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Original Research Article

Comparison of KOH, Calcofluor White and Culture for the diagnosis of Keratomycosis from a Tertiary Care Hospital

Kanishtha Sharma[1], Saba Choudhary[2], Varsha A. Singh[3]

Demonstrator[1&2], Professor and Head[3]

[1&2]Demonstrator, Department of Microbiology, Government Medical College, Jammu-180015, Jammu and Kashmir, India.
[3]Department of Microbiology, M.M. Institute of Medical Sciences and Research, Mullana, Ambala- 133207, Haryana, India.

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ABSTRACT

Background: Mycotic keratitis is an important ophthalmic problem. Scarring of cornea as a result of keratitis is one of the preventable causes of blindness and carries usually unfavourable prognosis and requires immediate specific therapy. Worldwide, the reported incidence of mycotic keratitis is 17% to 36%. Microbial culture and direct microscopic detection using 10 KOH% and Calcofluor white stain always supplements the clinical diagnosis. This study involves comparison of KOH, Calcofluor white and Culture for the diagnosis fungal corneal ulcer. **Material and Methods:** This cross-sectional study was conducted in the Department of Microbiology & Ophthalmology, MMIMSR from Oct 2013 to Oct 2014 on 60 corneal scraping samples from clinically suggested cases of infectious keratitis**.** Corneal scrapings were collected aseptically and subjected to direct microscopy by 10 KOH% and Calcofluor white stain for detection of fungal elements and fungal culture. **Result:** Out of 60 suspected cases of corneal ulcer, culture positivity was 43.3% which included 8 (30.76%) bacterial, 16 (61.53%) fungal and 2 (7.69%) mixed isolates respectively. Out of the 24 fungal pathogens, 100% were detected by microscopy and 75% by culture. 100% and 87.5% fungus was detected by Calcofluor white and KOH respectively**.** *Aspergillus* species was the most prevalent pathogen followed by *Fusarium solani* and *Curvularia geniculata*. **Conclusion**: Calcofluor white stain was found to be an excellent method in detection of fungal agents. KOH wet mount was also found to be a good alternate method in resource-poor settings. Mycological culture was the least efficient method however it may be used in routine for the identification of species.

INTRODUCTION

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes. [1] Bacteria and fungi are among the frequent etiological agents responsible for suppurative corneal ulcers. Fungal keratitis is an ulcerative and sight threatening infection of the cornea that sometimes leads to loss of the eye. [2] The incidence of fungal corneal infection has increased remarkably in the recent years with the increased use of broad spectrum antibiotics and corticosteroid. [3]

A large number of fungi have been incriminated to be the cause of mycotic keratitis. Although frequency and spectrum of fungal members differ from place to place largely based upon climatic, geographic and socioeconomic conditions. [4] The diversity in clinical presentations observed in each case and also emergence of new cases each year pose a diagnostic and therapeutic challenge to ophthalmologists. The definitive diagnosis of ulcers caused by multiple organisms can only be arrived by microbiological evaluation.

Laboratory diagnosis of Keratomycosis relies on the direct microscopic examination of the fungal elements by KOH and calcofluor white stain in the clinical sample and mycological culture of the fungus. 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 therapy. [2]

With this objective in mind, this study was undertaken to compare the efficacy of commonly employed laboratory methods for the diagnosis of clinically suspected cases of fungal keratitis. It will be helpful in the development of better methodology for the timely detection of cases so that appropriate therapy may be started at the earliest.

MATERIALS AND METHODS

Study design

This study was carried out in the Department of Microbiology & Ophthalmology, MMIMSR, Mullana, Ambala on 60 patients attending Ophthalmology OPD and IPD in clinically suggested cases of infectious keratitis for a period of 1 year i.e. October 2013 to October 2014.

Sample collection[5]

A detailed clinical history of the patient was noted. The effected eye was locally anesthetized after washing with normal saline. Scrapings from base and edges of the corneal ulcer were collected using a sterile Bard Parker blade no.15 or Kimura's Spatula and send immediately to the Microbiology laboratory.

Sample processing

Laboratory methods- Corneal scrapings of the patients were examined by microscopic examination.

1. Potassium hydroxide wet mount

A drop of 10% KOH was placed on a slide and a small amount of the scraping was added to the drop. A clean coverslip was placed over it. It was allowed to act for 5-20 minutes. The slide was then carefully observed under 10x and 40x objective lens to detect fungal elements.

2.Calcofluor white (CFW) stain wet preparation

A one drop of calcofluor white stain was added to the scraping on a clean grease free slide and covered with a cover slip. The slide was then left to stand for 10 minutes and was examined under fluorescence microscope using blue light excitation (300-400 nm for the emission wavelength with excitation at around 355nm).

4. Culture of isolates

The scrapings were inoculated on Blood agar and Sabourand's dextrose agar and processed further as per the standard laboratory procedures for bacterial and fungal identification.

RESULTS

Out of 60 suspected cases of bacterial and fungal keratitis, 26(43.3%) showed growth. Of these fungal isolates were 16(61.53%), bacterial isolates were 8(30.76%) while 2(7.69%) showed mixed growth.

Out of the 24 fungal agents detected, 24 (100%) were detected by direct microscopy and 18 (75%) by culture. [Table 1].

The direct microscopy was done by KOH and calcofluor white stain. KOH detected 21 (87.5%) as compared to calcofluor white which detected 24(100%) fungal agents. [Table 2]

Out of the 21 KOH positive samples, 15 (71.42%) were both KOH and culture positive whereas 6 (28.57%) were KOH positive but culture negative. Out of the 24 CFW positive samples, 16 (66.66%) were both CFW and culture positive whereas 8 (33.33%) were CFW positive but culture negative. Among 18 culture positive cases, 3(16.66%) by KOH and 2 (11.11%) by CFW were negative by microscopy. [Table 3]

Study the morphological forms of fungus, out of 21 KOH positive samples 20(95.2%) showed hyphae and 1(4.76%) showed yeast like cells and out of 24 Calcofluor white positive samples 22(91.6%) showed hyphae and 2(8.3%) showed yeast like cells. [Table 4]

Among the various fungal isolates Aspergillus species was most predominant constituting 44.44% then came in line Fusarium solani, Curvularia

geniculata and Candida albicans with 11.11%. [Table 5]

DISCUSSION

Scarring of the cornea developed as a result of suppurative corneal ulcer is the second commonest cause of preventable blindness after unoperated cataract among people in Asia, Africa and in the Middle East. [6] Microbial culture and direct microscopic detection always supplements the clinical diagnosis and provide supportive evidence for planning appropriate therapy

In the present study of the total 24 fungal agents detected, 24(100%) were detected by microscopy and 18 (75%) by culture. (Table 1) (p value=0.531 which was statistically not significant) This was in accordance with Satpathi and Satpathi [7] which reported 100% positivity by microscopy and 78.9% by culture. Higher rate of positivity by microscopy (100%) in the present study may be because of using combination of CFW with KOH which appeared to have significant advantage in detection by microscopy.

In the present study CFW and KOH detected 100% and 87.5% fungus respectively. (Table 2) (p value= 0.074 which was statistically not significant). Similar results were reported by Shokohi T et al. [8] (KOH 71.4%, CFW 100%), Zhang et al. [9] (KOH 81%, CFW 96.6%). Higher rate of detection of fungus by CFW (100%) showed that it was a more sensitive stain in detection of fungal aetiology as compared to KOH (87.5%). It can be used both for early and advanced fungal keratitis. It is a fluorescent stain that offers excellent visualization of morphology of pathogenic fungi, particularly when clinical material is very scanty.

14 samples in the present study i.e. 6 (28.57%) by KOH and 8 (33.33%) by CFW remained sterile on culture despite positive direct microscopic findings. (Table 3) (p value= 1.00 which was statistically not significant). This is in accordance with Anusuya devi. D et al. [2] reported 16 samples i.e. 7(34.2%) by KOH and 9 (36.7%) by CFW remaining sterile on culture despite positive direct microscopic findings. The reasons for cultures to be sterile even when the direct microscopy was positive could be that the patients were already using topical steroids or antifungal agents before the corneal scraping samples were taken. Another reason could be many a time the sample was so insufficient in quantity that only direct microscopy was feasible and inadequate material was left for establishing cultures.

It was also observed in the present study that out of total 18 culture positive cases, 3 (16.66%) by KOH and 2 (11.11%) by CFW were negative by microscopy. This is comparable to Satpathi and Satpathi [7] and Chander et al. [10] which reported 18.2% and 17.65% samples negative by microscopy but positive by culture. It shows that cases examined by direct microscopy alone should also be cultured otherwise these cases can be missed. Culture is not only important for species identification but also for diagnosis of cases.

The present study showed that out of the 21 KOH positive samples 20(95.2%) showed hyphae and 1 (4.76%) showed yeast cells and of the 24 Calcofluor white positive samples 22 (92%) showed hyphae and 2(8%) showed yeast like cells. (Table 4) It may be because detection of hyphae by KOH is difficult because of its confusion with epithelial cell wall border due to its refractility.

Aspergillus species (44.44%) was the most prevalent pathogen in the present study as the cause of fungal keratitis followed by Fusarium solani, Curvularia geniculata and Candida albicans with 11.11%. (Table 5) This was comparable to Basak et al. [11], Saha S et al. [12]. Moulds with enteroblastic conidia adhering in dry chains as in Aspergillus spp. were more frequently isolated from patients in the north of the country where the environment usually drier and dustier. Also the spores of Aspergillus spp. can tolerate hot, dry weather conditions.

CONCLUSION

Suppurative keratitis continues to be an important cause of ocular morbidity. In the present study fungal etiological agents were predominant than bacterial. Calcofluor white was found to be an excellent method in detection of fungal agents from clinically suspected keratomycosis cases. In resource poor settings, KOH wet mount serves as an alternate method for early detection of fungal elements with good results. Mycological culture was the least efficient of the three methods. It is the only method in routine use for the identification of

species however its disadvantage is that it's a time-consuming procedure.

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Table 1. Distribution of fungal agents detected by microscopy and culture.

Total no of fungal pathogens detected	Microscopy	Culture	*p value
24	24 (100%)	18 (75%)	0.531

Table 2. Detection of fungus in relation to KOH and Calcofluor white staining.

Result of microscopy	KOH	Calcofluor white	*p value
24	21(87.5%)	24 (100%)	0.836

Table 3. Correlation of KOH, Calcofluor white and culture for fungal pathogens.

Culture status	Positive by KOH	Positive by Calcofluor white	*p value
Culture positive	15(71.42%)	16 (66.66%)	0.815
Culture negative	6(28.57%)	8(33.33%)	1.00
Total	21	24	

Table 4. Comparison of direct microscopy by KOH and Calcofluor white for detection of morphological forms of fungus.

Microscopic finding	KOH	Calcofluor white	*p value
Hyphae	20 (95.2%)	22 (91.6%)	0.921
Yeast cells	01 (4.76%)	02(8.3%)	0.502
Total	21	24	

Table 5. Species wise distribution of fungal isolates

Fungal Species	No. of isolates	Percentage %
Aspergillus flavus	5	27.80
Aspergillus fumigatus	2	11.11
Aspergillus niger	1	5.55
Fusarium solani	2	11.11
Candida albicans	2	11.11
Curvularia geniculata	2	11.11
Helminthosporidium	1	5.55
Mucor species	1	5.55
Pseudallescheria boydii	1	5.55
Drechslera species	1	5.55
Total	18	100

Corresponding Author: Dr. Kanishtha Sharma, Department of Microbiology, Government Medical College, Jammu-180015, Jammu & Kashmir, India.
E-mail: kanishtha13@gmail.com

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