

MYCOLOGICAL STUDY OF ASPERGILLUS INFECTIONS IN KERATOMYCOSIS

Jayant M. Deshmukh¹, Swati M. Bhise², Asmita Band³

HOW TO CITE THIS ARTICLE:

Jayant M. Deshmukh, Swati M. Bhise, Asmita Band. "Mycological Study of Aspergillus Infections in Keratomycosis". Journal of Evidence Based Medicine and Healthcare; Volume 1, Issue 6, August 2014; Page: 391-397.

ABSTRACT: BACKGROUND: In recent years, fungi have been emerging more commonly as infectious agents in patients of tertiary care centers. **AIM:** The present study was undertaken to find out the prevalence of aspergillus infection causing keratomycosis and to determine the predisposing factors associated with these isolates. **METHODOLOGY:** One hundred twenty nine corneal scrapings from patients with corneal ulcers were subjected to KOH wet mount preparation. Twenty six samples which were positive for fungal elements on direct microscopic examination were also inoculated on Sabouraud's dextrose agar(SDA) medium. **RESULTS:** Incidence of aspergillus in corneal ulcers was maximum in the age group of 31-50 years (74%). Agriculturists constituted the largest group (42%) & history of trauma was present in 79% of the patients. Aspergillus fumigatus was the commonest species isolated (63%). **CONCLUSION:** Agricultural activity and related ocular trauma were the principal causes of mycotic keratitis. KOH wet mount preparation is an essential tool in the diagnosis of ocular fungal infections since it is a very simple and sensitive method. However, isolation is important to detect the prevalent strains. **KEYWORDS:** *Corneal ulcer, keratomycosis, Aspergillus fumigatus, agriculturists, trauma.*

INTRODUCTION: Corneal infection is a leading cause of ocular morbidity worldwide.^[1] Corneal ulceration is a major reason of ocular blindness in developing countries. Surveys in Asia and Africa have confirmed these findings.^[1] A recent report consistently lists corneal scarring second only to cataract as the major etiology of blindness and visual disability in Asia.^[2] Members of the Genus Aspergillus are ubiquitous organisms. Spores, the infective elements are distributed everywhere from the tropics to the arctics and are present on all objects, including household dust. Infections are encountered all over the world and seen in all age groups, both sexes and all races.^[3] Aspergilli are considered as conditional pathogens since various underlying conditions and factors contribute to the disease process.

Corneal infection of fungal etiology (keratomycosis) is very common and represents 30-40% of all cases of culture-positive infectious keratitis. Moreover, fungi have replaced bacteria as the predominant cause of infectious keratitis in developing countries.^[4]

The present study was conducted to find out the prevalence of Aspergillus responsible for corneal ulceration and associated predisposing factors in this part of the country.

METHODOLOGY: Our study was carried out for two years in the department of Microbiology, Government Medical College and Hospital, Nagpur. One twenty nine cases with clinical suspicion of Aspergillus or fungal infection such as traumatic ocular infections and corneal ulcers were included in the study [Figure 1].



Fig. 1: Corneal ulcer

The eye was cleaned with sterile normal saline to remove all necrotic exudates. Bark-Parker blade No. 15 was used to collect scraping from the cornea. First 2-3 drops of topical anesthetic solution (4% Xylocaine) were used and scraping was done 4-5 minutes after instillation of anesthetic drops. The entire base of the ulcer as well as edges was thoroughly scraped to obtain as much material as possible.

All specimens were subjected to KOH wet mount preparation and Gram staining. History of trauma, age and occupation of the patients was recorded. These samples were inoculated on two slopes of Sabouraud's dextrose agar (SDA) with antibiotics for isolation of fungi. One slope was incubated at 37°C and another at room temperature. The slopes were observed every alternate day for appearance of growth. The growth was identified by standard procedures.^[5]

The characteristics considered in fungal identification of the isolate by macroscopic examination of culture tubes were texture, color and growth rate. The slide culture technique with Lactophenol cotton blue (LPCB) mount showed characteristics such as mycelia, conidia types and hyphae more clearly. [Figure: 2]

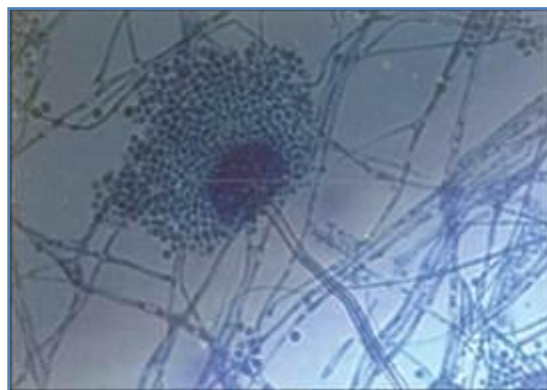


Fig. 2: Lactophenol cotton blue tease mount showing A. fumigatus sporing head

ORIGINAL ARTICLE

Statistical analysis: Statistical analysis was performed using the Statistical Package Social Sciences (SPSS). 'X²' test was done by taking $p < 0.05$ as statistically significant.

RESULTS: The study enrolled 129 clinically suspected cases of Aspergillus keratomycosis comprising of eighty (62%) males and 49 (38%) females. Out of 129 specimens processed, 23 were positive for fungus. Only those samples that were positive by both microscopy and culture were included. [Table: 1]

Microscopy (10% KOH)	Culture Positive for Aspergillus		Total
	Positive	Negative	
Positive	19 *	3	22
Negative	4	103	107
Total	23	106	129

Table 1: Correlation between microscopy and culture in aspergillus infection

* Included in the study.

Aspergillus spp. were isolated in 19 (15%) cases (13 males and 6 females), yeasts in 3% and fusarium species in 1% specimen. [Figure: 3]

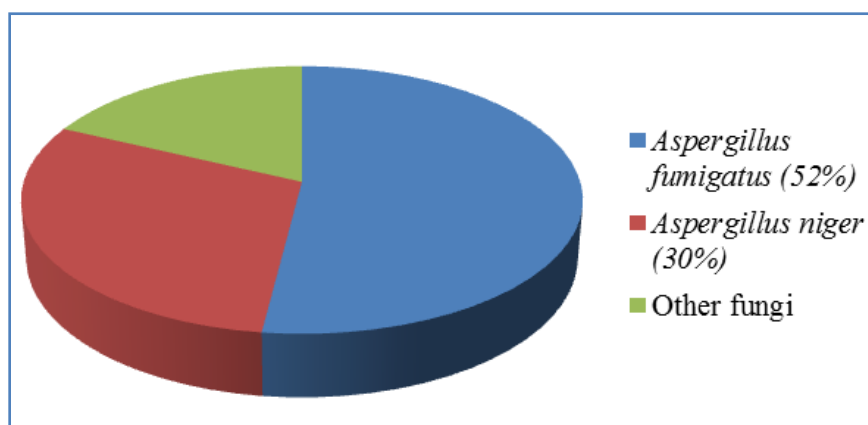


Fig. 3: Fungal isolates in corneal ulcer

The incidence of Aspergillus infection in corneal ulcer was maximum in the age group of 31–50 years (14/19 i.e. 74%) followed by 10.5%, in the age group of 50–69 years, whereas in the age group of 0–19 years it was only 5%. [Graph: 1] Aspergillus fumigatus was the predominant species isolated in 12 cases (63%) followed by Aspergillus Niger in 7(27%). Agriculturists (cultivators, farmers and laborers) constituted the largest group, i.e. 42%, mostly from rural area (79%). The incidence of 68% of corneal ulcers in males and 32% in females was

ORIGINAL ARTICLE

recorded in our study. Most common predisposing factor leading to aspergillus infections was corneal trauma (79%) followed by steroid/antibiotic eye drops (53%) beside others [Table: 2]

Predisposing factors	Corneal ulcer cases (129)	Aspergillus Positive (19)	Aspergillus Negative (110)	Odds ratio	X ²	P value
Age group (31-50 years)	69 (54%)	14 (74%)	55 (50%)	-	-	-
Farmers	35 (27%)	8 (42%)	27 (25%)	-	-	-
Rural area	73 (57%)	15 (79%)	58 (53%)	-	-	-
Trauma	107 (83%)	15 (79%)	92 (84%)	4.178	3.748	<0.005*
Topical Steroid/ Antibiotic drops	21 (16%)	10 (53%)	11 (10%)	10.8	4.67	<0.005*
Oil drops	2 (1.6%)	0	2 (1.8%)	-	F**	>0.005
Herbal juices	3 (2.3%)	0	3 (2, 7%)	-	F**	>0.005
Diabetes Mellitus	11(8.5%)	4 (21%)	7 (6.4%)	0.111	0.351	>0.005
Contact lens	9 (7%)	0	9 (8.2%)	-	F**	>0.005
No specific factor	13 (10%)	0	13 (12%)	-	F**	>0.005

Table 2: Showing Different predisposing factors associated with Aspergillus keratomycosis

X²: Chi -square test; **: Fisher exact test; *: Statistically significant.

DISCUSSION: In the current study, incidence of fungal ulcers was 74% in the age group of 31-50 years, whereas it has been shown to be 67% by Rehman et al [6] and 53% of other workers.[7] Higher percentage of Aspergillus corneal ulcer between 40-60 years is reported by Verenkar et al; 1998[8] Further, the incidence of Aspergillus in this study was quite high (83%) as compared to other studies who have reported an incidence of 52.26%.[9] This might be because of the prevalence of Aspergillus spores in this region. Aspergillus fumigatus was the most common species in our study (63%), same as reported by others,[7, 8] however, in the study of Narayan Shrihari et al (2012) Aspergillus flavus was the predominant isolates followed by Aspergillus fumigatus.[10]

The occupation of the patient may have a bearing both on corneal ulcer as well as Aspergillus infection. Trauma to the eye by vegetable matter,[11] field dust, the swish of a cow's tail while milking, twigs, thorns, dusty grains, corn stalks, grass, reeds favor mycotic keratitis as all of them have a generous number of fungal spores.[8,12] Fungal infections are thus more common in those who have close contact with soil, vegetable matter and prone to injury as compared to other occupational groups. Vegetative trauma as the most important predisposing factors is reported by other authors.[8, 11, 12]

In the current study the incidence of trauma in relation to fungal corneal ulcer was 79%, which coincides with the study of other workers.[13,14] The underlying stroma becomes excessively hydrated and possibly altered in such a way to constitute a more favorable site for fungus to

ORIGINAL ARTICLE

grow. Keratomycosis caused by filamentous fungi is an occupational hazard of farmers and agricultural workers.^[15, 16]

Prolonged use of topical steroids facilitates fungal infection by altering local immune response and promoting conjunctival colonization by fungi. Additionally, it indirectly promotes fungal replication and corneal invasion by interfering with host's inflammatory response. The systemic use of corticosteroids may predispose to fungal keratitis by causing immunosuppression.^[17]

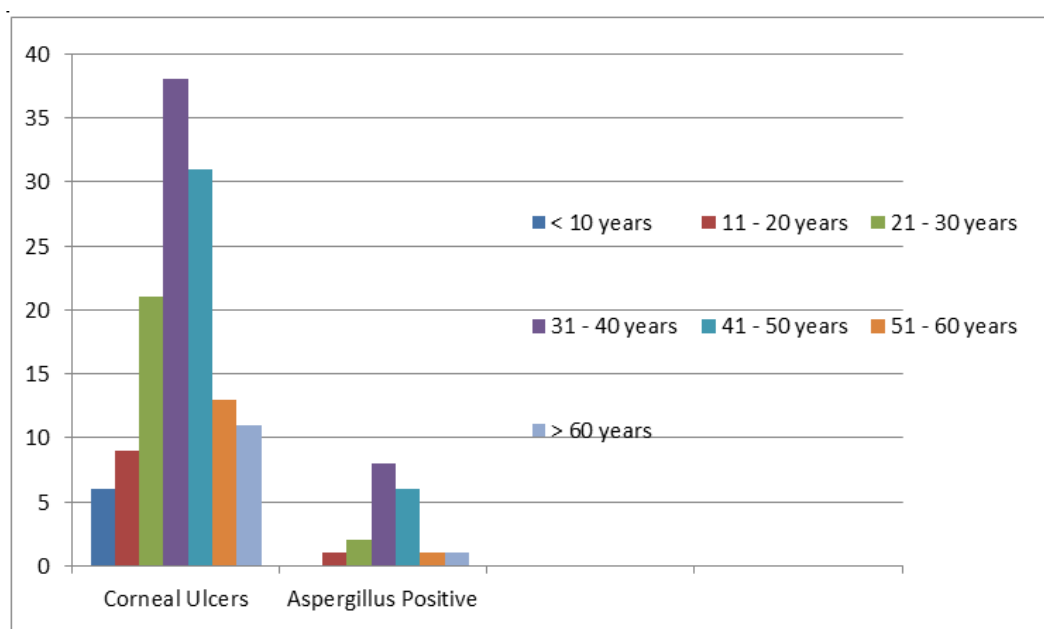
CONCLUSION: Keratomycosis is one of the most difficult to diagnose and treat form of microbial keratitis for the ophthalmologist. The diagnostic work up is tedious and the topical anti-fungal drugs are not as effective as antibiotics in bacterial keratitis. The treatment is prolonged and is often complicated by secondary glaucoma and medical failures. However, a high index of clinical suspicion and a judicious management approach will go a long way in reducing ocular morbidity & visual disability.

REFERENCES:

1. Khan MK, Haque MR, Khan MR. Prevalence and causes of blindness in rural Bangladesh. *Ind J Med Res* 1985; 82: 257-62.
2. Thylefors B, Negral AD, Segaram PR. Available data on blindness (Update 1994). *Ophthalmic Epidemiology* 1995; 2: 5-39.
3. Wald A, Wendy L, Jo-Anne Yan Burik Raleigh A Bowd. (1997) Epidemiology of Aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. *J Inf Dis* 1997; 175 (6): 1459 – 1466.
4. Sengupta S, Rajan S, Reddy PR, Thiruvengadkrishnan K, Ravindran RD, Lalitha P, Vaitilingam C M. Comparative study on the incidence and outcomes of pigmented versus non pigmented keratomycosis. *Indian J Ophthalmol* 2011; 59(4): 291-296
5. Chander J. Routine mycological techniques. *Textbook of Medical Mycology*, Ed. II, Mehta Publishers, New Delhi, 2002. pp. 391-93.
6. Rahman MR, Minassian DC, Srinivasan M. Trial of Chlorhexidine gluconate for fungal corneal ulcers. *Ophthalmic Epidemiology* 1997; 4: 141-49.
7. Kotigadde S, Ballan M, Jyothiratha, Kumar A, Rao S, Shivananda PG. (1992). Mycotic Keratitis A study in coastal Karnataka. *Indian J. Ophthalmology* 1992; 40 (1): 31-33
8. Verenkar MP, Borkar S, Pinto MJW, Naik P (1998). A study of mycotic keratitis in Goa. *Indian J. medical Microbiology* 1998; 16(2): 58-60.
9. Kumari N, Xess A, Shahi SK. A study of keratomycosis, our experience. *Indian J Pathol Microbiol.* 2002; 45(3): 299-302.
10. Narayan S H, Kumudini T.S, Mariraj.J, Krishna.S. The Prevalence of Keratomycosis, Dermatophytosis and Onychomycosis in a Tertiary care Hospital. *Int J Med Health Sci* 2012; 1(3): 25-30.
11. Dutta LC, Dutta D, Mahanty P, Sharma J. 1981 Study of Fungus Keratitis. *Indian J. Ophthalmology* 1981; 29: 407-409.

ORIGINAL ARTICLE

12. Sandhu DK, Randhawa IS, Singh D (1981). The Correlation between environmental and ocular fungi. Indian J. Ophthalmology 1981; 29: 177-182
13. 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.
14. Shokohi T, Dailami KN, Haghighi TM, Fungal Keratitis in patients with corneal ulcer in Sari, Northern Iran, Archives of Iranian Medicine, 2006; 9(3): 222-227.
15. Sudan R, Y R Sharma. Keratomycosis: Clinical diagnosis, Medical and Surgical Treatment. JK science. 2003; 5(1) pg 1-10.
16. Usha A, Aruna A, Vijay J. Fungal Profile and Susceptibility Pattern in Cases of Keratomycosis. JK SCIENCE 2006; 8(1) pg 39- 41.
17. Mitsui Y. Hanabusa J. Corneal infections after cortisone therapy. 131' Ophtha11110/1955; 39: 244-50.



GRAPH 1

ORIGINAL ARTICLE

AUTHORS:

1. Jayant M. Deshmukh
2. Swati M. Bhise
3. Asmita Band

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, Dr. Ulhas Patil Medical College & Hospital, Jalgoan, M.H.
2. Assistant Professor, Department of Microbiology, Government Medical College, Nagpur. M. H.
3. Microbiologist Incharge in Bombay Hospital, Mumbai.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Jayant Mahadeorao Deshmukh,
Associate Professor,
Department of Microbiology,
Dr. Ulhas Patil Medical College & Hospital,
Jalgaon Khurd, Post: Nasirabad – 425309,
Jalgaon District, Maharashtra State.
E-mail: jayantmdeshmukh@yahoo.co.in

Date of Submission: 31/07/2014.
Date of Peer Review: 01/08/2014.
Date of Acceptance: 07/08/2014.
Date of Publishing: 12/08/2014.