IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 9 Ser.8 (September. 2019), PP 18-22
www.iosrjournals.org

Study of Ocular Fungalinfections

Dr .Shruti Agarwal[1], Dr. Arun kumar Gupta[2], Dr.R.P.Singh[3], Dr. Huma Naim[4], Dr. Rahul Kumar Goyal[5]

[1](AssistantProfessor),
[2](Associate Professor, corresponding author,
[3](Professor and Head of Department Microbiology,F.H. Medical College),
[4](Assistant Professor),
[5](Professor SRMS.IMS)

Abstract

OBJECTIVE: -*Fungi are common inhabitant of environment and are generally harmless, of low virulence & considered non pathogenic. They often don't cause disease in persons having good immunity. Their significance has considerably increased in present scenario as numberof patients with compromised immunity are increasing day by day. Beside common fungal infections, ocular infections are of greater significance as they can cause severe complications if not diagnosed and managed properly. Objective of the study is to make clinicians aware of serious nature of fungal ocular infections.*

MATERIAL AND METHODS: - *Various samples such as corneal scrapings, conjunctival swabs and vitreous and aqueous aspirates were collected with aseptic technique & sent to microbiology lab for processing. 50 patients with suspected fungal eye infections were considered for this study. Samples were examined by KOH wet mount & subjected to culture on SDA media. Culture tubes were incubated at 25°C for 3 weeks before being declared negative for fungus.*

RESULTS:- *Preponderance of fungal eye infections was more in males (64%) than females (36%). In most of the patients there was history of trauma followed by those having serious systemic disease and wearing of contact lenses.KOH examination as well as culture positivity was maximum with corneal scrapings i.e, 33 % & 67% respectively.Fusarium was commonest fungus grown in culture, followed by A. flavus, A. fumigatus, Curvularia sp. & Candida.*

Date of Submission: 03-09-2019 Date of Acceptance: 18-09-2019

I. Introduction

Fungi are commonly found in the environment & most of them occur as harmless commensals,contaminants or nonpathogenic agents.[1]Sincefungal infections are notnotifiable,they are not given much attention and therefore diagnosis is established quite late.Approach to identify fungal infections is generally on the basis of morphology of the lesion especially in the developing countriesand also because of nonavailabilityof labs equipped with the facility for their isolation and identification,it becomes difficult to establish fungus as it's etiology.

The overall incidence and prevalence of fungal infections is increasing for the last few decades due to increase in the number of patients with compromised immunity in conditions such as AIDS,cancers & chronic ailments requiring steroids for long periods.[2]Candida and Aspergillus are two most common fungal pathogens implicated with variety of clinical conditions. Incidence of invasive fungal infections is also on the rise due to increase in patients with compromised immunity.[3]

This demands increased awareness about fungal diseases and their accurate diagnosis.A correct and early diagnosis helps clinicians to institute early & specific treatment of fungal diseases which may prove to be life savingor at least lessen the chances of complications produced by such fungal infections.

Fungal eye infections are known to causeblindness,although not very common.Fungi are associated with variety of ocular infections such as-keratitis,scleritis& endophthalmitis and most of the cases are found in developing countries.[4]In India,about 30 to 50% of patients with keratitis are known to be caused by fungi.

Risk factors associated with the fungal ocular infections include long term use of corticosteroids or immunodeficiency diseases,ocular surgery & wearing of contact lenses although ocular trauma is major predisposing factor for fungal ocular infections. Fusarium sp.,Aspergillus and Candida are the most commonly isolated fungifrom patients withfungal keratitis.[5]Candida albicans is most common fungus implicated in endogenous ophthalmitis.[6]Trauma, particularly vegetable matter is the common risk factor & is documented as it's cause in more than 50% of cases.[7]Even within India, variety of organisms causing fungal keratitis is

different among different regions.Fusarium sp is more commonly isolated in southern India while Aspergillus sp. is reported frequentlyfrom northern & eastern India.

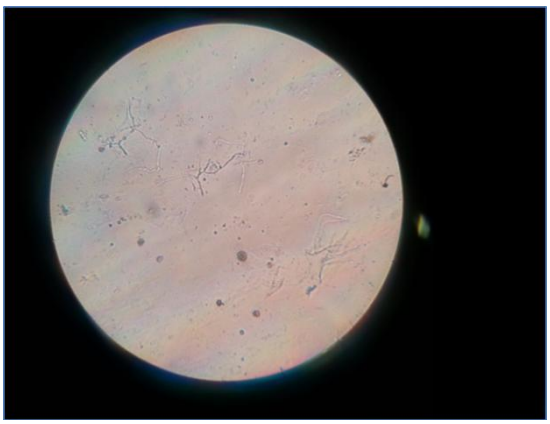
Ocular infections may be caused by bacteria,viruses, parasites & each of these may produce symptoms similar to those caused by fungi & therefore pose challenge to determine the causative agent with conviction.[8]Ophthalmologists & clinicians must be knowledgeable & aware of fungal infections & must think of fungal etiology in differential diagnosis of chronic eye infections.

II. Material & Methods

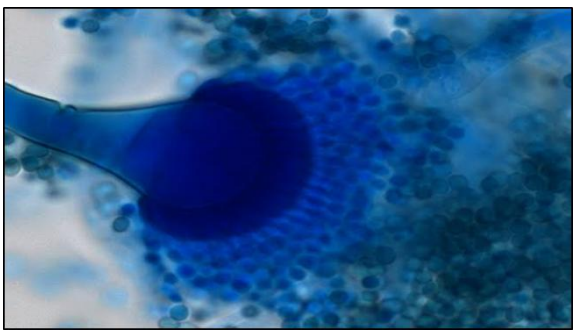
Various samples such as Cornealscrapings, Conjunctival swabs, Iris tissue and Vitreous and Aqueous aspirates(wherever possible) were sent to microbiology deptt.of Shri Ram Murti Smarak Institute of Medical Sciences,Bareilly U.P. a Tertiary Hospital. 50 patients withsuperficial fungal eye infection were included in the maintaining study. Samples were collected by ophthalmic surgeons taking all the possible precautions & maintaining sterility during the procedure of collecting samples.Before taking samples, presumption of etiology was made on the basis of history and morphology of lesions.Generally those patients were selected for the study who had lesions in the eyesnot responding to conventional treatment for long time and had history of trauma.

Samples were collected and subjected to KOH wet mount and fungal culture.KOH wet mount of each sample was made and examined under microscope for presence of fungal hyphae and budding yeast cells and inoculated on plane Sabouraud's Dextrose Agar & SDA with Chloramphenicol.Cultures tubes were incubated at 25°C.

They were examined every 48 hours for the appearance of any growth till 21 days. Lactophenol Cotton Blue wet mount were made from culture tubes showing growth to study the morphology of isolate for identification.



MICROSCOPIC PICTURE OF KOH



L.P.C.B MOUNT SHOWING A.FUMIGATUS

III. Result

Study was carried out in the dept. of Microbiology SRMS-IMS Bareilly. 50 samples from patients attending ophthalmic OPD werecollected with correlated symptoms, history of trauma with foreign body. Samples were analysed for fungal pathogens on the basis of KOH examinations and culture.

Patients were divided into four groups on the basis of their age. Age wise distribution is shown in Table No.1

Table No.1

Sr. No	Age groups(Years)	Number of samples	Percentage of samples
1	15-30	9	18
2	31-45	16	32
3	46-60	15	30
4	>60	10	20

Maximum number of patients were under the age group of 31 to 45 years followed by 46 to 60 years, then more than 60 year and minimum number of patients belonged to age group of 15 to 30 years.
Gender wise distribution of patients is depicted in Table No.2

Table No. 2

Gender	No. of Cases (50)	Percentage
Male	32	64
Female	18	36

Table No.2 shows that preponderance of infections in male patients(64%) is more than female patients(36%).As far as predisposing factors are concerned, there are many factors which make a patient susceptible to develop fungal infections.These include Ocular trauma with vegetable matter or any other foreign body, contact lens ulcers and patients with impaired immunity such as AIDS, Diabetes, Cancers, and those on steroids for a long time. Table No.3depicts association of predisposing factors and number of patients.

Table No.3

S.No	Predisposing factors	No. of samples	Percentage of samples
1	Trauma	25	50
2	Contact lens	5	10
3	Systemic disease	10	20
4	Unknown cause	10	20

Patients with history of trauma were maximum(25) followed by patients having predisposing factors such as wearing contact lenses and with systemic disease. There was no obvious cause in 25% of cases.
Samples from different sites were received in the lab. Its Distribution is shown in Table No.4.

Table No. 4

S. No.	Site of samples	No. of Samples	Percentage of Samples
1	Corn.scraping	30	60
2	Conj.swab	7	14
3	Vitreous fluid	5	10
4	Iris tissue	5	10
5	Aqueousfluid	3	6

Maximum number of samples(30) sent to lab were corneal scrapings followed by conjunctival swab(7), Vitreous fluid(5), Iris tissue(5) & minimum no. of samples were Aqueous fluid.

KOH preparation was made of all the samples to look for fungal elements. Only 14 samples were positive for presence of fungal hyphae or budding yeast cells.The overall positivity was 28%. Maximum no. of positive KOH preparation was seen in corneal scraping(33%), followed by conjunctival swab(14%), Vitreous fluid (20%), iris tissue(20%), Aqueous fluid (33%) respectively.TableNo.5 explains the sample wise positivity.

Table No.5

S. No	Sample	No.of samples	KOH positive	% KOH positive	Culture positive	% culture positive
1	Corneal scraping	30	10	33	20	67
2	Conj. swab	7	1	14	2	29
3	Vitreous fluid	5	1	20	3	60
4	Iris tissue	5	1	20	1	33
5	Aqueous fluid	3	1	33	2	40

10 samples of corneal scraping, 1sample each of aqueous fluid & vitreous fluid showed septate branching hyphae. 1iris tissue sample was also positive for fungal hyphae. Only1conjuntival swab out of 7 showed budding yeast cell.

As far as isolation and identification of fungal culture is concerned, growth was obtained from 28samples out of 50 samples. Positivity of cultures was 56%.

Fungal isolation was maximum from corneal scraping followed by Iris tissue, Vitreous fluid, Aqueous fluid & Conjunctival swab respectively. Fungal growth on culture media was identified on the basis of their colony morphology on SDA& Lactophenol Cotton blue wet preparation. They were identified as Fusarium species, A. flavus, A. fumigatus, Curvularia sp& Candida sp.

Table No. 6 showing the fungal profile of culture positive cases.

Table No.6

S.No	Fungus	Total no ofculture+ve cases	Positivity
1	Fusarium sp	28	13
2	A.flavus	28	5
3	A.fumigatus	28	4
4	Curvularia sp	28	4
5	Candida sp	28	2

Fusarium sp. was isolated from 13 samples followed by A.flavus(5), A.fumigatus(4), Curvularia(4) & Candida sp.(2) respectively.

Association of fungal isolates with sample source is shown in Table No.7. Fusarium sp is most common isolate from Corneal scrapings while Candida sp.is grown fromConjunctival swab.

Table No:7

Sample	Fusarium	A.flavus	A.fumig.	Curvu.	Candida	Total
Corn. scrape	12	4	2	2	-	20
Conj. swab	-	-	-	-	2	2
Iris	-	1	2	-	-	3
Aqueous fluid	1	-	-	-	-	1
Vitreous fluid	-	-	-	2	-	2

IV. Discussion

Fungal infections are becoming common in the community due to various reasons.General population has become more prone to acquiring fungal infectionsdue to diseases which lower the immunity such as diabetes,cancers & chronic ailmentsrequiring steroids for their treatment.

Fungal eye infections are generally due to traumain the eye with foreign body especially when it is accompanied by vegetable material.

Eye infections are commonly considered as bacterial in origin by the clinicians &fungal etiology is often missed which results in delayed diagnosis and treatment. This also leads to occurrence ofcomplications and poor outcome of the treatment.

High degree of suspicion for infections associated with fungal etiologyis required on the part of clinician, general practitioner and ophthalmologist so that specific treatment may be started in time that will not only cure the patient but will also prevent the development of complications.

Previously the diagnosis of fungal infections was difficult and cumbersome but now a daysbasic facilities for identification of fungi directly in the sample and its confirmation by culture are available in most of the laboratories. Fungal culture is considered asgold standardfor identification of fungi & most of the fungal infections can be diagnosed by this method along with KOH preparation.

In our study,more fungal cases of eye infections were found in the age group of 31- 45yrs(Table No.1)as compared to other group ofpatients.Probably due to more involvement in theoutdoor and agriculture activity which makes them prone to traumatic injury. In the study by D.khokhar et al,[9]majority of patients were found to be suffering from keratitis in the age group of 40yrs and above followed by 18-39 yrs of age and less common in 3-11 yrs of age.Work done by Tananuvat et al[10] showed mean age of patient to be 54 yrs.

Similarly more cases of fungal eye infections were noticed in males (Table No.2) due to same reasons. In another study byMA Salem et al,[11] there was male preponderance (67.8%). Majority of them were farmers and day labourers (53.5%). N. Tananuvat et al[10] found in his work 19(63%) males & 11(37%)females.

In our study, trauma was main predisposing factor (50%), followed by patients with systemic diseases (20%), & wearing of contact lenses(10%), while in 20% of patients there was no specific reason (Table No.3). Study conducted by MA Salem et al[11]showed that history of trauma was main predisposing factor which was 19(33%) out of 56 cases.

Maximum no. of fungal isolates werefound to be associated with corneal scrapings. This can be attributed to the reason ofbeing outermost layer of the eyeball, hence were more prone to direct injury. Since it is avascular component of the eye, it is more prone to damage. N. Tananuvat et al[10] conducted similar study & found that corneal trauma(67%), topical steroids(7%), splashed water(7%) & diabetes mellitus (7%) were the

main predisposing factors. In the present study also, maximum number of fungal isolates were identified in corneal scrapings(60%).

To diagnose all the suspected cases,samples were directly examined by wet mount (KOH preparation) & subjected to culture for confirmation.

In few studies conducted by D. Khokhar et al,[9] N. Tananvivat et al[10]& Vengayil et al[12]KOH wet mount showed positivity in 12 out of 103(12%), 6 out of 30(20%), 16 out of 40(40%) cases respectively.Gopinath et al[13]emphasised the diagnostic utility of KOH preparation to demonstrate fungal element in cases of fungal keratitis.He noticed fungus in 91% (1219) cases.

In our study KOH positivity was found to be 28% & culture positivity was 56%. This can be explained by receiving less amount of samples which could not be directly examined by KOH preparation & in some samples direct finding of fungal element could have been missed.

In our study Fusarium was isolated in maximum number of cultures(46%), followed by A.flavus(18%), A. fumigatus (14%), Curvularia(14%) and lastly Candida (7%). Studies conducted by various workers such as N. Tananvivat et al[10]culture positivity was 40%& most common fungus was also Fusarium(42%), followed by Dematiaceous fungi(33%), Candida(8%), A. fumigatus(8%), Curvularia(8%).

Vengayil et al[12] found in his study that Aspergillus sp. was predominant isolate. A. flavus was more commonly isolated than A. fumigatus(4:1). It was followed by Fusarium sp.(40%) and lastly Curvularia(10%).

Culture positivity was 43% in a study conducted by M.A Salem [14]& the maximum isolation was of A.fumigatus(33%) followed by Fusarium(21%), Mucor(17%), A. Flavus(8%), A. Niger(8%), Rhizopus (8%) & unidentified fungus(4%).

On comparing between the two conventional methods,it was seen that diagnostic sensitivity of wet preparation (KOH) was found to be 46.4% while culture was found to be 95.4% specific.

Sharma etal[15] reported KOH sensitivity as 68% & specificity 91%.Chaudharyet al[16] found sensitivity of 62% & specificity 97%.

However diagnosis by culture method has a big limitationfactor i.e., it takes quite a long time to become positive but the biggest advantage is in its ability to detect fungus in the sample even if it's presence is scanty.

Considering pros & cons of both the methods,KOH wet mount should be used as screeningmethod,which is rapid, cheap & less labour intensive while culture should be used for confirming the identity of the fungus.

References

[1]. Anthony Amend, GaetanBurgaud, Michael Cunliffe, Virginia P. Edgcomb, Cassandra L. Ettinger, M. H. Gutiérrez, Joseph Heitman, Erik F. Y. Hom, Giuseppe Ianiri, Adam C. Jones,MaikoKagami, Kathryn T. Picard, C. Alisha Quandt, SeshagiriRaghukumar, MertixellRiquelme, Jason Stajich, José Vargas-Muñiz, Allison K. Walker, Oded Yarden, Amy S. Gladfelter. Questions and Fungi in the Marine Environment: Open Unsolved Problems..DanielleA. Garsin, Editor.DOI: 10.1128/mBio.01189-18.

[2]. J Bras Pneumol.Fungal infections in immunocompromised patientsChapter 8 2010 Jan-Feb;36(1):142-7.

[3]. Chian-Yong Low[1,2] and Coleman Rotstein21876720Emerging fungal infections in immunocompromised patients.F1000 Med Rep. 2011; 3: 14. Published online 2011 Jul 1. doi: 10.3410/M3-14

[4]. Kalkanci A[1], Ozdek S.Ocular fungal infectionsCurr Eye Res. 2011 Mar;36(3):179-89. doi: 10.3109/02713683.2010.533810. Epub 2010 Dec 15.

[5]. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. Indian J Ophthalmol 2003;51:315-21.

[6]. Khan FA[1], Slain D, Khakoo RACandida endophthalmitis: focus on current and future antifungal treatmentoptions.Pharmacotherapy. 2007 Dec;27(12):1711-21

[7]. Chang peggy, Han-ying MD, Chodosh, James MD. Diagnostic and Therapeutic Considerations in Fungal Keratitis. IntOphthalmol Clin. 2011; 51(4): 33-42.

[8]. Sharma S. Diagnosis of Infectious Disease of the Eye .Eye(2012); 26: 177-184.

[9]. Khokhar ND, Mulla SA, Shah LN, Vaghela GM. Evaluation of diagnostic sensitivity of wet preparation microscopy using KOH for detection of fungal agents from keratitis patients. Natl J Community Med 2013; 4(2): 357-360.

[10]. Tananuvat N et al. Prospective Comparison between Conventional and Microbial work-up vs PCR in the diagnosis of Fungal keratitis. Eye (2012); 26: 1337-1343.

[11]. Salam MA et al. KOH wet preparation for the Laboratory Diagnosis of Suppurative Corneal Ulcer. Bangla JOL. 2010; 9(1).

[12]. Vengayil S, Panda A, Satpathy G, Nayak N, Ghose S, Patanaik D, Khokhar S. Polymerase Chain Reaction–Guided Diagnosis of Mycotic Keratitis: A Prospective Evaluation of Its Efficacy and Limitations. India. 2008

[13]. Gopinathan U, Garg P, Fernandez M, Sharma S, Athmanathan S, Rao GN. The epidemiological features and laboratory results of fungal keratitis: A 10-year review at a referral eye care center in South India. Cornea. 2002; 21: 555-559.

[14]. Laila A, Salam MA, Nurjahan B, Intekhab R, Sofikul I, Iftikhar A. Potassium Hydroxide (KOH) Wet Preparation for the Laboratory Diagnosis of Suppurative Corneal Ulcer. Bangladesh Journal of Medical Science. 2010; 09(1): 27-32.

[15]. Sharma S, Silverberg M, Mehta P, et al. Early diagnosis of mycotic keratitis: predictive value of potassium hydroxide preparation. Ind J Ophthalmol. 1998; 46(1): 31–35.

[16]. Chowdhary A, Singh K. Spectrum of fungal keratitis in North India. Cornea. 2005; 24(1): 8 –15.