Original Article

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Microbiology Section

Mycotic Keratitis in Solapur (A Two Years Study)

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ABSTRACT

Introduction: Corneal ulcer is the second most common cause of visual disability and blindness in developing countries after cataract. Corneal scarring due to keratitis can be prevented. This therefore necessitates the knowledge of its etiology for early intervention.

Aim: To study the occurrence, etiology and predisposing factors of mycotic keratitis at a tertiary care centre in Solapur, Maharashtra.

Materials and Methods: The retrospective study was carried out for duration of 2 years (April 2013 to March 2015). All aseptically collected corneal scrapings from clinically suspected cases of fungal keratitis were included. Conventional methods i.e. KOH mount, Gram's stain and fungal cultures on a pair of Saubaraud Dextrose Agar were used for diagnosis. Slide cultures on Corn Meal Agar with tween 80 were used for confirmation of moulds and germ tube tests done for yeasts.

Results: Hundred corneal scrapings were studied out of which fungal hyphae were identified in 20% of the KOH mounts and in 17% of Gram's stained smears. Fungal culture was positive in 24 samples. Predominant isolate was *Fusarium* spp (29%. 7/24) followed by *Alternaria* spp (21%, 5/24) and *Aspergillus* spp (17%, 4/24) and 8% (2/24) each of *Cladosporium* spp, *Penicillium* spp, *Bipolaris* spp and *Candida albicans*. Ocular injury was the most commonly encountered predisposing factor.

Conclusion: Fungal aetiology constitutes a significant proportion of corneal ulcers, out of which *Fusarium* spp is the most common isolate in Solapur, Maharashtra followed by *Alternaria* spp unlike majority of studies in the country where *Aspergillus* spp are the most common isolates.

Keywords: Corneal scrapings, Fungal culture, Fungal isolates, Microscopy

INTRODUCTION

Keratitis is a condition in which the cornea becomes inflamed by infectious organisms like virus, bacteria, fungus and parasites or non-infectious agents [1]. In developed countries viral infections are common whereas bacteria, fungi and Acanthamoeba are more common causes in developing countries [2]. The condition is marked by moderate to intense pain, impaired eyesight, photophobia, red eye and a gritty sensation and often results in corneal scarring and opacification ultimately leading to blindness [3].

Retinal diseases (40–54%) are important cause of blindness in developed nations while it is cataract (44–60%) and corneal diseases (8–25%) for developing countries [4]. Certain conditions like injury to the eye and therapy with antibiotics and corticosteroids render the eye susceptible to infection with various fungi especially in tropical parts of the world [5].

The incidence of fungal keratitis is higher in tropical regions of the world especially India [6], *Aspergillus flavus* is the most common isolate in Northern India (22.5%) [2], Eastern India (15.85%) [5], Western India (45.29%) [1] and Mumbai 14.7 % [6], *Aspergillus fumigatus* in Chennai 40% [7], *Aspergillus* spp in Chandigarh (41.18%) [8], and *Fusarium* spp in South India (42.%) [9]. Yeast isolation from corneal ulcers is generally low in India [10,11].

It is expected that individuals suffering from unilateral corneal blindness in India will be 10.6 million by 2020 [10] .Corneal scaring due to keratitis can be prevented [12]. Hence, this study aims to find out the occurrence of fungal keratitis, its etiology and predisposing factors with respect to the city of Solapur.

MATERIALS AND METHODS

The retrospective study was carried out for duration of 2 years (April 2013 to March 2015), in the Department of Microbiology Dr V.M Government Medical college Solapur, Maharashtra, India. Based on inclusion and exclusion criteria sample was collected from 100 corneal ulcers scrapings and were studied.

Inclusion criteria

All corneal scrapings from clinically suspected cases of fungal keratitis received in the Microbiology Department Dr VMGMC, Solapur were included in the study.

Exclusion criteria

Clinically suspected bacterial and viral keratitis was excluded from the study.

Procedure

The corneal scrapings were collected aseptically by Ophthalmologists and sent to Microbiology Laboratory. They were then inoculated on to one Chocolate Agar and two Sabouraund's Dextrose Agar (SDA) by making "C" or "S" streak to ensure fungal growth in the form of streak was from inoculum and not a laboratory contaminant [13]. Due to paucity of samples and for better yield minimum media was used for inoculation. The remaining were taken on to the centre of clean glass slide to be used for direct microscopic examination.

The Chocolate Agar and one SDA were incubated at 37^{0}C and the other SDA at 25^{0}C and were observed daily for growth. Confirmation of moulds was done by putting slide cultures on Corn Meal Agar (CMA) with tween 80 and germ tube tests done for yeasts [14]. The glass slides were used for performing KOH mount and Gram's staining for the demonstration of fungal hyphae. Detailed relevant history including age, sex, occupation, address, history of ocular trauma/injury, duration of symptoms, previous treatment, predisposing ocular conditions, use of topical medicines / steroids and contact lens was recorded [Table/Fig-1,2].

TESTS	POSITIVE	PERCENTAGE
1. KOH Mount	20	20%
2. Gram's Stain	17	17%
3. Culture	24	24%

[Table/Fig-1]: Test positivity

PREDISPOSING FACTORS		PERCENTAGE
1. Age	<14 yrs	4%
	15 yrs - 24 yrs	10%
	25 yrs - 34 yrs	38%
	35 yrs - 44 yrs	31%
	>45 yrs	17%
2. Gender	Male	83%
	Female	17%
3. Address	Rural	77%
	Urban	33%
4. Occupation	Children/ Student	4%
	Adult Unemployed	9%
	Agricultural Worker	69%
	Labourer	6%
	Professional	12%
5. Previous Treatment	Topical Antibiotics	20%
	Steroids	16%
	Contact Lens	6%
6. Predisposing Ocular Conditions	Trachoma Sequels	0
	Entropion	0
	Prior Corneal Surgery	0
7. Systemic Diseases	Diabetes Mellitus	32%
8. H/O Ocular Injury		71%

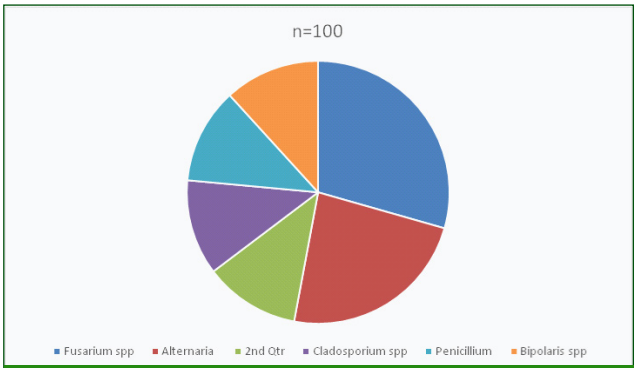
[Table/Fig-2]: Predisposing factors associated with Mycotic Keratitis.

Reading of Results

Fungus identification was done macroscopically based on the texture, growth rate, pigmentation, colony morphology on SDA slants, reverse and obverse surface colorof SDA slant and microscopically based on features such as mycelium and conidia types on Lactophenol cotton blue (LPCB) mounts of culture positive fungi.

RESULTS

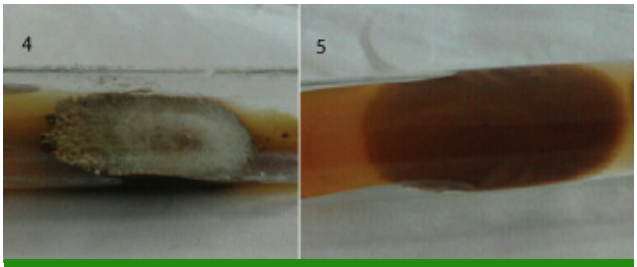
100 corneal scrapings were studied out of which fungal hyphae were identified in 20% of the KOH mounts and in 17% of Gram's stained smears. Ocular injury was found to be the most commonly encountered predisposing factor. Predominant isolates were *Fusarium solani* (6) and *Fusarium chlamydosporum* (1) followed by *Alternaria alternata* (5) as shown in [Table/Fig-3]. Pictures of *F. solani* and *Alternaria alternata* grown on SDA are shown in [Table/Fig-4,5] respectively.



[Table/Fig-3]: Distribution of fungal isolates.

STATISTICAL ANALYSIS

KOH mount showed evidence of fungal hyphae in 20% of corneal scrapings examined. With culture taken as standard test,the sensitivity and specificity of KOH mount and Gram's stain were calculated as shown in [Table/Fig-6,7] respectively. Kappa factor was found out to have a value of 0.901 in the



[Table/Fig-4]: *Fusarium solani* on SDA.
[Table/Fig-5]: *Alternaria alternata* on SDA.

KOH MOUNT	DISEASE PRESENT	DISEASE ABSENT	TOTAL
Positive	a = 20	c = 0	a+c = 20
Negative	b = 4	d = 76	b+d = 80
Total	a+b = 24	c+d = 76	
Sensitivity		83.33%	
Specificity		100%	
Positive Predictive Value		100%	
Negative Predictive Value		95%	

[Table/Fig-6]: Values KOH mount in study.

GRAM'S STAIN	DISEASE PRESENT	DISEASE ABSENT	TOTAL
Positive	a = 17	c = 0	a+c = 17
Negative	b = 7	d = 76	b+d = 83
Total	a+b = 24	c+d = 76	
Sensitivity		70.83%	
Specificity		100%	
Positive Predictive Value		100%	
Negative Predictive Value		91.57%	

[Table/Fig-7]: Values of Gram's stain.

	KOH Positive	KOH Negative	Total
Gram's Staining Positive	17	0	17
Gram's Stainng Negative	3	80	83
Total	20	80	100

[Table/Fig-8]: Variables for calculation of kappa factor.

study [Table/Fig-8]. This reveals that the degree of agreement is quite high for the two tests. The above tables revealed that KOH mount preparation and Gram stained smears are simple and sensitive methods for early diagnosis.

DISCUSSION

In Solapur, young adults (around 35 years) were most commonly affected and males showed higher positivity rates as compared to females. This could be due to more outdoor activity of males in their early adulthood. 77% of positive cases hailed from the rural areas where the occupation was mostly agricultural work in the fields. This again could be due to more exposure to environment in daily activity from which the fungal sources were contracted. These findings are similar with majority of studies on the topic all over India, though variation in percentage do exits. Winter months are associated with more cases as during this time as sugarcane harvesting starts and farmers are exposed to husks produced. Summer time arrived in Solapur with winds which also play a role in spreading the infectious fungi as well as in exposure to the population.

The predominant isolate in our study was *F.solani* in contrast to most of the studies and what the textbooks mention as *Aspergillus* Spp. Being the most common isolate, this suggest that there can be variation in isolation of fungal

PLACE OF STUDY	SOLAPUR, 2015	NORTH INDIA, Chandigarh, 2003 [8]	EAST INDIA, Hyderabad,2011 [5]	SOUTH INDIA, TamilNadu, 2003 [10]	WEST INDIA, Mumbai, 2008 [6]	SAUDI ARABIA [15] 2011
Predominant Age Group	25-34 yrs	21-50 yrs	50-60 yrs	21-50 yrs	35 yrs	55 yrs
Sex Predominance	Male	Male	Male	Male	Male	Male
M.C. Predisposing Factor	Ocular injury with vegetative material	Ocular injury with vegetative material	Ocular injury with vegetative material	Ocular injury with vegetative material	Ocular injury with vegetative material	Ocular injury with vegetative material
M.C. Occupation	Agricultural workers	Agricultural workers	Agricultural workers	Agricultural workers	Labourers	Not mentioned
Predominant Isolate	*F.solani*	*Aspergillus* spp	*Aspergillus* spp	*F.solani*	*Aspergillus* spp	*Aspergillus* spp
Seasonal Variation	Peak in winter	Peak in June. Sept. and Nov	Not mentioned	Peak between June and Sept	Not present	Not mentioned
Moulds	95%	100%	98.5%	100%	91%	89%
Yeasts	5%	0	1.5%	0	9%	11%

[Table/Fig-9]: Comparison of findings with similar studies.

isolates depending on geographical aspect. Moulds were the commonly isolated fungi in the various studies as mentioned in [Table/Fig-9] which comprises of 95-98% which a small proportion of yeast were also found to be the causative factor in our study.

LIMITATION

Some of the genus like *Cladosporium* and *Penicillium* were not identified to genus level. Antifungal susceptibility testing was not performed in our study.

CONCLUSION

A significant proportion of corneal ulcer in Solapur is of fungal origin (24%). This proves that many ocular complications including corneal opacification and blindness can be avoided by prompt management of mycotic keratiis. *Fusarium* spp. being the most frequent isolates reveals that the geographical distribution of fungus varies even within the country. The most frequently encountered predisposing factor is ocular trauma. Hence, history of ocular trauma should raise a high suspicion of mycotic keratitis.

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