77)

Table III. Factors associated with mycotic keratitis in both filamentous fungi and yeasts in patients (2000 to 2004)

Associated factors	Fungi	
	Filamentous N=58 (%)	Yeasts N=16 (%)
Topical antibiotic	8 (13.79)	2 (12.5)
Topical corticosteriods	10 (17.24)	2 (12.5)
H/O of trauma	20 (4.48)	4 (25)
Use of therapeutic contact lens	0 (0)	0 (0)
Immunosuppression	2 (3.44)	3 (18.75)
Keratoplasties	7 (12.06)	1 (6.25)
Use of herbal medicine	2 (3.44%)	——
No significant history	9 (15.51)	4 (25)

in 6 each (7.79%). The frequency of isolation of both filamentous fungi and yeasts was greater in the year 2004 (Table II).

Occupationally 30 of the 77 cases (38.96%) of mycotic keratitis were agriculturists/farmers, 7 housewives (9.09%), 7 students (9.09%), 12 labourers (15.58%) and the rest were from various walks of life like tradesman, drivers, merchants, factory workers and tailors.

Trauma appeared to be the most common predisposing factors associated with positive fungal isolations (Table II). However, association of

Table IV. Occurrence and most common fungal species involved in aetiology of fungal keratitis in India

Region	Total no. of cases examined	No. (%) positive for fungus	Most common fungal isolate (%)
Northern Zone:			
Chandigarh			
Talwar *et al* 1978[9]	100	34 (34)	*Aspergillus flavus* (17)
Chander J *et al* 1993[4]	632	47 (7.43)	*A. fumigatus* (16)
Patiala			
Sharma *et al* 1981[10]	100	19 (19)	*Aspergillus* spp. (52.6)
Western Zone:			
Udaipur			
Kulshrestha *et al* 1973[11]	52	23 (44.23)	*Aspergillus* spp. (35)
Mumbai			
Deshpande *et al* 1999[19]	1010	387 (38.31)	*A. fumigatus* (11.11)
Eastern Zone:			
Gauhati			
Dutta *et al* 1981[12]	100	32 (32)	*Aspergillus* spp.(69)
Southern Zone:			
Karnataka			
Kotigadde *et al* 1992[13]	295	67 (22.71)	*A. fumigatus* (28.36)
Hyderabad			
Reddy *et al* 1972[14]	600	36 (6)	*Aspergillous* spp. (50)
Pondicherry			
Dasgupta *et al* 1973[15]	175	50 (28.57)	*Aspergillus* spp. (47)
Tamil Nadu			
Venugopal *et al* 1989[16]	698	322 (46.16)	*A. flavus* (64)
Barathi *et al* 2002[1]	1618	522 (32.26)	*Fusarium* (45)
Barathi *et al* 2003[17]	3183	1095 (34.4)	*Fusarium* (42)
Madurai			
Srinivasan *et al* 1997[18]	434	139 (46.8)	*Fusarium* (47)

Superscript numerals denote reference numbers

filamentous fungi with predisposing factors verses association of yeasts with predisposing factors did not show any significant difference (Modules Exact test of SPSS 13.0; *P*=0.373).

Discussion

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes. The incidence of ocular fungal infections has increased in the last few years due to the improvement in microbiologic diagnostic techniques and because of introduction of new therapeutic measures such as widespread use of broad-spectrum antibiotics, immunosuppressive drugs and corticosteriods[7].

Prevalence of mycotic keratitis may vary depending upon the country of origin. In India it varies between 6-46.8 per cent in various regions (Table IV). In the present study the occurrence was 22.25 per cent, which was similar to that reported from northern India[8,9]. However, higher precentage positivity was reported from eastern, western and southern parts of India (Table IV). This regional variation could be because fungal keratitis was expected to be more common in the tropical and subtropical regions than in the temperate regions.

As has been reported worldwide as well as from India, *Aspergillus* species is the most common isolate in fungal keratitis[8]. Our results also correlated with this fact. However, *Fusarium* species was found to be the most common cause of fungal corneal infections in Southern United States, Florida, Brazil, Ghana, Nigeria, Paraguay and Columbia as well as from some parts of south India (Tamil Nadu)[1,17,19].

Mycotic keratitis may occur at any age, but highest incidence coincides with the period of maximal activity. In our study also the most affected age group was 31-40 yr, *i.e.*, the active age group and men outnumbered women. Men are involved more commonly than women, and agricultural workers and outdoor manual labourers constituted the largest affected occupational group in our study similar to other studies[11,15,18]. In contrast, Kotogadde *et al* from Karnataka[13] and Dutta *et al* from Gauhati[12] have reported higher incidences in females as compared to males.

Injury to the eye is an important predisposing factor. We have obtained a definite history of antecedent corneal injury in about one third of our patients, which was in agreement with the findings of other workers from north India[9]. In our study 3.5 per cent patients gave history of use of topical herbal medicine before ophthalmologic examination. It may be of interest to know that use of topical medicine in the form of breast milk, castor oil, seed oil, onion extract, extract from flower, honey, steam and even dropping of chicken blood have been reported from south India[18].

Some patients gave history of receiving topical antibiotics with corticosteroids empirically without any culture being done. It was only in the event of indolent and progressive nature of the ulcer that the patient was referred to a tertiary care hospital where cultures were obtained revealing the fungal aetiology of the infection. A survey carried out in Southern California revealed that about 50 per cent of patients with a clinical diagnosis of infective keratitis were treated with antibiotics without any cultures being obtained[20].

In conclusion, the key element in the diagnosis of mycotic keratitis is the clinical suspicion by the ophthalmologists and laboratory confirmation of the fungus before prescribing corticosteroids and antibacterial antibiotics. Therefore, precise identification of the causative fungus and institution of appropriate treatment strategy could save the eye from this preventable cause of blindness.

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References

1. Bharathi MJ, Ramakrishna R, Vasu S, Meenakshi R, Palaniappan R. Aetiological diagnosis of microbial keratitis in South India - A study of 1618 cases. *Indian J Med Microbiol* 2002; *20* : 19-24.

2. Anderson KL, Mitra S, Salouli R, Taylor HR. Fungal keratitis caused by *Paecilomyces lilacinous* associated with a retained intraocular hair. *Cornea* 2004; *23* : 516-21.

3. Foster CS. Fungal keratitis. *Infect Dis Clin North Am* 1992; *6* : 851-7.

4. Chander J, Chakrabarty A, Sharma A, Saini JS. Mycotic Keratitis: A five years study from Chandigarh. *Indian J Med Microbiol* 1993; *11* : 218-22.

5. Wilhelmus KR, Robinson NM, Font RA, Hamill MB, Jones DB. Fungal keratitis in contact lens wearers. *Am J Ophthalmol* 1988; *106* : 708-14.

6. Rippon JW. Medical mycology. *The pathogenic fungi and the pathogenic actinomycetes*. 2nd and 3rd ed. Philadelphia: W.B. Saunders Co.; 1982 and 1988.

7. Prats CH, Tello FL, Jose ABS, Otaolaurruchi JS, Baines JP. Voriconazole in fungal keratitis caused by *Scedoporium apiospermum*. *Ann Pharmacol* 2004; *38* : 414-7.

8. Upadhyay MP, Karmacharya PCD, Koirala S, Tuladhar NK, Bryan le, Smolin G. Epidemiologic characteristics, predisposing factors and etiological diagnosis of corneal ulceration in Nepal. *Am J Ophthalmol* 1991; *111* : 92-9.

9. Talwar P, Sehgal SC. Mycotic infection of the eye in Chandigarh and neighbouring areas. *Indian J Med Res* 1978; *67* : 929-33.

10. Sharma SL. Keratomycoses in corneal sepsis. *Indian J Ophthal* 1981; *29* : 443-5.

11. Kulshareshta OP, Bhargava S, Dutta MK. Keratomycosis - A report of 23 cases. *Indian J Ophthal* 1973; *21* : 51-5.

12. Dutta LC, Dutta D, Mohanty P, Sharma J. Study of fungus Keratitis. *Indian J Ophthal* 1981; *29* : 407-9.

13. Kotigadde S, Ballal M, Jyothirlatha, Kumar A, Rao S, Shivananda PG. Mycotic keratitis: A study in coastal Karnataka. *Indian J Ophthal* 1992; *40* : 31-3.

14. Reddy PS, Satyendra ON, Satpathy M, Kumar HV, Reddy PR. Mycotic keratitis. *Indian J Ophthal* 1972; *20* : 101-8.

15. Dasgupta LR, Gupta AK, Ghosh Roy B, Sunderraj T, Ramamurthy S, Lambo PA. Mycological studies in Keratitis. *Indian J Med Res* 1973; *61* : 165-8.

16. Venugopal PV, Venugopal TV, Gomathi A, Ramakrishna ES, Ilavarasi S. Mycotic keratitis in Madras. *Indian J Pathol Microbiol* 1989; *32* : 190-7.

17. Bharathi MJ, Ramakrishna R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. *Indian J Opthalmol* 2003; *16* : 58-60.

18. Srinivasan M, Gonzales CA, Georghe C, Cevallos V, Masearenhas JM, Asokan B, *et al*. Epidemiology and etiological diagnosis of corneal ulceration in Madurai, South India. *Br J Opthalomol* 1997; *81* : 965-71.

19. Deshpande SD, Koppikar GV. A study of mycotic keratitis in Mumbai. *Indian J Pathol Microbiol* 1999; *42* : 81-7.

20. Mc. Donnel PJ, Nobe J, Gauderman WJ, *et al*. Community care of corneal ulcers. *Am J Opthalmol* 1992; *114* : 531-8.

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