Original article

Potassium Hydroxide (KOH) Wet Preparation for the Laboratory Diagnosis of Suppurative Corneal Ulcer

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Abstract

Background: Suppurative 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 and cross sectional study was undertaken in order to evaluate the diagnostic sensitivity of wet preparation microscopy using KOH for detection of fungal agents from suppurative corneal ulcer patients. ***Methodology:*** Fifty six (56) consecutive clinically suspected patients of suppurative corneal ulcer attending Rajshahi Medical College Hospital (RMCH) during the period from July, 06 to June, 07 were included. Corneal swabs were taken aseptically for detection of bacteria in gram-stained smear and culture. Conventional mechanical corneal scrapings were collected under topical anesthesia and utilized for microscopic detection of fungal agents in KOH wet preparation and fungal cultures in the department of Microbiology of Rajshahi Medical College. ***Results:*** Culture yielded microbial growths in 47(83.93%) out of 56 samples of corneal ulcer that included 24 (42.86%) pure fungal growths, 14 (25.0%) pure bacterial growths and 09 (16.07%) mixed microbial growths (both bacteria and fungi). Direct microscopical examination using 10% KOH wet preparation detected 28 fungal agents out of total 33 fungal cases (combining both pure and mixed fungal growths in culture). Diagnostic sensitivity of wet preparation microscopy was found to be 84.85% by comparing its performance to fungal culture yields, which is the 'gold standard' for laboratory diagnosis. ***Conclusion:*** This limited study has revealed that wet preparation can be a tentative diagnosis of fungal keratitis and can be accurately relied upon for initiating prompt anti-fungal therapy and also recommended as a cost-effective method for laboratory diagnosis especially where culture facility is not available.

Key Words: Corneal ulcer, Fungi, Potassium hydroxide, Culture, Diagnostic sensitivity.

Introduction

Suppurative keratitis is an ocular emergency that requires prompt and appropriate management to ensure the best visual out come 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 suppurative corneal ulcers[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]. The widespread and sometimes injudicious topical application of cortisone and its derivatives combined with antibiotics may not only favor the growth of fungi but also causes invasive infection[4]. In order to

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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 corneal ulcers. 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 see the diagnostic sensitivity of 10% KOH wet preparation for fungal agents in comparison to fungal culture taken from suppurative corneal ulcers patients attending Rajshahi Medical College Hospital.

Materials & Methods

Patients

This prospective study included fifty six (56) consecutive patients of different age and sex with clinically suspected suppurative corneal ulcers attended at Rajshahi Medical College Hospital from July, 2006 to June, 2007. Inclusion of patients with corneal ulceration 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]. Clinically suspected viral corneal ulcer and degenerative corneal ulcer cases diagnosed by an ophthalmologist were excluded from this study.

Collection of samples

By using standard technique, one corneal swab and two corneal scrapings were collected aseptically from the base and edge of the ulcers from each patient by an ophthalmologist under operating microscope[15]. Corneal scrapings were taken after collection of corneal swab. Two drops of local anaesthetic (4% Xylocaine) eye drops were instilled in the conjunctival sac of the affected eye and then two corneal scrapings were taken by sterile Bard parker No. 15 scalpel blade.

Laboratory procedures

Smear was prepared from corneal swab and examined under microscope for Gram stained bacteria. The swab was also inoculated onto blood agar, MacConkey's agar and chocolate agar media and incubated aerobically at 37^0C for up to 48 hrs for bacterial culture. First corneal scraping was used for microscopical detection of fungal agents using 10x and 40x objectives of light microscope in wet preparation of 10% KOH. The demonstration of hyphae, pseudohyphae and yeast cells under microscope was considered as positive for fungal element. The second scraping was inoculated for fungus culture onto Sabouraud's dextrose agar medium (SDA). The technique of inoculation onto the medium consisted of "C" streaks on the plate for best assurance against mistaking air borne contaminants. Only presence of fungal growths on the

Table-I: Characteristics of patients of suppurative corneal ulcers

Characteristics		*No.*	*Percentage*
Male		38	67.86
Female		18	32.14
Occupations			
	Farmers	20	35.71
	Housewives	10	17.86
	Day laborers	10	17.86
	Service holders	04	07.14
	Others	12	21.43
Predisposing factors			
	Agricultural trauma	19	33.93
	Domestic trauma	09	16.07
	Other trauma	02	03.58
	Systemic disease	04	07.14
	Dacrocystitis	09	16.07
	Others	13	23.21
Sources of patients			
	Rural	39	69.64
	Urban	17	30.36
History of drug use			
	Antibiotic and antifungal drops	35	62.50
	Antibiotic and steroid drops	05	08.93
	Use of no drug	16	28.57

"C" streak was considered significant and out growth away from the "C" streak was discarded as contaminant[14]. SDA was incubated at 25^0C to observe the fungal growth daily for first 7 days and on alternate days for next 7 days for slow growing fungi. Plates did not show any growth after 14 days were discarded. 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 10% KOH was calculated by comparing its positivity with fungal culture-positive cases.

Ethical consideration

The protocol of this study was approved by the 'Institutional Review Board' of Rajshahi Medical College, Bangladesh for ethical issues related to this research. Informed written consent was obtained from each patient or legal guardian before sample collection.

Results

Characteristics of patients clinically diagnosed as suppurative corneal ulcers enrolled in the study are furnished in Table-I. There was male preponderance (67.86%), majority were farmers and day laborers (53.57%), with history of agricultural trauma as predisposing factor in 33.93% cases. Significant number of patients was from rural areas (69.64%) and vast majority (71.43%) of them had history

Table II: Rate of isolation of microbes in culture among corneal ulcer patients

Organisms	*No. of positive cases*	*Percentage*
Fungus	24	42.86
Bacteria	14	25.00
Mixed	09	16.07
Total	47	83.93

Table III: Fungal species isolated and identified among corneal ulcer cases by culture (n= 33)

Fungal species	*Pure fungal growth*	*Mixed growth*	*Total*
Aspergillus fumigatus	8	2	10(30.30)
Aspergillus flavus	2	1	03(09.09)
Aspergillus niger	2	0	02(06.06)
Fusarium	5	3	08(24.24)
Mucor	4	0	04(12.12)
Rhizopus	2	0	02(06.06)
Alternaria	0	1	01(03.03)
branching fungus (Unidentified)	1	2	03(09.09)
Total	24 (72.73)	09 (27.27)	33(100)

of use of drugs (antibiotics with antifungal or steroid eye drops) before collection of sample.

Rate of isolation of microbes in culture are shown in Table-II. Out of total 56 samples of corneal materials, microbial growths were yielded in 47 (83.93%) cases including 24 (42.86%) pure fungal, 14 (25.00%) pure bacterial and 09 (16.07%) mixed microbial (both fungi and bacteria) growths respectively.

Table-III shows the fungal species isolated and identified from corneal ulcer patients by culture. Number of total isolates was 33 including 24 (72.73%) pure fungal growths and 09 (27.27%) mixed growths. Aspergillus fumigatus (30.30%) and Fusarium (24.24%) were the leading fungi followed by Mucor (12.12%) and Aspergillus flavus (09.09%) detected among the clinical cases.

Rate of detection of fungal agents by KOH wet preparation microscopy against fungal culture is shown in Table-IV. Out of total 33 fungal cases, KOH microscopy detected 28 cases indicating its diagnostic sensitivity as 84.85%.

Table IV: Rate of detection of fungal agents by KOH wet preparation against culture

Methods	*Number detected*	*Percentage*
Culture	33	100.00
KOH preparation	28	84.85

Discussion

Corneal ulcer is a major cause of unilateral blindness in developing countries. The etiologies of corneal ulcer have been found to vary in different geographic locations, climate and also tend to vary over the time[15, 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 corneal ulcers are culture and direct microscopic examination of scrapes.

A KOH wet mount preparation of the corneal scrapings has been found to be a simple and sensitive method for diagnosis of fungal agents17. Considering fungal culture as gold standard for diagnosis, the diagnostic sensitivity of KOH microscopy for fungal elements has been revealed to be 84.85% in the present study. In a prospective study of 171 cases of clinically suspected fungal corneal ulcers, although cultures were positive in 88 eyes (51.46%), the fungus could be demonstrated by KOH preparation in 94.3% (83 of 88) of the culture-proved cases and 93.6% (160 of

171) of the overall eyes11. Gopinathan et al. 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-stains18. The smears established the fungal cause in 95.4% (1277 out of 1354) eyes. The KOH preparation alone revealed fungus in 91.0% (1219) eyes. So, the performance of KOH detection of fungal elements in our study are in very good accordance with all studies mentioned above and clearly establish its 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. More over, culture is a time consuming laboratory method which is not applicable to common clinical practice. In this context, Sharma et al. 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 examination12.

Considering the findings of KOH preparation for fungal keratitis in our study and similar studies both at home and abroad, we would like to emphasize that this is a very simple, rapid and cost-effective laboratory method with high diagnostic sensitivity. Further, it has been found to be very dependable for making decisions in the empirical treatment of fungal keratitis. We recommend that KOH wet preparation should routinely be done for all cases of suspected fungal infections including keratitis.

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