Fungal Profile of Occular Infection in Patients Attending In a Tertiary Care Hospital

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Aim and objective: *To identify the fungal etiology of Ocular infections in patients attending Ophthalmology department from the period Nov 2010 to Sep 2011 at Navodaya Medical College Hospital and Research Centre, Raichur.*

Methods: *102 patients attending Ophthalmology OPD/IPD in Navodaya Medical College Hospital and Research Centre were analysed from Nov 2010 to Sep 2011. Using predefined inclusion and exclusion criteria, samples were collected according to the standard protocol. These were cultured for microorganisms (fungal) and were identified.*

Results: *Out of 102 samples studied, 75 samples had growth and the isolation rate. only 23(26%) was fungal growth. KOH wet mount examination showed high specificity of 98.73% and PPV of 90.91% with very significant p value of < 0.0001. The most common fungal isolate were Fusarium spp (30.43%) followed by Aspergillus spp (26.04%).*

Conclusion: *Fungal etiology was mostly associated with corneal infections. Direct microscopic examination is an essential tool in the diagnosis of fungal infections. A simple and rapid KOH wet mount examination early during the disease result in significant progress of the disease in initiating early and specific therapy.*

Keywords: *Ocular infections, potassium hydroxide wet mount, Fusarium*

I. Introduction

Eye is the most important sensory organ concerned with the perception of vision.[1] Ocular infections can affect different eye structures and their presentation and treatment vary accordingly[1] and present as: blepharitis, conjunctivitis, canaliculitis, Dacrocystitis, keratitis, scleritis, orbital cellulitis, endopthalmitis, panophthalmitis and other infections which are responsible for increased incidence of morbidity and blindness worldwide, their morbidity vary from self limiting trivial infection to sight threatening infection.[2]

An estimated 38 million people worldwide are blind, and 110 million have low vision. Extrapolations from data summarized by the World Health Organization (WHO) and other sources suggest that nearly 10 million people are visually disabled as a result of infectious diseases, which account for about one fourth of the world's blindness.[3]

India is a tropical agricultural country having higher prevalence of fungal keratitis compared to European and other western countries.[4]

Keratitis is the most frequently encountered fungal infections, although the orbit, lids, lacrimal apparatus, sclera, conjunctiva and intraocular structures may also be involved.[5]

Timely institution of appropriate therapy must be initiated to control the infections and there by minimize the ocular morbidity. If they are not treated promptly, it may lead to sight threatening condition. For specific antimicrobial treatment identification of the causative agent is important.[6]

Culture and direct microscopy are the two important investigations that are widely used. Culturing of microbial pathogens is considered to be the gold standard whereas direct microscopic evaluation of smears provides immediate information about the causative organisms for initiating treatment.[7, 8]

II. Patients And Methods

The present study was undertaken at Navodaya Medical College Hospital and Research centre, Raichur which included 102 cases during the period November 2010 to September 2011. The subjects in this study included those who were clinically diagnosed cases of ocular infections attending Outpatient Department and Inpatient Department of Ophthalmology, Tertiary Care Centre (Navodaya Medical College Hospital and Research Centre), Raichur and patients who are not on antibiotics (either topical or systemic) or patients not responding to antibiotics.

Sample collection[9]

After clinical diagnosis of ocular infection made by Ophthalmologist, specimens were collected with the help of Ophthalmologist.

Eye lid swab was collected using sterile cotton tipped swab moistened with sterile peptone water which was rolled over the eye lid margin from medial to lateral side and back again.

Conjunctival swab was collected using dry sterile cotton tipped swab by asking the patient to look up, the lower lid was pulled down using thumb with an absorbing tissue paper and the swab was rubbed over the lower conjunctival sac from medial to lateral side and back again.

Pus from lacrimal sac was collected using dry sterile cotton tipped swab either by applying pressure over the lacrimal sac and allowing the purulent material to reflux through the lacrimal punctum or by irrigating the lacrimal drainage system with sterile saline called as Lacrimal Syringing and collecting the sample from the refluxing material ensuring that the lid margins or the conjunctiva were not touched.

In cases of acute lacrimal abscess on chronic Dacrocystitis pus was drained and taken on a dry sterile cotton tipped swab.

Corneal scrapings was collected after instilling 2 to 3 drops of local anesthetic into the conjunctiva, patient is asked to wait for 2 to 3 min and corneal surface was cleaned for debris and discharge using dry sterile cotton tipped swab and with the help of slit lamb the edge of the ulcer was scraped using sterile disposable scalpel blade no 15 taking care not to perforate the cornea.

The number of swabs and scrapings collected depended on the material obtained on swab stick or the blade, at least a minimum of 2 swabs or scrapings and maximum of 4 swabs or scrapings were collected.

The corneal button, the lacrimal sac, chalazion removed by surgery was sent to the microbiology laboratory in a sterile container filled with sterile normal saline immediately

The corneal button and the lacrimal sac tissue were labeled and processed after cutting into small bits using sterile scalpel blade and sterile forceps in a small sterile petridish following all aseptic precautions and processed immediately

Laboratory Diagnosis:
Direct smear examination:
KOH wet mount preparation: one swab was spread or sample material was placed on a clean grease free glass slide over which a drop of 10% KOH solution was placed and covered with a cover slip. After about 15 to 20 min the slide was examined under dry objectives for the presence of fungal elements like hyphae, pseudohyphae, yeast cells, spores, spherules or sclerotic bodies.
Gram stain: was done to look for gram positive budding yeast cell, hyphae, pseudohyphae.

Fungal culture:
The specimens (swab) was used to inoculate on two Sabouraud Dextrose Agar plates with antibiotics but without actidione in a "C" shaped streak and incubated at $25^{o}C$ and at $37^{o}C$, they were examined daily for any growth for the first week and twice a week for a period of four weeks and if any growth on SDA, the identification was done as below.

Isolation of fungi:
The growth was observed for the following -
Rate of growth, Morphology of colony, Texture, Surface pigmentation, Microscopic examination like LPCB mount and slide culture were done to identify the fungi.
No growth was observed even after 3weeks of incubation, the culture was considered as sterile and the plates were discarded.

Statistical Analysis:
Descriptive statistics was used such mean, standard deviation and proportion. Chi-square test or Fisher's exact test for small sample size was used to find out the relationship between categorical variables. A p value less than 0.05 were considered as significant and 0.01 as highly significant. Data were analyzed by SPSS v16.0 software.

Table 6: Total number of Bacterial and Fungal growth

Total isolates	87
Bacteria	64(74%)
Fungus	23(26%)

Of the total 87 isolates64 (74%) is bacterial growth; only 23(26%) is fungal growth.
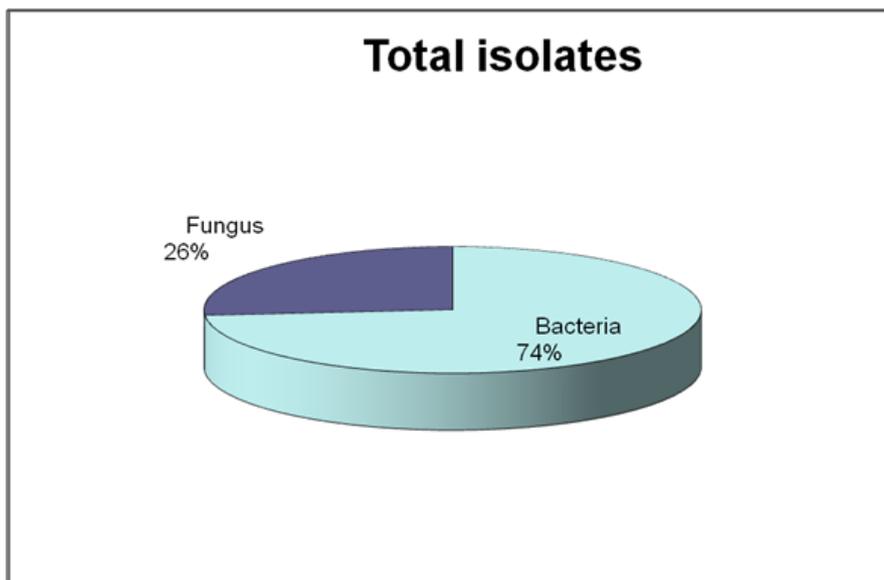
Graph 5 showing the distribution of bacterial and fungal isolates

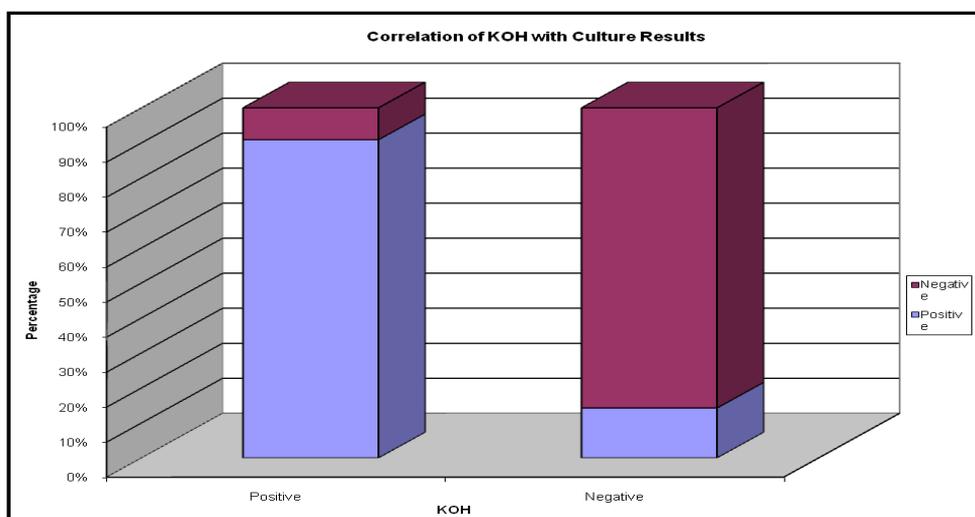**Table 8:** Correlation of KOH with Culture Results.

KOH	Culture		Total
	Positive	Negative	
Positive	10	1	11
Negative	13	78	91
Total	**23**	**79**	**102**

.X^2 = 28.75, df=1, p<0.0001

Sensitivity and specificity

Variable	Value
Sensitivity	43.48 %
Specificity	98.73 %
Positive Predictive Value	90.91 %
Negative Predictive Value	85.71 %

Out of 11 KOH positives, 10 correlated with culture results, out of 91 KOH negatives 13 was positive for culture results with high specificity of 98.73% and sensitivity of 43.48% with highly significant p value of < 0.0001.


Graph 7 showing the correlation of KOH results with culture results

Table 14: Distribution of fungal isolates according to their spectrum.

Fungal isolates	Number of isolates	Percentage
Aspergillus niger	1	4.35
Aspergillus fumigates	2	8.70
Aspergillus flavus	3	13.04
Fusarium spp	7	30.43
Rhizopus spp	4	17.39
Mucor spp	2	8.70
Alternaria spp	2	8.70
Unidentified filamentous fungi	2	8.70
Total	**23**	**100**

Out of 87 isolates, 23(26%) is the fungal isolates and the most common is *Fusarium spp* 7(30.43%) followed by *Aspergillus spp* 6(26.09%) and among this *Aspergillus spp*, *Aspergillus flavus* is 3(13.04%), *Aspergillus fumigatus* 2(8.7%) and *Aspergillus niger* 1(4.35%), *Rhizopus spp* is 4(17.39%), *Mucor sp, Alternaria spp* and unidentified filamentous fungi 2(8.7%) each.
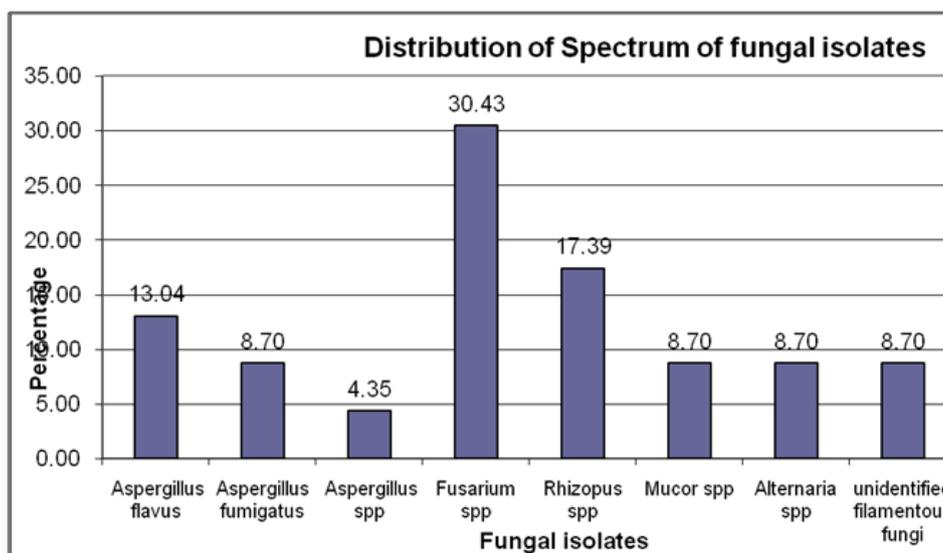
Graph 13 Distribution of organism wise fungal isolates

Table 15: Sample wise distribution of organisms

Organisms	Eye lid swab	Excised Chalazion tissue	Conjunctival swab	Pus from Dacrocystitis	Lacrimal Sac tissue	Corneal button	Corneal scrapings	Corneal swab	Pus swab from orbital cellulitis	Total
Aspergillus niger							1			1
Aspergillus flavus							2	1		3
Aspergillus fumigatus							1	1		2
Fusarium spp							4	3		7
Rhizopus spp				1			3			4
Mucor spp			1				1			2
Alternaria spp				1		1				2
Unidentified filamentous fungi							1	1		2
Total			1	2		1	13	6		23
PERCENTAGE			4.3	8.6		4.3	56.5	26		100

III. Results

Of the 102 samples studied, 23(26%) was the fungal growth among the total isolates of 87. Out of these 23 isolates 11 was positive for KOH wet mount. Out of these 11 KOH positives, 10 correlated with culture results and 13 was positive for culture results and KOH negative which showed high specificity of 98.73% and sensitivity of 43.48% with highly significant P value of <0.0001.

Among the patients with fungal isolates, 10 were female patients and 13 were male patients. The mean + SD age of the patients was + 15.84 (range 6yrs- 75yrs). 21 isolates were from corneal sample (corneal

scrapings, corneal swab, and corneal button). Only 2 isolates were from pus (chronic Dacrocystitis and canaliculitis abcess)

The most common fungal isolate was Fusarium species 7(30.43%) followed by Aspergillus species 6(26.09%). Among these Aspergillus species, Aspergillus flavus was 3(13.04%), Aspergillus fumigatus 2(8.7%) and Aspergillus niger 1(4.35%). Rhizopus species was 4(17.39%), Mucor species, Alternaria species and unidentified filamentous fungi 2(8.7%) each.

IV. Discussion

Ocular infections are one of the common reasons in ophthalmic clinics or hospital attendance. Bacteria are the common cause of ocular morbidity, whereas viral and fungal infections are much less common.

The growth characteristics of the fungus can result in superficial infection or invasion into deep tissues. Effective therapy of such infections must be selected from the small number of antifungal agents and requires recognition of the limitations of susceptibility testing, the importance of tissue penetration and absorption, and the need for protracted treatment. Because of these limitations, success of therapy primarily depends on early diagnosis of fungal infection and correct identification of particular fungus.[10]

Corneal infection of fungal etiology is very common and represents 30-40% of all cases of culture positive infectious keratitis in south India [11]

In the present study of the 102 clinically diagnosed cases of ocular infections fungal isolates were only 26% where as bacterial isolates was 74% this was in comparison to study by **Kunimoto et al[12], Garg et al[13], Dunlop et al[14]** and **Upadhyay et al[15]** where the bacteria: fungal isolates are 74% : 25.7%, 63.62% : 33.64%, 53.50% : 35.90%, and 63.2% : 6.7% respectively.

In other study by **Sherwal B L et al [16]**and **Leck et al[17]** fungal isolates were more than bacterial isolates, bacteria : fungal isolates are 20.84% : 32.50% and 23.9% : 38.6%.

In our study the fungal isolates were mostly from the corneal sample. The lower temperature of the cornea relative to the rest of the body and eye, and its exposure to potential trauma, may partially explain why keratomycosis is the most common ocular fungal infection.[10]

Among the 23 patients with fungal isolates 13(56.5%) were male and 10(43.4%) were female. There was not much difference in the sex ratio, though there was a male predominance. This correlates with the study of **Bharathi et al[18], Srinivasan M et al[19], Chowdhary et al [20]** and **Shokohi et al. [21]**

The mean age of the patients with fungal isolates was 48yrs (range 6yrs - 75yrs). This is comparable with the study of **Samar K Basak et al[8]**. The age distribution showed the incidence of fungal keratitis predominantly between the 4th to 6th decades, reflecting the active working period of life and hence the increased vulnerability to injury during outdoor activities.

In the present study KOH examination is positive for fungal elements in 11 samples whereas it is negative in 91 samples, KOH and culture results correlated in 88 samples showing high specificity of 98.73% and PPV of 90.91% with very significant p value of < 0.0001. In a study by **Sharma VK et al[22]**out of 16.9% KOH positive 15.4% was culture positive, in the study by **Laila A et al[23]** and **Vajpayee RB et al[24]** the diagnostic significance of KOH was 84.85% and 94.3% respectively.

In a study by **Gopinathan U et al[25]** KOH was positive in 91.0% of 95.4% fungal isolates, study by **Sharma S et al[26]**the sensitivity & specificity of fungal detection was 81.2% & 83.8% respectively. In another study by **Sharma S et al[27]** the sensitivity & specificity of fungal detection in early and late keratitis was 61.1% & 99.0% and 87.1% & 83.7% respectively. In a study by **Jain AK et al [28]** the sensitivity was 100% and specificity was 46.67%.

KOH wet mount preparation continues to be an ideal technique for revealing fungal elements in smears of corneal scrapings, considering the cost effectiveness, easy availability of reagents, ease of preparation of the reagents, sensitivity of method and rapidity of the test it helps in early introduction of appropriate antifungal drug by the ophthalmologist to prevent morbidity from corneal ulcers.[25]

In the present study the most common fungal isolate is *Fusarium spp* (30.43), followed by *Aspergillus spp* (26.09%), *Rhizopus spp* (17.39%), *Mucor spp, Alternaria spp*, and unidentified filamentous fungi 8.70% each.

In the study by **Gopinathan U et al[25] & Srinivasan M et al[19]**, *Fusarium* was the most common isolate with 37.2% & 47.1% isolation respectively and *Aspergillus spp* being the second common isolate with 30.7% & 16.1% isolation respectively. The present study is getting correlated with **Gopinathan U et al[25]** with *Fusarium spp* (30.43%).

In the present study 8.70% isolates were unidentified similar to other study by **Kunimoto DY et al[12]** where the percentage of unidentified spp was 9.5%.

In a study by **Laila A et al[23]**, the fungal spp isolated correlated with the present study & these fungal isolates were *Fusarium spp* (24.24%), *Aspergillus spp* (45.45%), *Alternaria spp* (03.03%), *Mucor spp* (12.1%), *Rhizopus spp* (06.06%) along with unidentified filamentous fungi (09.09%).

Isolation of *Aspergillus* was more compared to *Fusarium spp* in studies by **Sherwal BL et al[16]** & **Laila A et al.[23]**

In our study there was no yeast isolated because Candida infection predominates in colder climates. **Sharma S et al. [26]**

V. Conclusion

Ocular infections are the major cause of ocular morbidity and mortality which is a major public health problem in terms of visual compromise especially in developing countries like India. Fungal infections of the eye are seen mostly in the cornea with male predominance. KOH wet mount examination has good sensitivity and specificity for early diagnosis of fungal infections. Early and rapid identification of pathological organism is the key to ensuring successful medical therapy for fungal infections

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