



Fungal Profile and Susceptibility Pattern in Cases of Keratomycosis

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Abstract

The present study was undertaken to find out various fungi causing keratomycosis and to determine the antifungal susceptibility profile of these isolates. One hundred corneal scrapings from patients with corneal ulcers were subjected to KOH wet mount preparation. Thirty samples which were positive for fungal elements on direct microscopic examination were cultured on Sabouraud's dextrose agar medium (SDA). The isolates were identified and were subjected to drug susceptibility tests for ketoconazole and fluconazole by tube dilution method. Incidence of fungal corneal ulcers was maximum in the age group of 20-49 years (56.67%). Agriculturists constituted the largest group (53.33%), history of trauma was present in 90% of the patients. *Aspergillus* species were the commonest isolates (70%) followed by *Candida* (20%), *Alternaria* (6.70%) and *Penicillium* (3.30%). MIC of ketoconazole varied from 0.5mg/ml to 10 mg/ml. MIC of fluconazole varied from 0.5 - 10 mg/ml and 19 isolates of fungi did not show any sensitivity to fluconazole upto concentration of 10 mg/ml which was the upper limit of the test system. As agricultural activity and related ocular trauma were principal causes of mycotic keratitis, KOH wet mount preparation is an essential tool in the diagnosis of these infections. It is a very simple and sensitive method. Susceptibility pattern to antifungal drugs was determined to optimize therapeutic response in eye infections.

Key Words

Keratomycosis, *Aspergillus*, Minimum Inhibitory Concentration (MIC), Antifungal Agents.

Introduction

Corneal infection is a leading cause of ocular morbidity and blindness worldwide (1,2). Corneal ulceration is a major cause of monocular blindness in developing countries. Surveys in Asia and Africa have confirmed these findings (1,2) and a recent report on the causes of blindness worldwide consistently lists corneal scarring second only to cataract as the major etiology of blindness and visual disability in Asia (3). Due to a large agrarian population and environmental factors, fungi contribute

largely to the environmental list of infectious intruders of the cornea (4). Review of Indian literature reveals that *Aspergillus* and *Fusarium* are the common fungi isolated along with *Curvularia* and *Dreschlera* which are also leading causes (5). Effective usage of the available drugs is hampered by the inefficiency of currently available antibiotic sensitivity tests for fungal organisms (6). One of the key elements in this effort is a proper understanding of the microbiological and clinical characteristics of this

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disease entity and to perform the antifungal susceptibility test which will enable the ophthalmologist to initiate appropriate antifungal therapy. So the present study was conducted to find out the various fungi responsible for corneal ulceration in this part of the country. The study also evaluates antifungal activity of the drugs.

Material and Methods

One hundred patients having corneal ulcers were selected for the study. The corneal scrapings were subjected to KOH wet mount preparation. Thirty scrapings which were positive for fungal elements were processed further. History of trauma, age and occupation of the patients was recorded. These samples were cultured on Sabouraud's Dextrose Agar (SDA) with antibiotics and inoculated at 28°C. The appearance of growth was observed on alternate days. The growth was identified by standard procedures (7). Pure growth was subcultured in yeast nitrogen base with glucose and asparagine. Growth obtained was washed 3 times with distilled water. Washed mycelia were resuspended in yeast nitrogen base and fragmented with vortex shaker. It was standardized spectrophotometrically. Tube dilution method was opted for doing drug sensitivity test. Serial dilutions of ketoconazole and fluconazole were prepared i.e. 0.5 mg/ml of yeast nitrogen broth, 1 mg/ml of yeast nitrogen broth, 2 mg/ml of yeast nitrogen broth, 5 mg/ml of yeast nitrogen broth, 10 mg/ml of yeast nitrogen broth.

0.1 ml of inoculum was pipetted into each tube containing 2 ml of yeast nitrogen base along with drug and incubated at 30°C and observed on 2nd, 4th, 6th day. One positive and one negative control was put up along with. All the results were recorded taking end point for both ketoconazole and fluconazole which was defined as the lowest concentration in which the growth score was zero i.e. optically clear. This criteria was used to define the minimum inhibitory concentration (MIC) of the drug. Both the drugs were evaluated in vivo at random i.e. the clinical response to the therapy was noted on 1,2,3,4,5 & 7 days. The zone of infiltration, ulcer base, ulcer margin, hypopyon, circumcorneal congestion were observed. Diminution of these were necessary criteria for a healing

ulcer. In event of absence of clinical improvement after treatment for a week, patient was switched to the other antifungal drug.

Results

Fungal isolates were obtained in all the thirty samples which were positive for fungal elements on direct examination. The incidence of fungal corneal ulcer was maximum i.e. 56.67% in the age group of 20-49 years. From 0-19 years it was 13.34% whereas, in 50-69 years it was 30%. The incidence of 76.66% of corneal ulcers in males and 23.34% in females was recorded in our study. Agriculturists (cultivators, farmers and labourers) constituted the largest group i.e. 53.33%. History of trauma was present in 27 patients (90%). Total number of fungi isolated are shown in table No. I. MIC of ketoconazole and fluconazole is shown in table-II. It was observed that patients treated with fluconazole showed the clinical response better than the patients who were treated with ketoconazole. There were 3 cases who did not show clinical signs of improvement with both the drugs.

Table-1

Fungal Isolates in Corneal Ulcers

Fungal isolates	No. of cases	Percentage
	(n=30)	
1. Aspergillus spp.	21	70.00
Aspergillus niger	9	
Aspergillus flavus	12	
2. Candida Species	6	20.00
3. Alternaria	2	6.70
4. Penicillium	1	3.30
Total	30	100.00

Table-II

Minimum inhibitory concentrations of Ketoconazole and Fluconazole against Fungal isolates

Ketaconazole					
MIC's microgram / ml	0.5	1.0	2.0	5.0	10.0
No. of cases	4	2	4	7	13
%age	13.30	6.70	13.30	23.30	43.40
Fluconazole					
MIC's microgram / ml	0.5	1.0	2.0	5.0	10.0
No. of cases	1	2	2	2	4
%age	3.30	6.70	6.70	6.70	13.30

Discussion

In the current study, incidence of fungal ulcers was 56.67% in the age group of 20-49 years whereas it has been shown to be 53.12% by other workers (8). Rehman *et al* (9) reported an incidence of 67.40% in the age group of 30-49 years. The incidence of 76.66% of corneal ulcers in males and 23.34% in females coincides with the study of other workers (9,10).

In the current study the incidence of trauma in relation to fungal corneal ulcer was 90% which coincides with the study of other workers (11). In present study incidence of aspergillus (70%) was quite high as compared to other studies (12) who have reported 52.26%. This might be because of prevalence of aspergillus spores in this region. The incidence of Candida (20%) coincides with other studies (13). The incidence of penicillium (3.3%) is quite similar to the study of Kotigadde⁸ who have shown it to be 2.99%.

In the present study 17 isolates of aspergillus out of 21 did not show any sensitivity to Fluconazole upto the concentration of 10mg/ml which was the upper limit of the test system. Other workers (14) have also observed that aspergillus was resistant to Fluconazole *in vitro*.

In the present study the MIC of ketoconazole was similar to the study conducted by Isabel *et al* (15). ketoconazole was more effective than fluconazole *in vitro* but fluconazole was found to be better antifungal drug *in vivo*. Clinical efficacy of fluconazole might be because of its unique physiochemical and pharmacokinetic properties. This has been shown by Troke *et al* (16) that fluconazole is 15 folds more potent than ketoconazole in a model of vaginal candidiasis in mice despite its being 80 fold less active *in vitro*.

Thus it is concluded that there is region wise variation in the predominance of fungal corneal pathogens. The results of *in vitro* antifungal drug sensitivity testing were not consistent with *in vivo* results, therefore further studies are required. More refined methods need to be devised so that accurate interpretation of the antimycotic activity of these drugs can be done.

References

1. Chirambo MC, Tielsch JM, West KP. Blindness & visual impairment in Southern Malian. *Bull WHO* 1986; 64 : 567-72.
2. Khan MK, Haque MR, Khan MR. Prevalence and causes of blindness in rural Bangladesh. *Ind J Med Res* 1985; 82 : 257-62.
3. Thylefors B, Negral AD, Segaram PR. Available data on blindness (Update 1994). *Ophthalmic Epidemiology* 1995 ; 2 : 5-39.
4. Verenkar MP, Subhangi B, Pinto MJW. A study of mycotic keratitis in Goa. *Ind J Med Microb* 1998; 16 : 58-60.
5. Pankjaluxmi V, Taraluxmi V, Gomathi A, Kavarasi S. Mycotic keratitis in Madras. *Ind J Pathol Microbiol* 1989; 32 : 190-97.
6. Ganegoda N, Rao SK. Medical Research Foundation, Naidu, India..Antifungal therapy for keratomycosis. *Expert Opin Pharmacother* 2004 ; 5(4) : 865-74.
7. Chander J. Routine mycological techniques. Textbook of Medical Mycology, Ed. II, Mehta Publishers, New Delhi, 2002. pp. 391-93.
8. Kotigadde S, Ballal M, Jyothirath AK. Mycotic keratitis : A study in coastal Karnataka. *Ind J Ophthalmol* 1992 ; 40 : 31-33.
9. Rahman MR, Minassian DC, Srinivasan M. Trial of Chlorhexidine gluconate for fungal corneal ulcers. *Ophthalmic Epidemiology* 1997; 4 : 141-49.
10. Reddy RP, Shanthi N, Das H. A randomized single blind comparative study of topical fluconazole / natamycin in the treatment of fungal corneal ulcer. *Ind J Ophthalmol* 1997; 1 : 24-25.
11. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis; A three year study. *Ind J Ophthalmol* 2003 ; 51(4), 315-21.
12. Kumari N, Xess A, Shahi SK. A study of keratomycosis, our experience. *Indian J Pathol Microbiol*. 2002 ; 45(3) : 299-302.
13. Poria VC, Bharad VR, Dongre DS. Study of mycotic keratitis. *Ind J Ophthalmol* 1985; 33 : 229-31.
14. Odds FC, Cheesman SL, Abbott AB. Antifungal effects of fluconazole (UK 49858), a new triazole antifungal, *in vitro*. *J Antimicrob Chemother* 1986; 18(4) : 473-78.
15. Isabel P, Joan FB, Josep G. Effect of inoculum form on *in vitro* antifungal susceptibilities of aspergillus species. *J Antimicrob Chemother* 2001; 47 : 715-18.
16. Troke PF, Andrew RJ, Pye GW. Fluconazole and other azoles translation of *in vitro* activity *in vivo* and clinical efficacy. *Rev Infect Dis* 1990 ; 12 (Supp 3) 276-80.