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Evaluation of Impression Smear Technique in Microbiological Diagnosis of Infectious Microbial Keratitis

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Abstract

Introduction: Infectious keratitis is one of the leading cause of preventable blindness, proper and early diagnosis of infectious keratitis paves way for treatment and prevention of ocular morbidity. from corneal scrapings is considered to be the Gold standard in microbiological diagnosis of keratitis. Smear from impression technique can be used for laboratory diagnosis of keratitis. *Purpose*: This study was under taken to know the sensitivity and specificity of impression smear technique in diagnosis of infectious keratitis. *Materials and Methods*: Study was conducted at tertiary hospital, after taking all enrolled patients with infectious keratitis underwent clinical examination followed by smear examination by impression technique on filter paper and corneal scraping sent to lab for gram staining and KOH mount. *Results*: Out of 50 cases Male: Female ratio was 3: 1.1. Age group was 30-70. Grams stain was positive in 70% patients with impression smear technique and in 64% patients with corneal scrapings. Sensitivity and Specificity of impression smear technique gram staining was 96.8% and 77.77% , positive and negative predictive value were 88.50% and 93.33%, accuracy of test was 90.0%. KOH mount was positive in 20% patients with impression smear technique and in 22% of patients with corneal scrapings. Sensitivity and Specificity of impression smear technique for KOH mount was 81.82% and 97.4%, positive an negative predictive value was 90.0% and 95.00%, accuracy of test was 92%. Specific therapy was started based on initial smear examination. *Conclusion*: Impression smear technique is safe simple and atraumatic procedure in lab diagnosis of infectious keratitis, with good sensitivity and specificity, it is also helpful in diagnosis of smaller lesions.

Keywords: Corneal Ulcer; Keratitis; Corneal Scraping.

Introduction

Microbial keratitis continues to be a leading cause of ocular morbidity and blindness worldwide, more so in developing countries like India [1]. Bacterial and fungal corneal ulcer occur frequently and in equal frequency but regional variations of corneal pathogens may be seen [2]. The occurrence of corneal ulceration is significantly associated with lower socioeconomic status [3]. Thus, patients with microbial keratitis are commonly managed by community ophthalmologists who do not conduct microbiological investigations [1]. It is recommended that all suspected microbial keratitis be scraped for smear and cultures before initiating antibiotic treatment [4,5]. In tropical countries like India, fungal infection amounts to high of all cases of central corneal ulceration [1]. Hence, starting empirical antibacterial treatment in all cases could be dangerous The utility of smear examination results and their impact on therapeutic decisions have already been established in the literature [5]. Impression cytology was first introduced in ophthalmology by Egbert et al. who reported the

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possibility of removing the cells from the surface of the epithelium by pressing a cellulose acetate filter paper on the bulbar conjunctiva [6]. Sensitivity and specificity of grams stain is less as compared to KOH preparation in corneal scrapings [7]. KOH smear has got more diagnostic value as compared to infective keratitis and can be utilised in clinics [8].

Instead of smear from scrapings impression smear can be used as it is safe alternative. Impression cytology is a non or minimally invasive biopsy technique which samples the superficial layers of the conjunctival and corneal epithelium IC has become a useful research tool in both basic and clinical aspects for sampling ocular surface epithelium .Impression smear technique has been used in the aetiological diagnosis of various ocular surface disorders .utility of impression smear for the detection of micro-organism invasion of ocular surface is also seen. Arora et al used impression technique for debridement and found that impression debridement improves the quality and efficacy of corneal ulcer debridement without harming the surrounding tissue [9].

Aim

To evaluate the accuracy of impression smear technique in microbiological diagnosis of infectious keratitis and compare it with conventional scrapping method

Objectives

- To evaluate the accuracy of impression smear technique in microbiological diagnosis of infectious keratitis
- To compare impression smear technique with conventional scrapping method in microbiological diagnosis of infectious keratitis

Materials and Methods

- *Type of study*: Prospective Non Randomized comparative study
- *Duration of study* :December 2015- May 2016
- *Sample size*: 50

Inclusion Criteria

- Clinically suspected cases of microbial keratitis attending ophthalmology OPD at KIMS, Narketpally

Exclusion Criteria

- Patients clinically suspected as viral keratitis
- Patients clinically suspected as non infectious keratitis

Patients under anti fungal or anti bacterial treatment

Procedure

Informed consent was taken from all patients who were willing to participate in the study, and were included in the study. Detailed history was taken for all cases. All cases were subjected to clinical examination including, slit lamp biomicroscopic examination. Xylocaine (4%) eye drops were instilled in the conjunctival cul de sac. A 3 x 5 mm precut and autoclaved strip of cellulose acetate filter paper (GSWP 02500 Millipore, 0.22 mì pore size) was applied over the lesion. The filter paper strip was gently pressed for five seconds with a blunt end of a glass rod, filter paper was gently peeled off using blunt forceps and immediately transferred on the surface of an autoclaved glass slide, leaving the impression on the slide. slides were stained with 10% KOH and Gram stain and the impression was observed under a standard light microscope . After taking impression Corneal scrapings were taken from the base and edge of the ulcers aseptically, with a sterile Bard-Parker blade (No 15), using local anesthetic solution (4% xylocaine) and were seen under direct microscopy with 10% KOH and Gram Stain. Corneal scraping was taken as golden standard for diagnosis of microbial keratitis, True Positives, True Negatives, False Positives and False Negatives were identified and statistical analysis of the test done with MedCalc Version 17.5.3 software.

Results

50 cases of suspected microbial infectious keratitis were enrolled in the study. Most common age group was 30-70 years with male to female ratio of 3.1:1. Grams stain was positive in 35 (70%) patients with impression smear technique and in 32 (64%) patients with corneal scrapings. sensitivity and specificity of impression smear technique in comparison to corneal scraping in gram staining was 96.8% and 77.77%, Positive Predictive Value was 88.50% and Negative Predictive Value was 93.33%, Accuracy of the test was 90.0% , Disease Prevalence was 64.0%. KOH mount was positive in 10(20%) patients with impression smear technique and in 11 (22%) patients

with corneal scrapings. Sensitivity and Specificity of impression smear technique in comparison to corneal scraping in KOH mount was 81.82% and 97.4%, positive predictive value was 90.0% and negative predictive value was 95.00%, accuracy of the test was 92%, disease prevalence was 22.0%. 5 Patients were negative for KOH and Gram Stain in both corneal scrapings and impression smears. Specific therapy was started based on initial smear examination.

Table 1: Age distribution of the study population(n=50)

Age	No. n=50
10-30	5(10%)
31-50	22(44%)
52-70	20(40%)
>71	3(6%)

Table 2: Sex distribution in the study population(n=50)

Sex	n =50	%
Male	38	76%
Female	12	24%

Table 3: Comparison of Impression smears and conventional scraping smears stained in KOH mount (n=50)

KOH Mount	Positive	Negative	Total
Corneal Scrapings (Gold standard)	11	39	50
Impression Smear	10	40	50

KOH Mount (Impression smear)	KOH mount (corneal scrapings) n=50 Positive	Negative	Total n=50
Positive	09 (TP)	1(FP)	10 (Test Positive)
Negative	02(FN)	38(TN)	40(Test Negative)
	11 (Total Disease -TD)	39 (Total Non Disease)	50 (T)

Disease Prevalence (TD/T)X100 = 22.00%(95% CI) 11.53% to 35.96%
Sensitivity (TP) /(TP+FN) X 100 = 81.82%(95%CI - 48.22% to 97.72%)
Specificity (TN/FP+TN)X 100 = 97.4% (95%CI - 86.52% to 99.94%)
Positive Predictive Value (TP/TP+FP) X 100 = 90.0% (95%CI - 56.03% to 98.45%)
Negative Predictive Value 94.87% (TN/TN+FN)X100 = 95.00%(95%CI- 84.42% to 98.52%)
Positive likely hood ratio = sensitivity /100- specificity = 31.91(95% CI - 4.52 to 225.33)
Negative likely hood ratio = 100- sensitivity/specificity =0 .19 (95% CI 0.05 to 0.65)
Accuracy = (TP+TN)/(TP +TN +FP +FN)X100 = 92%

Table 4: Comparison of Impression smears and Conventional scraping smears stained with Gram stain (n=50)

Gram Stain	Positive	Negative	Total n=50
Corneal Scrapings (Gold standard)	32	18	50
Impression Smear	35	15	50

Gram Stain (Impression Smear)	Gram stain (corneal scrapings) Positive	Negative	Total
Positive	31(TP)	4(FP)	35 (Test Positive)
Negative	1(FN)	14(TN)	15 (Test Negative)
	32 (Total Disease)	18 (Total Non Disease)	50

Disease Prevalence (TD/T)X100 = 64% (95% CI) 49.19% to 77.08%
Sensitivity (TP) /(TP+FN) X 100 = 96.8 (95% CI- 83.78% to 99.92%)
Specificity (TN/FP+TN) X 100 = 77.77 (95% CI) 52.36% to 93.59%
Positive Predictive Value (TP/TP+FP) X 100 = 88.5 (95% CI) 76.52% to 94.85%
Negative Predictive Value (TN/TN+FN) X 100 = 93.33 (95% CI) 66.69% to 98.99%
Accuracy (TP+TN)/ (TP +TN +FP +FN) X 100 = 90.0% (95% CI)
Positive Likely Hood Ratio = Sensitivity /100- Specificity = 4.36 (95% CI) 1.83 to 10.37)
Negative Likely Hood Ratio = 100- Sensitivity/ Specificity = 0 .04 (95% CI) 0.01 to 0.28)

Discussion

Present study was under taken to compare the impression smear technique with corneal scrapings. KOH wet mount, Gram stain and Giemsa stain are widely used for rapid detection of microbes [9], Smear examination in early stages helps in starting empirical therapy in corneal ulcers which has got greater impact on the outcome of the disease [10]. Mc Donell et al. reported that only half of all corneal ulcers seen by community ophthalmologists in southern California were sent for microbiological analysis. A Survey of 30 yrs of laboratory experience concluded that the use of Gram Stain and culture in combination seems to yield the highest percentage of bacterial recovery [12]. Sharma S and Gopinathan et al [3] in their series of fungal keratitis, have reported the diagnostic utility of smears of corneal scrapings using KOH preparation, Calcofluor White (CFW), Gram and Giemsa-stains. KOH preparation alone revealed fungal cause in 91.0%. Sharma et al [7] in their series of 114 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%. These studies clearly establish the superiority of KOH smear of direct corneal scrapings over culture as gold standard in diagnosis of fungal keratitis. Nitin Goel and BL chowdhary have highlighted the sensitivity of KOH examination as a simple and rapid means of making a tentative diagnosis of fungal keratitis [11]. In the light of above studies, impression smear with KOH preparation were compared with corneal scrapings In the present study Keeping KOH positivity from mechanical corneal scraping as the gold standard, the sensitivity of impression smear technique was 81.8%, the specificity was 97.94%, Positive predictive value was 90.0%, Negative predictive value was 95.0% and the accuracy of the technique was 92% Which was similar to study by Arun K Jain et al in 2007 reported sensitivity of 97.14% and specificity of 92.86% in evaluation of impression smear technique in fungal keratitis [13].

In present study Keeping Gram Stain positivity from mechanical corneal scraping as the gold standard, the sensitivity of impression smear technique was 96.8%, the specificity was 77.7% with Positive predictive value 88.5% and Negative predictive value 93.3% and the accuracy of the technique was 90.0%. Sharma et al [7] in his study reported sensitivity of Gram Stain is better but less as compared to results of KOH mount in diagnosing microbial keratitis which is similar to studies by Levey et al[11] and Mc Leod et al [14].

Conclusion

In the present study, an attempt has been made to establish the use of impression cytology in the diagnosis of microbial keratitis. It is safe, simple and atraumatic procedure. It gives quick results and does not require expensive equipments. It is helpful in obtaining specimen from small lesions which is otherwise difficult by conventional scraping method .By the above study, we conclude that impression smear and mechanical scraping are equally sensitive and specific in making tentative diagnosis of microbial keratitis. However, further studies are needed to substantiate the safety of this newer procedure.

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