ISOLATION OF FUNGI FROM CORNEAL ULCERS IN PATIENTS ATTENDING TERTIARY CARE HOSPITAL

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ABSTRACT: Blindness is a major challenge to the modern world in which the majority is due to corneal ulcers. The importance of early diagnosis of keratomycosis is stressed for the eyes not treated in time, may lead to complications and blindness. 102 corneal scrapings were processed for fungi as study group and 100 healthy persons were included as control group. In the study direct microscope by 10% KOH Wet mount, LPCB, and GRAM staining revealed fungi in 18 cases, and in 28 cases culture yielded growth on SDA. *Aspergillus* was the predominant causative agent (39.2%), followed by *Fusarium* (17.85%), *Curvularia* (10.71%), *Candida* (7.14%), *cephalosporium* (7.14%), *Rizopus* (3.57%) *Penicillium* (3.57%) and *mucor* (3.57%). In the present study 53.57% were associated with trauma of the eye. Majority of cases are from the rural areas (19cases), than urban areas (9cases) and large number of patients were agricultural workers. Seasonal variations were also observed. Majority of the cases were affected in the months of September and October.

KEYWORDS: Mycotic keratitis, Trauma, 10% KOH mount, Agricultural workers.

INTRODUCTION: In the tropical, agricultural, and developing countries like India, fungal infection of eye is one of the major problems. It is not uncommon to find infection of cornea with varied fungi of soil in South India. It has been observed that conjunctival flora vary according to the environment and population. Fungi, with few exceptions, are essentially free living saprophytes in nature. Fungal diseases are usually not contagious. Mycotic infections in man and animals follow traumatic implantation of the fungi as commensals and are capable of causing endogenous disease under special circumstances like lowered immunity, metabolic disorders and malignancy etc.¹

The cornea, outer most and transparent coat of the eye, plays a vital role for vision, the most precious gift of the nature. Cornea, being always exposed to the environment during the most of the working hours. Many a time, the injurious material carries diverse infective agents like bacteria, viruses and fungi along with it. In most occasions; the minor trauma occurring in day to day life heals by itself due to normal repairing mechanisms of the body. But in some cases, when the body's resistance is lowered or the dose and virulence of the microbial agents are more, even a minor injury can result in the formation of corneal ulcer.

In our country, such infections have been one of the main problems. In the last 4 decades, however, the incidence of fungal infection of the eye mostly corneal, has increased considerably, a phenomenon which seems to be due to two factors:

1. An upset of normal symbiosis between bacteria and fungi since the introduction of antibiotics into eye and.
2. An alteration of the resistance of the tissue from the use of corticosteroids.

These two factors allow saprophytic fungi to become facultative pathogens.

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The importance of early diagnosis of Keratomycosis is stressed, for the eyes not treated in time, may lead to complications and blindness. The microbiologist should be aware of fungi as possible pathogenic agents whenever the material is processed from eye infection. Increase in the incidence of Keratomycosis during the past few years has been due to better diagnostic methods and the recognition that fungi which have been considered as non-pathogenic do have the ability to infect eye. Furthermore, absolute increase in the number of fungal infections has probably resulted from the widespread and indiscriminate use of antibiotics and corticosteroids in ocular therapy also. As the line of treatment in fungal ocular lesions is different from that of bacterial infections, it is felt that detection of diseases like mycotic keratitis is important. It will go a long way in the formulation of therapy. Hence, the present study is made to assess the incidence of fungi that causes keratitis in Rayalaseema area.

MATERIAL AND METHODS: 102 patients with corneal ulcer were analyzed for fungi, as aetiological agents. A control group was included, comprising of 100 healthy human beings from whom, conjunctival swabs were collected and analyzed.

Cornea was anaesthetized with topical 4% lignocaine and exposed by universal speculum. Corneal scrapings were taken from margin and base of the ulcer and processed as follows:

A. DIRECT WET MOUNT PREPARATION, WHICH INCLUDED:

- I. KOH wet mount preparation.
- II. Lacto Phenol Cotton Blue, wet mount preparation.
- III. GRAM's staining.

KOH WET MOUNT PREPARATION:

- i. A portion of scraped material was transferred on a sterile glass slide and a drop of 10% Potassium Hydroxide was added to it. A cover slip was placed on it and left undisturbed for 10 to 15 min. then it was examined under low power and high power objective.² Lacto Phenol Cotton Blue, wet mount preparation.

LACTO PHENOL COTTON BLUE WET MOUNT PREPARATION: A drop of stain was placed on a clean glass slide. The scrapings from the ulcer were placed in the stain and mixed. A cover slip was placed over the mixture with little pressure avoiding air bubbling formation. Then it was observed under low power and high power objective.^{2,3}

GRAM STAINING (HUCKER'S MODIFICATION): Hucker's Modification of Gram staining was followed where crystal violet was used as primary staining.

B. CULTURE FOR FUNGI IN SABORAUD'S DEXTROSE AGAR MEDIUM: Sabouraud's dextrose agar medium was used for isolation of fungi. The material was inoculated into four slants of Sabouraud's dextrose agar medium, two with chloramphenicol and two without chloramphenicol. Two slants were incubated at room temperature and other two slants incubated at 37°C.

An uninoculated medium was exposed by the side of the eye for few seconds and incubated along with the inoculated media as control. The inoculated slants were observed daily for evidence of

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growth. Earliest growth of fungus appeared within one week but slants were kept for four weeks before being discarded as negative. The growths from slants were identified by their cultural characters and microscopic examination.

In case of *Candida*, the following procedures were carried out to identify the species:

1. Grams Staining.
2. Germ Tube Formation.
3. Chlamydospore Formation.
4. Sugar Fermentation Tests.

SLIDE CULTURE TECHNIQUE: Once growth was observed on Saboaraud's dextrose agar slide culture technique was followed for more detailed morphological identification of fungi.

A slide was placed on a bent (U shaped) glass rod in the bottom of a Petridish, covered and sterilized. Saboaraud dextrose agar prepared in plates of about 15 ml of agar per plate was allowed to solidify. Blocks of about 1 cm agar per plate was allowed to solidify. Blocks of about 1 cm square and 2 – 3 mm depth were taken out from the agar plate using all sterile techniques and placed on the sterile slide in Petridish. Centers of four sides of agar block are inoculated with the culture. The inoculated block was covered with a sterile cover slip. 8 cc of sterile water is added to the bottom of sterile Petridish, covered and incubated at room temperature. This was examined periodically for growth. If there was evaporation of water from Petridish, water was added again and further incubated until growth was observed.²

When sporulation was well developed, the cover slip was lifted completely and placed on a drop of lacto phenol cotton blue and observed under microscope.

RESULTS: Corneal scrapings, from 102 cases suffering from corneal ulcer and conjunctival swabs from 100 healthy persons were processed for fungi. Former group constituted the study group, and the latter control group.

Group	Males	Females	Direct Microscopy	Culture positive		Culture Negative
				Males	Females	
Study (102)	63	39	18	19	9	74
Control (100)	50	50	-	5	3	92

TABLE 1: STUDY GROUP

In the study group, direct microscopy revealed fungi in 18 cases, and culture yielded fungi in 28 cases. In 74 cases culture yielded no growth.

KOH		KOH	Positive	KOH	Negative
LPCB	Positive	LPCB	Negative	LPCB	Negative
GRAM		GRAM	Negative	GRAM	Negative
	16		2		10

TABLE 2: DIRECT MICROSCOPY FINDINGS

In this study group, fungal elements were seen in 18 cases. All the three i.e. KOH, LPCB, GRAMS showed positive for fungi in 16 cases, in 2 cases KOH only showed fungal elements.

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SL.NO.	FUNGI	NO. OF CASES	PERCENTAGE (%)
1.	Aspergillus	11	39.28%
2.	Fusarium	5	17.85
3.	Curvularia	3	10.71%
4.	Candida sp	2	7.14%
5.	Cephalosporium	2	7.14%
6.	Rhizopus	1	3.57%
7.	Penicillium	1	3.57%
8.	Mucor	1	3.57%
9.	Unidentified	2	7.14%
	Total:	28	100.00%

TABLE 3: FUNGI ISOLATED

In this study group, *Asperigillus* was the predominant causative agent of corneal ulcer (39.28%) followed by *Fusarium* (17.85%), *Curvularia* (10.71%), *Candida* (7.14%), *Cephalosproium* (7.14%), *Rhizopus* (3.57%), *Pencillium* (3.57%), *Mucor* (3.57%) and Unidentified (7.14%), as shown in the above table.

In the study group, *Aspergillus flavus* was the predominant causative agent (63.63%) followed by *Aspergillus fumigates* (27.27%), and *Aspergillus Niger* (9.09%).

A high incidence of 36.84% was noted in the age of group of 41–50 yrs in males and in the females 28.57% was noted in the age group of 51 – 60 yrs.

History suggestive of trauma was the leading predisposing factor in the present study (53.57%) followed by local and systemic use of antibiotics and corticosteroids (21.42%).

The incidence of Kerato mycosis was highest in the agricultural workers in the present study with an incidence of 57%.

A high incidence of 38.09% was noted in the month of October, followed by September with an incidence of 35.71%.

100 conjunctival swabs were collected from control group, processed for fungi.

Total cases	Male	Female	Culture positive	
			Male	Female
100	50	50	5 (10%)	3 (6%)

TABLE 4: CONTROL GROUP

In the control group, culture yielded growth in 8 cases (8%). 5 of males and 3 of females.

Fungi	Males	Females
Aspergillus	3	1
Penicillium	1	1
Rhizopus	1	0
Candida sp	0	1

TABLE 5: FUNGI ISOLATED FROM CONTROL GROUP

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DISCUSSION: In the present study, among 28 mycotic corneal ulcers, *Aspergillus* species were isolated in 11 cases with an incidence of 39.28%, in 7 cases *Aspergillus flavus* was isolated, followed by *fumigates* in 3 cases and in one case *Aspergillus niger* was isolated.

Many workers in their studies stated that *Aspergillus* species were the predominant causative agents. Subbannayya from Manipal⁴ 34%, Poria Dogre⁵ 27%, Anitha Panda⁶New Delhi 39.5% and savitri sharma⁷

In the present study, *Fusarium* species were isolated in 5 cases (17%), *Candida* in 2 cases, *Rhizopus*, *Penicillium* and *Mucor* in each one case and in 3 cases fungi were not identified.

In the present study, *Fusarium* species were isolated in 5 cases with an incidence of 17%. Similar studies by several workers revealed an overall incidence of ranging from 8.5% to 32.3%.

In the present study, *curvularia* species were isolated in 3 cases from 28 with an incidence of 10.7%, this coincide with Anitha Panda ⁶from Delhi (7.9%).

An interesting feature in the present study was *Candida*, which were isolated in two cases with an incidence of 7.14%. This also coincides with Siva Reddy,⁸ PoriaDogre,⁵ Anitha Panda⁶ and other earlier workers. *Candida* had not been reported as a major corneal pathogen in most studies in tropical countries¹.

In the present study, *Cephalosporium* species are isolated in two cases with an incidence of 7.14%. Sunitha and Deshpande⁹ with 2.33% and Anitha Panda with 3.7%.⁶

The incidence of keratomycosis in the present study was more in males than in females. Males are affected in 19 cases with an incidence of 67% and females were affected in 9 cases with an incidence of 33%.

Trauma plays an important role in keratomycosis. In the present study, 15 cases were due to trauma of eye. In the present study 53.57% cases were associated with trauma. Dasgupta et al¹⁰ with 54%, Siva Reddy ⁸et al. 52.8%, Anitha Panda⁶ with 55.3%, Taiwar and sahgal,¹¹ Zimmermann¹²

In the present study trauma of the eye was usually due to vegetable matter like paddy seeds, wooden stick of plants, thorn, lash of cow tail, falling sand. Among 28 patients, 8 cases (25.57%) were due to drugs used locally (antibiotics and corticosteroids)¹³ for more than a month.

Out of 28 patients of keratomycosis, the incidence was more common in the rural area when compared to the urban area. 19 cases from rural areas and 9 cases from Urban were reported. The people in the rural area were mainly agricultural workers and these were the people who used drugs indiscriminately.

In the present study, large number of patients was agricultural workers with an incidence of 57.14%.

There was a seasonal variation of mycotic ulcers according to the climatic conditions.¹⁴ In the present study the maximum incidence was mainly in cool and humid months. A high incidence of 38.09% was noted in the month of October followed by September with an incidence of 35.7%. Lower incidence was seen in November, December, January, February, March and April. Least incidence was noticed in May and June. From July to October 20 cases were reported out of 28 cases. This was due to the harvesting season. Various factors like humidity, temperature and rush of field work may account this seasonal variation.

CONTROL GROUP: In the control group fungi were isolated in 8 cases from a total of 100 cases with an incidence of 8%.

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Similar studies by several workers revealed an overall incidence ranging from 6.7% to 18.4% in India, Sinha and Das⁼¹⁵ 13.4%.

CONCLUSION: Mycotic keratitis can easily be confused with other forms of microbial keratitis. In order to save the vision, mycotic keratitis should be suspected in every patient with corneal ulcers and should be ruled out by proper laboratory methods before commencing steroids and antibiotic treatment.

Aspergillus species were observed to be one of the predominant organisms in air and hence, it can easily infect injured cornea.

Lack of a broad spectrum antifungal medicine for ophthalmic use was a depressing observation. It is our sincere desire that more research be conducted to discover effective antifungal medicine for ophthalmic use. Steps to improve ophthalmic care and treatment in the periphery and rural areas should also be taken to lower the incidence of keratomycosis along with other ophthalmic diseases.

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Full maturation of Fusarium showing pink colour

Photomicrograph of cephalosporium from slide culture showing unbranched septate hyphae with cluster of conidia at the tip of conidiophore

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