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TEN YEAR STUDY OF FUNGAL KERATITIS - TERTIARY CARE HOSPITAL

N. Shanmuga Vadivoo[1], K. Sujatha[2], N.V.K. Sridevi[3], R. Sharda[4], M. Niranjana[5]

ABSTRACT

Objective: To analyze and understand the epidemiologic features of fungal keratitis as seen in a tertiary care hospital.

Materials & Methods: A ten year retrospective review of 228 patients with diagnosis of fungal keratitis was done. Demographic pattern, risk factors and fungal etiology were evaluated.

Results: Corneal scrapings from 228 patients with corneal ulcer were evaluated. Fungi grew in43.42 % of the specimens. Of the culture proven cases, 71 % of patients were male and the Mean age was 21 to 50 years. History of trauma was present in 74 patients while 25 patients had only symptoms. Fungal elements were seen in direct microscopy in 41.6% of specimens - KOH mount 84 %, calcoflour white mount 84%, grams stain 82% and Lacto phenol cotton blue mount 74%. These were all filamentous fungi. Yeasts or yeast like fungi were significantly absent. From 40% of the samples only fungi were isolated, while 16% had only bacterial growth, 3% had both. There was no growth in 41% of samples. The most commonly isolated fungi were - *Fusarium* species (49.49%), followed by *Aspergillus spp* (22.2%). Dematiacious fungi and coelomytes accounted for 18 % & 2% respectively.

Conclusion: Our results confirm to the existing literature on fungal keratitis in India in that keratomycosis is predominant in adult males in the mean age group of 21-50 years, and filamentous fungi especially *Fusarium*, are significant etiological agents for fungal keratitis. Trauma with vegetative matter is the major predisposing factor indicating that filamentous fungal keratitis is more commonly seen in persons living in rural areas involved in outdoor occupation.

Key Words: *Fungal keratitis, Retrospective study, India*.

INTRODUCTION

Infective keratitis due to fungal etiology is very common and represents 30% to 40% of all cases of culture proven infective keratitis[1, 2, 3, 4, 5.] Fungal keratitis occurs in healthy young males engaged in outdoor occupation[6,11]. The principal predisposing factor appears to be trauma. Filamentous fungi are the reason for large proportion of corneal infection particularly following trauma with vegetative matter [1, 3, 4, 6, 7, and 11]. *Fusarium & Aspergillus* species are the etiology of 70 % of cases. The purpose of this study is to report the epidemiologic features of fungal keratitis as observed in a tertiary care hospital in South India.

MATERIALS AND METHODS

Patients: A total of 228 patients with corneal ulceration, seen at Department of Ophthalmology over a ten year period - 1998 to 2008, were evaluated. Demographic, clinical and laboratory details on these patients were collected through retrospective Chart review.

Specimen Collection and Microbiological methods

Corneal scrapings were collected following standard recommended methods [6,8,9.] Under local anesthesia, without preservative, the edge of ulcer was firmly scraped using Bard parker blade number-9, after removal of debris (or) discharge in the vicinity. The aid of slit lamp/ operating microscope was made use of for the procedure. Several scrapings were collected and used in sequence to prepare smears and inoculate culture media. The scraped material obtained from the edge & the base of ulcer was initially inoculated on to solid media such as Sheep Blood Agar (BA), Chocolate Agar (CA), and Sabourauds Dextrose Agar (SDA) in a row of 'C 'shaped streaks. Then the scraping material was spread on to labeled slides in a thin even manner for examination with 10% KOH mount, Lacto phenol cottonblue mount(LCB) [10], and Grams stain. Meticulous care was taken in collection of material and its aseptic transfer to the appropriate culture media. On 10 % KOH mount, after allowing the material for digestion, a drop of calcoflour white stain was also added and then mounted. The inoculated BA

[1]Assistant Professor, Department of Microbiology, Annapoorna Medical College & Hospital, Periyaseeragapadi, Salem, Tamilnadu.
[2,3,4,5] Technical Assistants, Department of Microbiology, Sri Sathya Sai Institute of Higher Medical Sciences, Puttaparthy, Andrapradesh.

and CA were incubated at 37^{0}C for seven days, while the SDA was incubated at 27^{0}C for three weeks.

Interpretation of results of Smears & culture

Smears were observed and reported for the presence or absence of fungal elements including yeast like organisms. Cultures were examined everyday and the growth was considered significant if

1. The same organism has been isolated on more than one occasion.
2. It had been isolated in two or more media inoculated with corneal scrapings.
3. It was consistent with the clinical signs.
4. Growth occurs only on the 'C' streaks. (Growth away from the 'C' mark is considered a contaminant) A subculture of the fungal growth on primary isolation media was done on Potato Dextrose Agar (PDA) slide culture mounts & incubated for seven to ten days to facilitate sporulation. Following adequate growth of fungal isolate on PDA slide culture mounts, identification was done based on its macroscopic and microscopic features.

Table -- 1: Demographic details of the 99 patients with proven fungal keratitis

Demography	Particulars	No (%)
Gender	Males	70 (71%)
	Females	29 (30%)
Age in years	< 21 years	10
	21—30 years	16
	31---40 years	27
	41---50 years	23
	>50 years	17
Predisposing factor	**Pts with H/o Trauma**	
	Vegetative matter	**74 (75%)**
	Stone/sand	40 (41%)
	Dust/dirt	10
	Cow/dog tail	18
	Pinprick	2
	Splinter/wood	1
	Patients with only Symptoms & Agent not known.	3
		25

Table –2: Microbiology of corneal scrapings over the study period

Total Number of Corneal Scrapings	228 No	%
Total culture Positive	***135***	***59***
Pure Fungal isolates	92	40
Pure bacterial isolates	36	16
Mixed Fungal &Bacterial isolates	7	03
Total Culture Negative	***93***	***41***

Table --3: Culture proven fungal keratitis

Year	No of corneal scrapings	Culture Positive No	(%)
1998	01	01	100
1999	25	10	40
2000	04	0	0
2001	08	02	25
2002	05	02	40
2003	07	04	57.1
2004	24	10	41.6
2005	26	10	38.46
2006	33	11	33.33
2007	44	25	56.81
2008	51	24	47.05
Total	**228**	**99**	**43.42**

Table - 4: Correlation between direct microscopy and culture

Methods	MICROSCOPY POSITIVE	MICROSCOPY NEGATIVE	Total
CULTURE POSITIVE	79	20	99
CULTURE NEGATIVE	16	113	129
Total	95	133	*228*

Table –5: Fungal isolates over the 10 year period

Filamentous fungi	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	total
Hyaline fungi												79
Fusarium spp	01	07	-	02	01	02	04	04	05	10	13	49
Aspergillus's spp	-	03	-	-	-	02	01	02	03	04	07	22
Acremonium spp	-	-	-	-	-	-	01	01	-	02	01	05
Sedosporium spp	-	-	-	-	-	-	-	01	-	-	-	01
Cylindrocarpon spp	-	-	-	-	-	-	-	-	-	01	-	01
Unidentified hyaline	-	-	-	-	-	-	-	-	-	01	-	01
Dematiaceous fungi												18
Bipolaris spp	-	-	-	-	-	-	-	-	01	01	01	03
Lasidiotheobrome diplopia	-	-	-	-	01	-	-	-	-	-	-	01
Cladosporium spp	-	-	-	-	-	-	01	-	-	-	-	01
Exserohilum spp	-	-	-	-	-	-	01	-	-	-	-	01
Curvalaria spp	-	-	-	-	-	-	-	-	-	01	-	01
Helminthosporium sp	-	-	-	-	-	-	-	-	-	01	-	01
Stemphylium spp	-	-	-	-	-	-	-	-	-	-	01	01
Unidentified [UID]							02	02	02	02	01	09
Coelomycetes												02
Colletotrichum Sp	-	-	-	-	-	-	-	-	-	02	-	02
Total	01	10	0	02	02	04	10	10	11	25	24	99

Table: 6: Comparison of Direct Microscopic Methods

Name of Investigation	Result	Number	Fungal growth		Sensitivity
			Positive	Negative	
KOH wet mount	Positive	80	67	13	68%
	Negative	148	32	116	
	Total	**288**	**99**	**129**	
Grams Stain	Positive	79	70	09	71%
	Negative	149	29	120	
	Total	**288**	**99**	**129**	
LCB Wet mount	Positive	71	64	07	65%
	Negative	157	35	122	
	Total	**288**	**99**	**129**	
CFW Wet mount	Positive	80	67	13	65%
	Negative	148	32	116	
	Total	**288**	**99**	**129**	

RESULTS

The results are summarized in tables 1-6.

***Table-1:** gives the demographic details of the patients with proven fungal keratitis.

***Table-2:** -provides information on the microbial growth from the corneal scrapings over the study period. A total of 99 filamentous fungal isolates were recovered from 228 corneal scrapings. Ninety seven specimens had single species of fungal isolate and one specimen had two species of fungal isolate.

***Table-3:** The number of corneal scrapings submitted for

fungal culture during the study period (1998-2008), and the number & percentage of fungal growth each year.

***Table-4:** gives the correlation between direct microscopy and culture.

***Table-5:** indicates the genera of fungi isolated over the ten year period. Out of 99 fungal isolates 89 could be identified. Ten were unidentified fungi.

***Table-6:** indicates the sensitivity of Different Direct microscopic methods used to identify the fungal elements

DISCUSSION

Fungal keratitis continues to be a major cause of vision loss in developing countries. A hot humid climate and an agricultural based occupation of a large population make fungal keratitis more frequent in tropical countries. The predominance of fungal corneal infections in the younger age group (21-50 years) and among males could be attributed to their involvement in outdoor activities. Thus they are more prone to corneal injury with external agents. A few investigators have made similar observations.[1,6,11]

Our study also substantiates these details where males (80%) were predominantly involved and in the age group of 21-50 years (Table-1). The commonest predisposing factor in our series was ocular trauma (75%), most commonly with vegetative matter-41% (Table-1). This is in accordance with previous data from our subcontinent[1,3,4,6,7,11]

During the Ten year period, the major etiology of Keratitis in our Hospital was fungus which is supported by many studies from India. [1, 2, 3, 4, 5.] From 40% of the samples only fungi were isolated, while 16% had only bacterial growth, 3% had both bacteria and fungi .There was no growth in 41% of samples (Table-2). There was a gradual increase in the number of specimens and also higher rate of isolation in our tertiary care centre which was probably due to increasing awareness and also development of diagnostic methods in mycology (Table-3).

When correlation was done for fungal identification methods between direct microscopy & fungal isolation in our study (Table-4), fungal elements were seen in direct microscopy in 95 specimens (41.6%) and culture positive was seen in 99 of samples (43.42 %). This data differ from a study by Bakshi et al [1] where direct microscopy revealed fungal filaments in 90.9% specimens and Culture positivity was seen in 57.3%.

Fusarium was the commonest fungus isolated in our series (49.49%), followed by *Aspergillus spp (*22.22%) as shown in Table-5. This is in accordance with series by Gopinathan *et a*l and Bharathi *et al*[4, 7] but contrast to the other studies [1, 3, 5, 11] where *Aspergillus* spp was the most common isolate. Dematiacious fungi contributed to 22.2% of culture positive cases in our series. This is almost similar to the study by Chowdry *et al* & Bharathi *et al* [5,7], where dematiaceous fungi accounted for 29% and 28.07 % respectively. In our study Coelomycetes accounted for 0.2% of isolated fungi. Around 20 reports of *Colletotrichum* infection have been recorded in medical literature and only a few of them have been reported from India. Seven cases were reported by Kaliamurthy et al in 2004 [14], one report by Joseph et al in 2004 [15], and one in 2005 by Menderitta et al [16]. We report two cases of fungal keratitis caused by *Colletotrichum* spp. Yeasts or yeast like fungi were significantly absent in direct microscopy and not isolated as well. They are more commonly isolated in temperate climatic condition. [13]

In this series direct microscopy with Gram stain showed better sensitivity i.e 71% for detection of fungal elements compared to the other microscopic methods , i.e. KOH wet mount (68%), Calcofluor staining (68%) and LPCB staining (65%) respectively (Table-6). This is in contrast to other previous data where KOH was more sensitive[3,5,12]. This difference in results could have been due to the difference in quantity of material collected and sampling from different sites of the ulcer of varying severity.

CONCLUSION

Our results conform to the existing literature on fungal keratitis in India in that keratomycosis is predominant in adult males the mean age group being 21-50 years, and filamentous fungi especially Fusarium, are significant etiological agents for fungal keratitis. Trauma with vegetative matter is the major predisposing factor indicating that filamentous fungal keratitis is more commonly seen in persons living in rural areas involved in outdoor agricultural activity. Education for protection from occupational injury might help to reduce the incidence of fungal keratitis in this population.

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