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Research Article

Comparative study of impression smear with conventional mechanical corneal scrapping by potassium hydroxide (10%KOH) in diagnosis of fungal keratitis

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ABSTRACT

Background: Diagnosis of fungal keratitis remains a challenge to an ophthalmologist due to variety of presentation of patients. If a prompt, aggressive and early treatment is not started disease turns indolent resulting in sloughing of cornea, serious loss of vision, possible perforation and loss eye.
Methods: This observational study was conducted in upgraded department of ophthalmology, BRD Medical College, Gorakhpur, India among 50 patients of suspected fungal keratitis.
Results: Fungal keratitis is more common in young males of rural area. Most of fungal corneal (>5 mm) with presence of hypopyon, pigmentation and satellite lesion as consistent features. KOH smear with impression smear technique was positive in 36 (72.0%), out of total 50 cases. KOH smear with conventional mechanical scrapings was positive in 35 (70.0%), out of total 50 cases. Sensitivity of impression smear technique as compared to conventional scraping was 100.0%. Sensitivity of mechanical scraping technique as compared to impression smear technique was 92.11%.
Conclusions: Impression cytology is a recent diagnostic tool in fungal keratitis. It has various advantages over mechanical scraping like no expensive equipments like slit lamp and operation microscope required. Impression smear KOH mount is comparative to conventional mechanical corneal scraping KOH examination in making and tentative diagnosis of fungal keratitis and can be accurately replied upon for initiation antifungal therapy.

Keywords: Impressing smear, Fungal keratitis

INTRODUCTION

Keratitis is an inflammation of the cornea (the clear, front part of the eye) and is often caused by bacteria, viruses, amoeba, fungi. There are two types of keratitis infectious ulcerative keratitis (IUK), caused by infectious pathogens and noninfectious ulcerative keratitis (NIUK), which has a clinical presentation similar to that of IUK but no known infectious cause and is a diverse disease associated with systemic disorders. Fungal keratitis is an inflammation of the cornea caused by a fungus. Fungal keratitis was first described by Leber in 1879.[2,3] According to the World Health Organization report, it is estimated that ocular trauma and corneal ulceration result in 1.5 to 2 million new patients of corneal blindness annually, posing a major public health problem for developing countries.[4]

Fungi cannot penetrate the intact corneal epithelium and do not enter the cornea from episcleral limbal vessels. The principal routes of inoculation are introduction concurrent with a penetrating or perforating wound,

either mechanical injury or surgery and introduction through an epithelial defect.

In tropical climates as in South Florida, Bangladesh, South India and Nepal, fungal keratitis is reported to be from 17-40%.[5] Incidence of fungal keratitis in now increasing because of non-judicious use of topical antibiotic-steroid combinations especially by Quacks in any form of eye diseases, increasing outdoor activities, increase use of contact lens for cosmetic purpose, increase incidence of diabetes and other immuno-compromised diseases. Weather conditions, work habit and distribution of organism in warmer climate have led to increased incidence of keratitis due to filamentous fungi. Fungal keratitis is mostly encountered in patient who comes from rural setting, because of more change to get injured by vegetative matter or injury by animal tail, common sufferers being field workers especially during harvesting season.

Visual outcome after fungal keratitis are often unsatisfactory. One study reported 25% evisceration rates in eye with this infection. This poor outcome is usually due to difficultly arising in making correct diagnosis, establishing the clinical characteristics of fungal keratitis and obtaining confirmation from the microbiology and non-availability of specific antifungal drugs.

As the outcome of fungal keratitis is often unsatisfactory rapid and correct diagnosis and prompt treatment is of great importance. There are studies establishing the efficacy of KOH (10%) smear of mechanical scraping over culture as gold standard for diagnosis of fungal keratitis.[6,7]

Impression cytology is a recent method used to diagnosed dry eye conditions due to vitamin A deficiency, contact lens wearer keratitis and fungal keratitis. Impression cytology though, its role in diagnosis in fungal keratitis is not well established, is a safe, simple, repeatable and non-traumatic process. It does not require expensive equipment like slit lamp, operating microscope. It is helpful in obtaining specimen material from small lesion which is otherwise difficult by the conventional techniques of mechanical scraping. Also there is less chance of perforation in cases of impending corneal perforation.

This study was undertaken to establish the role of impression smear in diagnosis of fungal keratitis and to compare impression cytology/smear with convention mechanical corneal scraping in diagnosis of fungal keratitis.

METHODS

This one year observational study was conducted among patients attending the out-patient department (OPD) of Ophthalmology at, BRD Medical College. Patients with at least three of the following criteria present were selected for the study.

- Patient with history of trauma of eye with vegetative or organic matter
- Patient with clinical sings more than symptoms
- Ulcer with irregular and feathery margins
- Ulcer with satellite lesion
- Presence of an endothelial plaque, fibroid aqueous reaction and hypopyon
- Dry looking ulcer
- Any pigmentation

Detailed history regarding particulars of the patient, symptoms in chronological order with duration and history of trauma, contact lens use, initial therapy and use of corticosteroids was obtained.

General examination, systemic examination and detailed ocular examination was done.

- Forehead- for wrinkling
- Lids-Edema, redness, ectropion, entropion, trichiasis, tylosis and lagophthalmos, etc.
- Palpebral fissure-normal/narrow/wide
- Conjunctiva- congestion, chemosis, lustre, scar, growth, pigmentation, degenerative conditions and foreign body.
- Cornea-surface, transparency, texture, sensation, vascularization, opacity, pigmentation, endothelium.
- Details of corneal ulcer-location, size, shape, depth, edge, presence of slough, immune ring, satellite lesions and any pigmentation.
- Anterior chamber- depth and contents (hypopyon), fibroid aqueous reaction.
- Lacrimal sac- by regurgitation test and syringing
- Visual acuity- by Snellen's chart
- IOP-by digital tonometry
- Nasal examination was also done.

Investigations like Slit lamp examination

Details of corneal ulcer (size, depth, hypopyon, satellite lesion, pigmentation), complete blood counts and blood sugar level was assessed.

10% KOH mount preparation by conventional mechanical scraping and 10% KOH mount preparation by impression smear technique was done.

RESULTS

Out of the total 50 cases, 5 cases (10.0%) were between 11-20 years, 26 cases (52.0%) were between 21-40 years, 14 cases (28.0%) were between 41-60 years and 5 cases (10.0%) were between 61-80 years. Thirty eight (76.0%) were males and 12 (24.0%) were females. Forty two cases (84%) belonged to rural background.

Fourteen cases (20.0%) had small size ulcer (<2 mm), 20 cases (70.0%) showed medium size ulcer (2-5 mm) and 16 cases (32.0%) had large sized ulcer (>5 mm).

Table 1: Ulcer noticed with or without hyopopyon, satellite lesions and pigmentation.

Ulcer (n=50)	No. of cases	Percentage (%)
Hypopyon		
Present	40	80.0
Absent	10	20.0
Satellite lesions		
Present	34	68.0
Absent	16	32.0
Pigmentation		
Present	12	24.0
Absent	38	76.0

Forty cases (80.0%) presented ulcers with hypopyon, 34 cases (68.0%) presented ulcers with satellite lesions and 12 cases (24.0%) presented ulcers with pigmentation. Out of total 50 cases, 12 patients (24.0%) gave a definite history of eye trauma with vegetative materials. Sixteen patients (32.0%) gave history of fall of dust in eye. No significant history was obtained in 22 patients.

Table 2: Comparison of KOH mounts by impression smear and KOH scarping results.

KOH(impression smear	KOH scrapings			
	By impression cytology		By mechanical scraping	
	No.	%	No.	%
Positive	36	72.0	35	70.0
Negative	14	28.0	15	30.0

Out of 50 cases, 36 patents were positive for KOH smear from impression smear technique and 35 patients were positive for KOH smear by mechanical scraping.

Table 3: KOH mount negative cases by both methods.

KOH mount negative cases	No. of cases	Percentage (%)
Responding to antifungal drugs	11	84.62
Non-responding to antifungal drugs	02	15.38
Total	13	100.0

Out of those 13 cases (26%) negative for KOH mount by impression cytology and conventional mechanical scraping method, 11 cases (84.62%) responded to antifungal drugs and 2 cases (15.38%) cases showed corneal melting and were eviscerated. Out of all 14 cases, negative for impression smear 13 cases were negative by conventional scraping as well. One case reported negative by impression smear was found to be positive by mechanical scraping.

Table 4: Impression smear negative cases.

Impression smear negative cases	No.
Cases reported negative by impression smear as well as by mechanical scraping	13
Cases reported negative by impression smear but positive by mechanical scraping	01
Total	14

Out of all 15 cases, negative for mechanical scraping 13 cases were negative by impression smear as well. Two cases reported negative by mechanical scraping was found to be positive by impression smear method. All 50 cases were of fungal keratitis on clinical basis and response to antifungal drug. The sensitivity for impression smear as compared to mechanical scrapping was 100.0%. The sensitivity for mechanical scrapping as compared to impression smear was 92.11%.

Table 5: Mechanical scraping negative cases.

Mechanical scraping negative cases	No.
Cases reported negative by mechanical scraping as well as by impression smear	13
Cases reported negative by mechanical scraping but positive by impression smear	02
Total	15

DISCUSSION

Fungal keratitis is one of the most serious form of microbial keratitis because of delay in making early diagnosis and treatment. In this study, out of 50 patients 52% were between 20-50 years of age. So, it generally effect young active population as they are more prone to trauma to eye with vegetative matter.[8] As far as sex in concerned it is more common in males than female as they are more engaged in outdoor activities and hence are more prone to eye trauma with vegetative matter.

This was clearly evident from the fact that out of 50 cases 38 (76%) were males. Fungal keratitis is mostly seen in rural population as they are more prone to trauma with vegetative matter.[9] Out of total 50 cases, 42 cases (84%) patients were from rural background. In this study, out of 40 cases 28 cases (56%) had either eye trauma history of fall of dust as compare to 22 cases (44%), who showed no significant history.

In fungal keratitis there is either history of trauma to eye with vegetative matter or animal tail or fall of dust if thorough and proper history is taken.[8] Out of total 50 cases, most of the ulcer 36 cases (72%) were either medium size (2-5 mm) or larger in size (>5 mm). Since symptoms are less in fungal keratitis so by the time patient comes to ophthalmologist the size of ulcer

become large. Out of total 50 cases, 40 cases (80%) presented with hypopyon. Hypopyon is a consistent feature of corneal ulcer.[10] Satellite lesion is also a prominent feature of fungal corneal ulcer, as in this study also 68% cases showed satellite lesion.[11] In this study 13 cases of suspected fungal corneal ulcer (on clinical bases) showed negative result with both the methods. This could be inadequate sampling or deeper penetration of fungal hyphae.

Out of these 13 cases, negative by both methods, 11 responded to immunotherapy with 5% natamycin supported the above stated reason and evisceration was done in 2 cases, 5% natamycin is the main stay of treatment of fungal keratitis which is consistent with previous studies Pranja NV et al.[12]

Ten percent KOH wet mount preparation of the corneal scrapings is a simple and sensitive method for diagnosis. In a study by Arora et al, impression debridement of corneal lesions has been done for various types of corneal ulcers, removal of superficial foreign bodies, filaments of filamentary keratitis and superficial infiltration around cataract-related exposed corneoscleral sutures.[13,26] 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 culture- proved cases and 93.6% (160 of 171) of the overall eyes. 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-stains.[14] The smears established the fungal cause in 95.4% (1277 out of 1354) eyes. Sharma et al, in their series of 144 patients of early keratitis and 363 cases of late keratitis, found that in early keratitis, KOH with CFW stain had a sensitivity of 61.1% and a specificity of 99.0%.[15] The KOH with CFW stain in advanced keratitis had sensitivity of 87.1% and a specificity of 83.7%.

These studies clearly establish the superiority of the KOH smear of direct corneal scrapings over culture as the gold standard for the diagnosis of fungal keratitis though culture is required for species identification. Sharma et al have recommended resorting to 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 clement is unlikely to be misinterpreted.[15]

In present study, an attempt has been made to establish the use of impression technique in the diagnosis of fungal keratitis. It is safe, simple, repeatable and non-traumatic procedure It gives quick results and does not require expensive equipment like a slit-lamp or an operating microscope. It is helpful in obtaining specimen materials from small lesion, which is otherwise difficult by the conventional techniques of mechanical scraping. With mechanical scraping the infected cells as well as some surrounding normal cells are inadvertently removed and there is risk of corneal perforation, especially in impending perforations. There is risk of injury to the basement membrane, bowman's layer and superficial stroma in superficial ulcers and risk of perforation.

The impression smear technique relies on cellular adherence to the filter paper rather than mechanical abrasion of the sub epithelial areas. Therefore, there is little chance of damage to these structures or to the adjacent epithelium. When the filter is applied and peeled off from the normal cornea, the cohesive forces of epithelial cells to the underlying structures counteracts the adhesive force to the filter paper, which results in removing only the most superficial epithelial layers. In contrast, when the filter paper is applied to the corneal lesion, only the affected cells are removed. The cohesive force of degenerated cells to the underlying structures is reduced. It has a valuable place as an adjunct therapy because it removes some lesions, removes necrotic material, inflammatory cells, organisms and degenerating epithelium from most corneal ulcers removes necrotic material, inflammatory cells, organisms and degenerating epithelium form most corneal ulcers. For convention all techniques greater magnification is needed to stay within the confines of the lesion and to avoid areas of imminent perforation. The material obtained may not spread well on slides and cells may get distorted and crushed with loss of spatial relation. With impression smear technique all of these disadvantages are overcome, especially if the sampling is done in community practice setting or in cases of fungal keratitis in the hands of an ophthalmologist as it maximally reduces fungal load and enhances drug penetration.

CONCLUSION

Impression cytology is a recent diagnostic tool in fungal keratitis. It has various advantages over mechanical scraping like no expensive equipment like slit lamp and operation microscope required. Impression smear KOH mount is comparative to conventional mechanical corneal scraping KOH examination in making the tentative diagnosis of fungal keratitis and can be accurately replied upon for initiation antifungal therapy.

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