



THE INCIDENCE OF VARIOUS FUNGI IN PATIENT WITH CORNEAL ULCER IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Microbial keratitis is predominantly an opportunistic, serious ocular infectious disease that can lead to significant vision loss and ophthalmic morbidity.¹ Fungi are one of the most common infective organism responsible for this morbidity. Corneal scraping of the patients suspected of fungal keratitis attending ophthalmology OPD and those admitted in ophthalmology ward were studied.

KEYWORDS : fungal keratitis , mycotic keratitis , corneal ulcer

INTRODUCTION

Microbial keratitis is predominantly an opportunistic, serious ocular infectious disease that can lead to significant vision loss and ophthalmic morbidity.¹ Fungi are one of the most common infective organism responsible for this morbidity.² Fungal infection of the cornea (keratomycosis, mycotic keratitis or fungal keratitis) was described for the first time by Leber in Germany in the year 1879.³ Since then it has been recognised as a major public health problem in the tropical parts of many developing nations including India. Corneal infection of fungal etiology may represent 40-50% of all cases of culture proven infectious keratitis.⁴ If not treated early, this condition may lead to corneal blindness.

MATERIAL AND METHODS

Study type: This was a prospective, non-randomized, cross-sectional study.

Material of study: Corneal scraping of the patients suspected of fungal keratitis attending ophthalmology OPD and those admitted in ophthalmology ward of J A Group of Hospitals, Gajra Raja Medical College were studied.

Inclusion Criteria: Patients who were having corneal ulcer and were suspected of fungal keratitis attending Ophthalmology OPD and those admitted in ophthalmology ward in the J. A. Group of Hospitals during the study period of one year.

Exclusion Criteria:

- 1) Child <2 yrs of age
- 2) Patients who were not willing to participate in the study.

Specimen collection: Written consent from the participants or their guardians included in the study was obtained after providing full explanation of the current study in their local language. The study was submitted to Institutional Ethical committee and got the approval for proceedings. All the data collected were kept confidential.

Corneal scrapings were collected from patients of corneal ulcer.

Procedure -

1. Patient was made to lie down comfortably on a couch.
2. The affected eye was cleaned with sterile saline using sterile swabs.
3. Sterile 2% Paracaine was applied to the eye taking care not to apply too much of it as it may inhibit the growth of the organism.
4. Care was taken to see that the eyelids did not contaminate the specimens. Eye speculum was used whenever necessary.
5. Patients were given relevant instructions regarding position

and restriction of eyeball movement during the scraping procedure.

6. No.15 Bard Parker blades were used to scrap the ulcer.^{6,8} A new sterile blade was used for each patient.

7. Materials were obtained from leading edge and base of ulcer Scrapings were taken and processed as follows.⁶

Transportation: All collected samples were transported to the laboratory as soon as possible in aseptic condition along with requisition form.

Processing of sample: Two samples were collected from each patient for culture and staining purpose.

Samples were further processed as follows -

- Direct microscopy by KOH mount and Gram's staining.^{6,35}
- Culture on Sabouraud's dextrose agar and blood Agar.⁶

1. DIRECT MICROSCOPIC EXAMINATION - First sample was used for direct microscopic examination which included the following-

I. KOH Wet mount:⁵⁷

The scraping material was transferred onto a clean glass slide and one or two drops of sterile 10% KOH was applied over that and covered with clean coverslip. Slide was then examined under low and high power objective for the presence of hyphal elements, conidial forms of the fungal isolates. KOH digests proteinacious material and retain the polysaccharide fungal cell wall facilitating the demonstration of fungal elements. The results were correlated with culture report later.

ii. GRAM'S STAIN:

Principle:¹⁵³

This differential stain divides the majority of bacteria into two groups- the gram positive and the gram negative bacteria. Bacterial cells are stained with crystal violet; treated with iodine to form a crystal violet/iodine complex within the cell; washed with acetone; and stained again with the red counterstain safranin.

Reagents:

1. Crystal violet

Crystal violet	: 10 gm
Absolute ethanol	: 100 ml
Distilled water	: 1000 ml

(Preparation: The dye was dissolved in the alcohol, filtered through filter paper and distilled water was added.)¹⁵⁴

2. Gram's iodine

Iodine	: 10 gm
Potassium iodide	: 20 gm
Distilled water	: 1000 ml

(Preparation: 20 gm potassium iodide was dissolved in 250 ml water; 10 gm iodine was added to it. When iodine dissolved completely, remaining volume of distilled water was added to make 1 litre of solution.)¹⁵⁴

3. Acetone (100 %)

4. Safranin solution:

Safranin O	: 2.5 gm
Absolute ethanol	: 100 ml
Distilled water	: 1000 ml

(Preparation: The dye was dissolved in the alcohol, and distilled water was added to make final volume.)¹⁵⁴

Procedure:¹⁵⁴

- The corneal scraping material was transferred onto a clean, grease free, glass slide with a drop of sterile normal saline.
- The smear was made using a sterile bacteriological loop. The smear was allowed to air dry and heat fixed by passing over a flame 3-4 times.
- Slide was then covered with crystal violet (primary stain) and left to act for 1 min.
- Slide was tilted with the help of forceps and iodine was poured from upper end to wash away the crystal violet.
- Fresh gram's iodine was added for 1 minute (to act as mordant).
- The slide was washed with running tap water.
- Acetone was poured on slide, allowed to act for 2-3 seconds and washed off immediately with tap water.
- Slide was then covered with safranin for 30 seconds and rinsed with water.
- It was allowed to air dry.
- Gram stained smears were examined under oil immersion objective and observed for presence of polymorphs, mononuclear cells, epithelial cells, bacteria (Gram positive & Gram negative), yeast like cell and fungal elements like hyphae, if present their nature and relative number were noted.

CONCLUSION

The following conclusions were derived from the present study on keratomycosis.

- Keratomycosis, an important cause of ocular morbidity was found mostly in patients residing in rural area and those who were involved in agricultural and outdoor activity.
- History of trauma was found to be an important predisposing factor and strongly correlated along with the clinical features.
- It was seen commonly in the middle age group with male preponderance. Male of this age group are often the sole earners of the family and so ocular morbidity due to this can cause grave economic consequences for them.
- Precise identification of the causative organisms is very important as early institution of antifungal therapy may limit ocular morbidity.
- Although culture helps in definitive diagnosis and identification, microscopy for detection of fungal elements in corneal scraping permits a rapid diagnosis and 10% KOH mount was found to be a sensitive and reliable method for prompt diagnosis of mycotic keratitis.
- A variety of fungal isolates can cause infectious corneal ulceration in which *Aspergillus* spp was the most common fungal species found as this is one of the most common

fungus present in air and hence can easily infect damaged cornea

- From the present study, the vital role of microbiological evaluation in the management of infectious corneal ulcer is clearly evident, since the clinical features alone are not adequate to confirm diagnosis.

SUMMARY

- Total 106 samples were collected during the study period
- Out of 106 samples, 38 cases were confirmed to have fungal etiology depending upon direct microscopy of lesions and clinical features showing 35.84% incidence of keratomycosis in this study.
- Among the 38 cases found to have fungal etiology, 35 cases were found positive for fungal elements in 10% KOH mount
- Among these 35 cases positive on 10% KOH, 28 cases were positive on culture.
- Three cases with negative finding on 10% KOH were also found positive on culture.
- Total of 31 cases were found culture positive.
- Male preponderance was observed (70.96%) in this study.
- Patients in age group 41 and 60 years were the most common affected.
- Incidence of fungal corneal ulcer was more in rural population (74.19%) than urban population.
- Most of the patients were farmers by occupation (51.61%) followed by labourers.
- History of trauma was present in majority of cases (58.06%) and was found as the most common predisposing factor associated with fungal keratitis.
- Among trauma, vegetative matter traumawas present in 55.55% of cases.
- Long term use of steroid or antibiotic eye drops were the second most common predisposing factor after ocular trauma and was present in 29.03% cases
- History of ocular surgery was present in 12.90% cases and 9.67% cases had diabetes.
- Maximum cases have occurred in month of Mar-Apr (32.25%) followed by Nov- Dec (29.03%).
- Among the 31 fungal isolates majority of the isolates were *Aspergillus* (51.61%), followed by *Candida* species (19.35%) and *Fusarium* spp.(9.67%).
- Among the *Aspergillus* species, most of the isolates were *A.flavus* followed by *A.niger* and *A.fumigatus*.
- Candida albicans* constituted 66.66% among the *Candida* species isolated.
- Molds constituted 80.64% of the total fungi isolated.

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