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A Study To Find Out Diagnostic Accuracy of Calcoflour White Stain Among Patients (Mycotic Keratitis) Attending Ophthalmology Department of SMS Hospital Jaipur

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Background: Fungal keratitis is a suppurative, ulcerative and sight threatening infection of the cornea that some time leads to loss of the eye. Corneal infection of fungal etiology is very common.

Methods: The present study has been carried out on 100 patients of confirmed keratitis or suspected keratitis in the department of Microbiology and department of Ophthalmology, SMS Hospital, Jaipur (Rajasthan) from January 2015 to December 2016.

Results: In this study, positive fungal culture was taken as the gold standard showing a sensitivity of KOH+CFW for diagnosing mycotic keratitis was 69.23% and specificity was 96.82%.The PPV for CFW was 85.18% and NPV was 78.08%.The sensitivity of KOH was 58.97% and specificity was 93.44%.PPV for KOH was 93.1% and NPV was 78.08%.

Conclusion: Calcofluor white staining was found to be more sensitive as compared to KOH wet mount smear in demonstrating fungi in direct microscopy. In addition, Calcofluor white stain decreased the chances of false negative results. Calcofluor white stain is highly sensitive method for detection of fungi in mycotic keratitis and should be performed routinely before declaring the corneal scrapings negative for fungi.

Keywords: Mycotic keratitis,Calcofluor white stain,KOH.

Introduction

Ophthalmic infections of fungal origin have a worldwide distribution and its incidence is higher in tropical and subtropical countries[1]; particularly among agricultural workers in rural areas; following traumatic implantation of fungal spores from soil or plant matter into the corneal stroma.[2]

Fungal keratitis is a suppurative, ulcerative and sight threatening infection of the cornea that some time leads to loss of the eye. Corneal infection of fungal etiology is very common.[3] Mycotic keratitis presents as an important ophthalmic problem causing visual disability due to its protracted course and unfavourable responses. Corneal ulcer is a sight threatening disorder presenting in all age groups and either sex worldwide. It may resolve without any sequelae, progress to perforation and its resultant consequences, or leave behind an opacity which if central may lead to loss of vision.

Fungal infection of the cornea (keratomycosis, Mycotic keratitis or fungal keratitis) was described for the first time by Leber in Germany in the year 1879.[4] Since then it has been recognized as a major public health problem in the tropical parts of many developing nations including India.[5,6] Corneal infection of fungal etiology may represent 40-50% of all cases of culture proven infectious

keratitis.[7.] If not treated early, this condition may lead to corneal blindness.

The purpose of our study was to analyze the prevalence of specific fungal pathogens responsible for mycotic keratitis in this part of the country and to evaluate the efficacy of Calcofluor white stain for the diagnosis of fungal keratitis.

Material And Methods

The present study has been carried out on 100 patients of confirmed keratitis or suspected keratitis in the department of Microbiology and department of Ophthalmology, SMS Hospital, Jaipur (Rajasthan) from January 2015 to December 2016.

Patients which were attending ophthalmology OPD, after obtaining their informed and written consent and filling of Performa any eligible patients fulfilling inclusion criteria; patient willing to participate in study and with corneal ulcer eligible for providing corneal scraping in opinion of ophthalmologist were subjected for detailed socio-demographic & clinical history.

A sample of corneal scraping was taken by expert ophthalmologist and were submitted to the laboratory for microbiological investigations. The patients age, gender, occupation, history of any predisposing factor like trauma, past and current use of topical medicines or use of contact lens were meticulously recorded.

Corneal scrap were examined in 10% KOH wet preparation for fungal elements. scrap were also stained by caleoflour white stain with 10% KOH , two SDA agar slants were also inoculated with corneal scrapings to isolate fungi.

Results

Table 1 Total cases of fungal keratitis from OPD patients.

S.No.	Samples	No.	%
1.	Total corneal scraping samples collected for examination	100	
2.	Total no. of fungal culture positive cases	39	39%
3.	Total no. of KOH positive cases	25	25%
4.	Total no.of KOH+CFW positive cases	29	29%

Out of the 100 corneal scraping samples that were received during study period,39(39%) samples were fungal culture positive.Among the positive culture samples,25(25%) were KOH positive and 29(29%) were KOH+CFW positive.

Table 2 Direct demonstration of organisms.

Method	**Positive**	**Percentage**	**Negative**	**Percentage**
KOH	25	25	75	75
KOH+CFW	29	29	71	71

In this study,25(25%) corneal samples were positive after KOH examination and 29(29%) samples were positive after doing combined KOH and Calcofluor White staining.75(75%)samples were negative with KOH examination and 71(71%)samples were negative after KOH and Calcofluor White staining.

Table 3 Correlation of KOH and CFW with culture.

Method	Direct microscopy Positive Culture Positive	Direct microscopy Positive Culture negative	Direct microscopy negative Culture Positive	Direct microscopy negative Culture Negative	Total
KOH	21	4	17	58	100
CFW	27	2	10	61	100

Table 4 Comparison of sensitivity, specificity, positive predictive value and negative predictive value.

Method	Sensitivity	Specificity	PPV	NPV
KOH	58.97%	93.44%	85.18%	78.08%
KOH+ CFW	69.23%	96.82%	93.1%	83.56%

In this study, positive fungal culture was taken as the gold standard showed the sensitivity of KOH+CFW for diagnosing mycotic keratitis was 69.23% and specificity was 96.82%.The PPV for CFW was 85.18% and NPV was 78.08%.The sensitivity of KOH was 58.97% and specificity was 93.44%.PPV for KOH was 93.1% and NPV was 78.08%.

Discussion

The list of fungi isolated from patients of mycotic keratitis is long and varied. There is considerable fungal flora in normal eye and even greater in the diseased eye.[8]The patients in whom infection is substantiated by demonstration of fungal elements in the debrided materials or culture, organisms belonging to at least 30 genera have been isolated. Essentially, all of them are saprophytic fungi and are usually not associated with true infection in man. [9] Fungi are opportunists and hence it is not surprising to find colonization when natural defenses of the eye are abrogated by corneal trauma and use of topical corticosteroids.[10]Almost any bacterial/fungal species can infect the cornea if the integrity of the natural anatomic barriers or defense mechanisms is compromised.[11]Mycotic keratitis continues to be an important cause of ocular morbidity despite advances in the ocular treatment strategies.[12] As it leads to corneal blindness and sometimes loss of vision. Its incidence is reported to vary from 7%-40% in various parts of India.[13] Early and rapid identification of the pathological organism is the key to ensuring successful medical therapy for corneal infections and is particularly true for fungal keratitis. Thus, direct microscopic evaluation of corneal scrapings from patients with suspected fungal keratitis is of the utmost importance because early and rapid detection of fungal structures permits early initiation of antifungal therapy.

In the present study we identified the magnitude of fungal corneal ulcer and efficacy of Calcofluor White Stain from the corneal scraping samples received from patients attending ophthalmology OPD of a SMS hospital. Our study patients were subjected to complete ophthalmological examination and corneal scrapping was done for all suspected fungal keratitis. Part of the Corneal scrapping sample was subjected to KOH mount while remaining sample was used for fungal culture.

In our study the magnitude of fungal keratitis was 39% from OPD patients which were almost similar to study of Usha Gopinathan et al[14] and Verenkar et al[15] of 38.2% and 38.9% respectively.

The sensitivity of KOH used in the present study for diagnosing mycotic keratitis was 53.84% and is comparable to the studies by Tahereh Shokohi et al[16] and Renu Sahay et al[17].Calcoflour white was found to have a

better sensitivity as compared to KOH and similar findings have been reported by Savithri Sharma et al[18],Tahereh Shokohi et al[16],Zhang et al[19],Renu Sahay et al[20] and Usha Gopinathan et al[14].Specificity of KOH and CFW were 93.44% and 96.72% respectively.

Chander et al[21] in their study stated that CFW+KOH on direct microscopy detected fungi in 20(95.2%) patients in comparison with 15 (71.4%) patients by both KOH wet mount and culture. They confirmed the superiority of KOH+CFW in comparison of with KOH alone and culture. Renu Sahay et al[17] also confirmed the superiority of KOH+CFW stain in detection of fungal elements in corneal scraping. It is not only highly specific and sensitive but also has a high NPV and PPV for both early and advanced keratitis. Since they had seen some false positive results in advanced keratitis, they recommended resorting to antifungal therapy whenever a KOH+CFW stain smear is positive for fungus. Shokohi et al[16] in their study revealed a sensitivity of 71.4% for KOH and 42.9% for Gram stain. They concluded that KOH with CFW as the single most important screening test for rapid diagnosis of fungal corneal ulcer. Zhang et al[19] found out sensitivity of KOH+CFW was 96.6%. This combined staining method, enhanced the rate of correct diagnosis[21]

Conclusion

Fungal corneal ulcer continues to be an important cause of corneal morbidity. The key element in the diagnosis of mycotic keratitis is the clinical suspicion by the ophthalmologists and laboratory confirmation of the fungus before prescribing corticosteroids and antibacterial antibiotics. Therefore, precise identification of the causative fungus and institution of appropriate treatment strategy could save the eye from this preventable cause of blindness.

Calcofluor white staining was found to be more sensitive as compared to KOH wet mount smear in demonstrating fungi in direct microscopy. In addition, Calcofluor white stain decreased the chances of false negative results. Calcofluor white stain is highly sensitive method for detection of fungi in mycotic keratitis and should be performed routinely before declaring the corneal scrapings negative for fungi.

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