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Mycotic Corneal Ulcer in North India

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Abstract:
Aims& objective*: To report laboratory diagnosis of 100 cases of Keratomycosis diagnosed at Department of microbiology Maharani Laxmibai Medical College, Jhansi.*
Method*: 100 corneal scrapings were evaluated by 10% KOH, Gram stain and Calcofluor stain (CFW) and the samples will be inoculated on Fungal culture media.*
Result*: Out of 100 cases studied, KOH was positive in 43(43%), CFW was positive in 52(52%) patient and Gram stain was positive in 19(19%) patients. In the present study, positive fungal culture was taken as the gold standard highly sensitive of CFW in detection of fungal filaments compared of KOH and gram stain.*
Conclusion: *Direct microscopy with Calcofluor white smear was more sensitive in detecting fungal elements. This is a simple and rapid staining method for examination for corneal scraping. Early detection will be helpful in medical therapy for corneal ulcer.*
Keywords- *Corneal scrapings, Fungal keratitis, Calcofluor stain.*

I. Introduction

Fungal keratitis is a suppurative, ulcerative and sight threatening infection of the cornea that some time leads to loss of the eye. Corneal infection of fungal etiology is very common and represents 30%-40% of all cases of culture positive infection keratitis in South India.[1] WHO estimates global blindness about 20-40 millions. In India there are approximately 10 million blind persons and 3 million are corneally blind. The majority of cases occur among agricultural workers following corneal trauma with vegetative matter contaminated by fungi .These are opportunistic organisms and colonize when natural defences of eye are abrogated by corneal trauma, use of topical steroids or any other predisposing factors[2,3] . 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.[4]

As the background knowledge of these factors accumulate, it is becoming important to identify the fungal corneal ulceration and to standardize the laboratory diagnostic methods so that an accurate early diagnosis can be made. Hence, this study aims to identify the most common causative organisms and also compares the efficacy of Calcofluor White staining with other methods like 10% KOH wet mount and Gram stain for early diagnosis of keratomycosis.[4,5,9]

Objective of the study

1. Microbiological study of corneal ulcer patients.
2. Prevalence of corneal ulcer in Bundelkhand Region, Uttar Pradesh.
3. To identify the risk factors of corneal ulcer.
4. To isolate and identify the fungal pathogens using scalpel method on Sabouraud's dextrose agar

II. Material And Methods

The comparative study was conducted in Bundelkhand reasion from 1.1.2014 to 30.6.2014. This study included 100 patients with clinically suspected fungal corneal ulcers. The sample were collected from Department of Opthalmology Maharani Laxmibai Medical College, Jhansi.

Patients who had received antimicrobial therapy and not responding to treatment were included in this study.Patients with suspected (or) confirmed viral keratitis, bacterial keratitis, interstitial keratitis and sterile neurotropic ulcers were excluded.

Sample preparation

Multiple corneal scrapings were taken with the help of slit lamp using 26 gauge needle on a syringe. The corneal ulcer bed and margins were taken under topical Anaesthesia using 4% Lignocaine hydrochloride after removal of debris or discharge from the vicinity[6.7].

Direct microscopic examination

The materials from the corneal scrapings were examined under 10% KOH, Calcofluor white stain and Gram stain.

[1]. 10% KOH preparation: Wet mount preparation in 10% KOH was done to detect the presence of fungal elements.

[2]. **Calcofluor white stain**: One drop of calcofluor white stain and one drop of 10% KOH was added to the sample on slide. The slide was then left to stand for 10 minutes andwas examined under fluorescence microscopy using blue light excitation (300-400 nm for the emission wavelength with excitation at around 355 nm).

[3]. **Gram stain:** Gram's stained smears were prepared to look for hyphae, budding yeast cells and pseudohyphae.

Culture of specimens

The corneal scrapings obtained were inoculated directly onto Sabouraud's dextrose agar with antibiotics and incubated aerobically at 25°C and 37°C. The plates were examined daily during the first week and twice weekly during the next two weeks [4.5]. The isolates were identified by standard laboratory procedures. As the mycelial isolates, were identified by their colony characteristic on SDA, microscopic morphology on LPCB wet mount and Slide culture.

The yeast isolates were identified by germ tube test, Chlamydospore production on cornmeal agar[4]. No growth was observed even after 3 weeks of incubation the culture was considered as sterile and the plates were discarded[4,8,9].

III. Figures And Tables

Table-1: Occupational distribution

Occupation	Number of patients(%)
Student	7
Farmers	48
Housewifes	12
Manual labourers	12
Industrial workers	17
Business class	4
Total	100 (%)

Table- 2: Correlation of KOH, CFW, Gram with Culture

Method	Direct microscopy positive Culture Positive	Direct microscopy positive Culture Negative	Direct Microscopy negative Culture Positive negative	Direct Microscopy negative Culture negative negative	Total
KOH	34	09	4	53	100
CFW	38	14	0	48	100
Gram's Stain	13	6	25	60	100

Table -3: Distribution of isolates in fungal keratitis

Species	No of isolates	%
Fusarium species	17	44.73
Aspergillus fumigates	7	18.42
Aspergillus flavus	4	10.52
Aspergillus niger	2	5.26
Penicillium species	3	7.89
Candida albicans	5	13.59
Total	38	100%

IV. Result And Discussion

Out of 100 cases studied fungal keratitis was predominately seen in farmers (48%), followed by house wifes (12%), industrial workers (17%) and laborers (12%), respectively. Agriculture is the most common occupation predisposing to fungal corneal ulcer (table-1)

Out of 100 cases studies, KOH was positive in 43 patients, CFW was positive in 52 patients and gram stain positive in 19 patients. In the present study, positive fungal culture was taken as the gold standard shows sensitivity of CFW was higher in detection of fungal filaments compared of KOH and Gram staining.

Direct Microscopy positive and Culture Positive was seen in 34%, 38% and 13% with KOH, Calcoflour white staining and gram's staining respectively. In KOH mount slide, fungal filaments appear as

refractile hyphae with septate or aseptate, branching or non-branching filaments. Some filaments look brown due to melanin pigments in some species of fungi. On the other hand, yeast cells are oval or round and colorless and sometimes produce pseudohyphae in the KOH wet mount preparation.

Whereas, total 38 fungal species isolated were, Fusarium species in 17(44.73%) cases followed by Aspergillus fumigates 7(18.47%) Aspergillus flavus 4(10.52%) Aspergillus niger 2(5.26%) Penicillium species 3(7.89%) and Candida albicans 5(13.59%). This high prevalence of fungal pathogens in North India was not so different from that found in similar studies in Bangladesh (36%), Ghana (37.6%) and South Florida (35%).[19,20]

The majority of mycotic keratitis was due to filamentous fungi, namely Aspergillus and Fusarium species. Aspergillus species was the most common isolate in fungal keratitis reported by Chander et al. [16]. However, Fusarium species was found to be the most common cause of fungal keratitis from south India (Madurai and Tamilnadu) by Barathi et al. (2002, 2003) [21-22] and Srinivasan et al. (1997) [23] which is similar finding with the present study.

Direct microscopy is an important diagnostic modality in investigating microbial keratitis, and a highest sensitivity at 99% is reported [24]. The addition of calcofluor white (CFW) stain to the diagnostic armamentarium has significantly increased the sensitivity of smear examination on direct microscopy [27]. However, it is difficult to determine the genus of fungi from KOH mounts [24]. Preliminary identification of Fusarium and Aspergillus species using microscopic features in histological specimens has been reported [25]. Identification of Fusarium from scraping material is reported by the detection of adventitious sporulation. [26]

In conclusion, Central corneal ulceration leading to most often to uniocular blindness to be common opthalmological problem. Keratomycosis is an important cause of microbial keratitis with injury to the cornea being a leading predisposing factor. The key element in the diagnosis of mycotic keratitis is the clinical suspicion by ophthalmologists. Fungal corneal ulcer is common in India due to the tropical climate and a large agrarian population that is at risk. Various factors are involved, such as trauma and the injudicious use of topical antibiotics and corticosteroids. However, due to the potential serious complications from mycotic keratitis, direct microscopy with CFW is noted to be more sensitive in detecting fungal elements with fungal culture. So, it is important to know the exact etiology and Early and rapid diagnosis of organisms of corneal ulcer to institute appropriate therapy in time.

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