

Lab Diagnosis and Evaluation of Fungal Keratitis

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ABSTRACT

Objective: This study is aimed to evaluate the usefulness of Sabouraud dextrose agar in the confirmatory diagnosis of suspected cases of fungal keratitis.

Study Design: Pre-designed prospective study of corneal scrapings obtained after detailed slit-lamp examination and documentation from all patients seen for non-viral microbial infective keratitis.

Place and Duration of Study: This study was conducted in the Department of Ophthalmology, JPMC, Karachi from July 2008 to June 2011.

Materials and Methods: 128 cases of non-viral microbial infective keratitis. Corneal scrapings of 128 patients with microbial keratitis Smears of corneal scrapings were stained with Gram's Method and inoculated specimens on Sabouraud dextrose agar (SDA) and incubated for 3-4 days.

Results: In a series of suspected cases of fungal keratitis, 119/128 (75% patients) had positive results for fungus in corneal scrapings by direct microscopy using Grams staining method and culture on Sabouraud dextrose agar (SDA). 43% males and 32% females had Candidial keratitis and 11% males and 7% females had Fusarium infection. Other samples showed presence of Gram positive cocci on smears and were negative for SDA.

Conclusion: Fungal keratitis continue to be an important cause of ocular morbidity, Since it becomes difficult to clinically diagnose and differentiate between bacterial and mycotic keratitis in complicated cases. It is better to use a standard culture medium like Sabouraud agar (SDA) when confirming ocular mycosis.

Key Words: Fungal keratitis, Ocular mycosis, Sabouraud agar, Candidia and Fusarium

INTRODUCTION

Fungal infections of cornea "mycotic/fungal keratitis" have been reported from different parts of the world¹. In tropical region during hay forming season most common isolated fungi are Aspergillus, Fusarium and Candida². Candidial keratitis is closely related to insufficient tear secretion, defective eye closure or systemic illness like Diabetes Mellitus and is closely related to minor trauma including contact lens wear³. The stromal keratitis caused by Candida closely resembles bacterial keratitis clinically. Clinical features include irregular, feathery margins, a dry, rough texture, and satellite lesions⁴. Microbiological investigation must be carried out when ocular mycosis is suspected due to a risk of complete loss of vision⁵. Fungal keratitis accounts for 30-40% of all cases of microbial keratitis in developing countries^{6,7}. Treatment of microbial keratitis is aimed at rapid eradication of the infecting organisms with control of inflammation and tissue damage, thereby preserving the transparency of the cornea⁸. Effective treatment depends on efficient identification of the infecting microorganisms⁹. A major factor in the improved management of fungal infection has been the ability to detect fungus, thus facilitating the selection of appropriate therapy¹⁰. While some of the clinical features of fungal keratitis are suggestive of fungal infection, none of them can be considered absolutely pathognomic of a fungal

infection. Deep seated mycosis may end up as endophthalmitis with complete loss of vision and destruction of eye. Therefore, microbiology workup in keratitis is required before initiating any treatment¹¹. Despite the advent of many new techniques, culture remains the cornerstone of diagnosis of most ophthalmic mycoses. Sabouraud dextrose agar (SDA) has been the preferred culture medium for fungus by physicians¹².

MATERIALS AND METHODS

In a pre-designed prospective study was done at ophthalmic unit at JPMC, Karachi. The corneal scrapings were obtained after detailed slit-lamp examination and documentation from all patients seen for non-viral microbial infective keratitis. A common protocol for diagnosis was used in all cases. Corneal scrapings were obtained by qualified cornea specialists from the base and edge of the ulcer using a sterile surgical blade (No.15 on a Bard Parker handle) under topical anesthesia and slit-lamp magnification. Gram's stain was included as a part of the standard protocol for microscopic evaluation of corneal smears. Gram's stained smears were examined at $\times 400$ and $\times 1000$ magnification. Smears were examined by light microscope. Scrapings for smears were collected prior to those for culture. Sabouraud Dextrose Agar was used to inoculate the corneal scrapings. SDA was incubated at 25°C and colonies were studied after 4 days.

RESULT

Total number of patients enrolled in the study was 128, out of which 74 were males and 54 females. Among 128 suspected cases of fungal keratitis 119 (93%) showed grey white fungal colonies. 9 cases (7%) which had appeared to be Gram positive cocci on Gram's staining could not be cultured on SDA also as shown in table # 1. 96% cases had candidial keratitis which were 43% males and 32% females. 23% cases of fusarium out of which 11% were males and 7% females was also isolated from the studied population.

Table No.1: Percentage of Male and Female in different micro-organism

Micro-organism	Male	Female	Total patients=128
Candida group	55 (43%)	41(32%)	96 (75%)
Fusarium species	14 (11%)	09 (7%)	23 (18%)
Gram positive cocci	05 (4)	04 (5%)	09 (7%)

DISCUSSION

The stromal keratitis caused by *Candida albicans* mimics bacterial keratitis clinically¹³. Direct microscopic detection of fungal structures in ocular samples permits a rapid presumptive diagnosis but at time vague especially deep seated fungus in the stroma^{14,15}. Use of corticosteroids in such cases would aggravate the infection as growth of fungal colonies is augmented by topical steroids¹⁶. Once fungi enter the host tissue it becomes very difficult to treat it by medication alone and the chances of endophthalmitis are increased manifold. In such complicated and severe cases attempt is made to remove antigenic/infectious agent and necrotic debris along with antifungal prior and after surgery. Therefore microbiological investigation of suspected ocular mycosis is mandatory to reach conclusive diagnosis before starting topical corticosteroids^{17, 18, 19}. For *Candida*, *Fusarium* etc SDA is the recommended culture medium. The fungal colonies are visible on SDA after incubation of 3-4 days. Colonies of *Candida* appear as grey white on SDA²⁰. In our study we made early identification of fungal elements on staining corneal scrapings with Gram's stain and inoculated the samples on SDA for 3-4 day also. The most frequently isolated fungi are the *Fusarium*, *Aspergillus*, and *Candida*²¹. *C. albicans* is the most frequent cause of fungal keratitis²².

CONCLUSION

Fungal infections of the eye continue to be an important cause of ocular morbidity, particularly in the developing world. Since it becomes difficult to clinically diagnose and differentiate between bacterial and mycotic keratitis it is better to use a standard

culture medium like Sabouraud agar (SDA) when confirming ocular mycosis. Understanding ocular infections will improve the outcome and reduce chances of fatality like endophthalmitis.

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