Original article

EVALUATION OF DIAGNOSTIC SENSITIVITY OF WET PREPARATION MICROSCOPY USING KOH FOR DETECTION OF FUNGAL AGENTS FROM KERATITIS PATIENTS

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ABSTRACT

Background: Keratitis is a common ophthalmic condition mostly caused by fungi. Apart from fungal culture, wet preparation using 10% Potassium hydroxide (KOH) for microscopic detection of fungal elements is a rapid and accurate method of laboratory diagnosis.

Purpose: This prospective study was undertaken in order to evaluate the diagnostic sensitivity of wet preparation microscopy using KOH for detection of fungal agents from keratitis patients.

Methodology: 103 samples of clinically suspected patients of keratitis attending tertiary care hospital between march 2010 and june 2011 were included. Samples like corneal swabs, corneal scrapings, corneal button, and corneo-scleral rim were collected aseptically after slit lamp examination, then transported to microbiology laboratory. Samples were processed for direct microscopy (gram stain and 10% KOH wet mount preparation) and culture. Culture positive isolates were identified based on morphology and standard biochemical tests. Data entry and analysis was done statistically.

Results: From 103 samples, fungal culture was positive in 12%. Different fungus isolates include *Aspergillus flavus* (67%) and *Candida spp* (25%) were the leading fungi followed by *Curvularis* (8%). Direct microscopical examination using KOH wet preparation and gram's stain had detected fungal elements in 83% and 75% samples respectively against culture results.

Conclusion: *Aspergillus flavus* (67%), *Candida spp* (25%) and *Curvularia spp* (8%) was most common cause of fungal keratitis. Wet mount with KOH can be relied upon as the single most important screening tool for rapid diagnosis of fungal corneal ulcer and treatment should be dispensed on its basis.

Key Words*:* Keratitis, Fungi, Potassium hydroxide, Culture, Gram's stain.

INTRODUCTION

Keratitis is an ocular emergency that requires prompt and appropriate management to ensure the best visual outcome of the patient. Without adequate treatment, corneal infection leads to blindness through corneal scarring and endophthalmitis [1]. Bacteria and fungi are among the frequent etiological agents responsible for Keratitiss.[2] The incidence of fungal corneal infection has increased remarkably in the recent years with the increased use of broad spectrum anti-

biotics and corticosteroid.[3] The widespread and sometimes injudicious topical application of cortisone and its derivatives combined with antibiotics may not only favour the growth of fungi but also causes invasive infection.[4] In order to minimize the ocular morbidity, timely antimicrobial treatment must be initiated on the basis of clinical and microbiological evaluation.[5, 6] Culture and direct microscopic detection of causative organisms are the two important microbiological investigations that are used for laboratory confirmation of diagnosis. Although the specificity of cultures makes them indispensable for the confirmation of a diagnosis, direct smear examination of the specimen is of immense help in early diagnosis and treatment.[7] Potassium hydroxide (KOH) 10% wet mount is one of the oldest and principle modalities for demonstration of fungi not only in corneal scrapings but in other specimens too.[8,9] Despite being the isolation of microbial pathogens through culture is considered to be the gold standard, but due to lack of culture facilities especially for fungi its scope for routine laboratory diagnosis is limited. There are many studies establishing the efficacy of KOH smear of mechanical scraping over culture as gold standard for the diagnosis of fungal keratitis.[10,11,12] More over it is a very useful method not only for rapid and cost effective diagnosis but also helps in early introduction of appropriate antifungal drug by the ophthalmologist to prevent morbidity from keratitis. Although wet preparation microscopy for fungal agents is being practiced by clinical laboratories for long time but its diagnostic sensitivity has yet to be estimated especially in our settings. The present study was designed to access the diagnostic sensitivity of KOH wet preparation for fungal agents in comparison to fungal culture taken from keratitis patients attending tertiary care hospital.

MATERIALS & METHODS

Ethical clearance and approval for this study is given by human resource ethical committee of the institution.

Patients: Total of 130 ocular samples were processed for microbiological evaluation from the 93 clinically suspected cases of keratitis attended at tertiary care hospital between march 2010 and june 2011. Inclusion of patients with keratitis, which was defined as a loss of the corneal epithelium, with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.[13]

Collection of samples: By using standard technique corneal scraping from the base and edge of the ulcers, corneal swab, corneal button, corneosclera rim were collected aseptically after slit lamp examination and intraoperatively.[14] Corneal scrapings were taken after two drops of local anaesthetic (0.5% proparacaine hydrochloride) eye drops were instilled in the conjunctival sac of the affected eye and then corneal scrapings were taken by sterile bard parker no. 15 scalpel blade.

Laboratory procedures: For culture of fungus pathogen, material was inoculated on sabouraud's dextrose agar containing gentamycin. Primary inoculation was done at the site of sample collection. Two sets of sabouraud's dextrose agar medium (SDA) plates were inoculated, one incubated at 25 ^{o} C for isolation of filamentous fungi and the other at 37 ^{o} C for isolation of yeast form of fungi. Smear was prepared from corneal samples and examined under microscope in KOH wet mount preparations as well as gram's stain for the presence of fungal elements by using 10x and 40x objectives of light microscope. The demonstration of hyphae, pseudohyphae and yeast cells under microscope was considered as positive for fungal element. Cultures plates were examined for growth daily during first weeks and twice a week during next three weeks.[15] Plates that did not show any growth after 21 days were discarded and considering negative for fungal elements. Primary fungal isolates were subcultured onto SDA media for identification of species. The fungal species were identified on the basis of their gross colonial characteristics and microscopic morphology. The diagnostic sensitivity of wet preparation microscopy using KOH was calculated by comparing its positivity with fungal culture-positive cases.

RESULTS

Total 93 patients with keratitis were enrolled in the study. There was male preponderance with 66% as compared to female with 34%. Majority of patients had history of trauma 42% as predisposing factor, followed by foreign body exposure 14% in our study. Majority of patients suffering from keratitis were in age group of 40 years and above followed 18-39 year of age and less common in 3-11 years of age. Trauma was most

commonly associated risk factor contributes 42% followed by foreign body exposure in to the eye 15%, contact lens 11%, other ocular disease 6%, systemic diseases 5%. In 9% of cases occurrence of infection was idiopathic.

Table-I: Predisposing factors in patients with keratitis

Variables	Percentage
Gender	
Male	66
Female	34
Age in years	
0-2	0
3-11	1
12-17	0
18-39	43
≥40	56
Predisposing factors	
Trauma	42
Exogenous infection	2
Post operative	2
Foreign body	15
Contact lens	11
Other ocular disease	6
Systemic disease	5
Not Known	9
Donor	8

Rate of isolation of microbes in culture were shown in Table-II. Of the 103 samples, microbial growths were yielded in 34 samples including 12(12%) pure fungal, 21(62%) pure bacterial and 1(1%) mixed bacterial growths.

Table II: Fungal species isolated by culture among keratitis cases

Fungal species	Percentage
Aspergillus flavus	67
Candida spp.	25
Curvularia spp.	8

Table shows the fungal species isolated and identified from keratitis patients by culture. *Aspergillus flavus* (67%) and *Candida spp* (25%) were the leading fungi followed by *Curvularia* (8%).

Rate of detection of fungal agents by KOH wet preparation microscopy against fungal culture is shown in Table-III below. The sensitivity and specificity of KOH preparations was calculated by considering the culture as gold standard.

Table III: Correlation of KOH preparation and Culture positivity for fungus.

KOH preparations	Fungal culture (No.)	
	Positive	Negative
Positive	10	2
Negative	2	89

Total fungal positive cases were 14 (KOH+ culture). Out of total 12 fungal culture positive cases, KOH microscopy detected 10 cases. The sensitivity of KOH preparations was 83% (Confidence interval: 55.2- 95.3), where as specificity was 98% (Confidence interval: 92.34- 99.4). The positive predictive value was 83% (Confidence interval: 55.2-95.3) and the negative predictive value was 98% (Confidence interval: 92.34- 99.4).

DISCUSSION

Keratitis is a major cause of unilateral blindness in developing countries. The etiology of keratitis have been found to vary in different geographic locations, climate and also tend to vary over the time.[14,16] Methods for rapid detection of microbial agents and their confirmation are of paramount clinical importance especially in case management. Common laboratory techniques for identifying microbial agents causing keratitis are culture and direct microscopic examination of scraping. A KOH wet mount preparation of the corneal samples has been found to be a simple and sensitive method for diagnosis of fungal agents.[17] Considering fungal culture as gold standard for diagnosis, the diagnostic sensitivity of KOH microscopy for fungal elements has been revealed to be 83% in the present study. The results of present study were comparable with other study. In a prospective study Vajpayee RB et al[18] from 171 cases fungus could be demonstrated by KOH preparation in 94.3% of the culture-proved cases. Gopinathan et al[19] in their large series (1354 eyes) of fungal keratitis have reported, the diagnostic utility of smears of corneal scrapings using KOH preparation, calcofluor white (CFW), gram and giemsa-stains. The KOH preparation alone revealed fungus in 91.0% (1219) eyes. The performance of KOH detection of fungal elements has high diagnostic sensitivity which can be compared with culture. In fact culture is not available in all routine diagnostic laboratories and it really requires for species identification. Moreover, culture is a time consuming laboratory method which is not applicable to common clinical practice. In this context, Sharma et al[20] have recommended

introduction of anti-fungal therapy whenever a KOH+CFW-stained smear is positive for fungus because they believed that the gold standard of culture has its own limitations and a fungal element is unlikely to be misinterpreted under microscopic examination.

CONCLUSION

Considering the findings of KOH preparation for fungal keratitis in present study and other similar studies, all emphasize that this is a very simple, rapid and cost effective laboratory method with high diagnostic sensitivity. Further, it has been found to be dependable for making decisions in the empirical treatment of fungal keratitis. KOH wet preparation can be recommended routinely for all cases of suspected fungal infections including keratitis.

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