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ORIGINAL RESEARCH PAPER

Medical Microbiology

nalo **POSSIBILITY OF PREDICTING COVID-19 KEY WORDS:** Fungal ASSOCIATED FUNGAL INFECTION USING SWAB infection, Mucormycosis, Covid-19 complication. AND TISSUE. Dr. Manoj Assistant Professor, Dept. of Microbiology, Dr. V.M. Govt. Medical College, Vedpathak Solapur. Junior Resident, Dept. of Microbiology, Dr. V.M. Govt. Medical College, **Dr. Snehal Patil*** Solapur.*Corresponding Author Associate Professor, Dept. of Microbiology, Dr. V.M. Govt. Medical College, Dr. Nasira Shaikh Solapur. Service Resident, Dept. of Microbiology, Dr. V.M. Govt. Medical College, **Dr. Atif Patel** Solapur. During COVID-19 pandemic emergence of potentially lethal and COVID-19 associated invasive fungal infections highlights the need for the rapid diagnosis, to maximize the survival rate by early interventions. 10% Potassium hydroxide (KOH) wet mount is used for demonstration of fungal elements and it is not only useful for rapid diagnosis but also helps clinician to introduce appropriate antifungal drugs as early as possible. But adequate and suitable specimen with early transportation to the laboratory is mandatory. Hence, we aim to evaluate the possibility of predicting fungal ABSTRACT infections among COVID-19 patients by using swab and tissue for KOH. A retrospective study was conducted between May 2021 to September 2021 from Mycology laboratory, Department of Microbiology, Dr.V.M. Govt. Medical College, Solapur. Swab and/or tissue samples received in laboratory were subjected to 10% KOH mount to observe the fungal element and cultured on SDA slant for fungal growth. Samples from 359 patients were analyzed. Of these, either swab and/or tissue was received from 359 patients and found that the possibility of predicting fungal elements by 10% KOH mount differs significantly (p value < 0.01) between these

two kinds of samples. Both types of samples (tissue and swabs) were received from 50 patients and compared with culture which is considered as a gold standard and observed that tissue (91.66 %) had greater positive predictive value than swab (40 %). Hence observing fungal elements in tissue samples was better correlated than swabs in suspected fungal infections.

INTRODUCTION

The health scenario from the last couple of years (Since December 2019) has been dominated globally by a novel strain of severe acute respiratory syndrome corona virus-2 SARS-CoV-2, commonly known as COVID-19. [1] As the consequences of this, there was notable rise in the incidence of invasive fungal infections (IFIs). [1] Despite of having potentially lethal angioinvasive nature, mucormycosis remains unrecognized while Pulmonary aspergillosis (CAPA) received attention.[2,3] Also emerging reports from India highlight the importance of early and rapid diagnosis of mucormycosis to maximize survival rate by urgent intervention.[3,4,5]

Rapid diagnosis by 10% Potassium hydroxide (KOH) wet mount is very useful method which demonstrate the fungal elements.[6] But it is essential to collect adequate and suitable specimen with early transportation to the laboratory.[7]

Theoretically there should be advantages of tissue sample over swab sample.[8,9] But statistically possibility of predicting fungal elements in a tissue compared to swab was not found to be documented in literatures. Hence, we aim to evaluate the possibility of predicting fungal infections among COVID-19 patients by using swab and tissue for KOH.

METHODOLOGY

A retrospective analysis was conducted from May 2021 to September 2021 from Mycology laboratory, Department of Microbiology, Dr. V.M. GMC, Solapur. Samples from 359 patients were received of which either tissue or swab was received from 309 patients and both samples from same site were received from remaining 50 patients. In this study only those patients samples were included who had active COVID-19 infection or recovered and clinically suspected to have fungal infection. These samples were subjected to 10% KOH mount to observe for the fungal elements. Each sample was cultured on two Sabouraud dextrose agar (SDA) slant of which one was kept at 25°C and other at 37°C. These SDA slants were observed for fungal growth up to 3 weeks.

Considering culture as a gold standard we were evaluate the possibility of predicting fungal infections using swab and tissue by 10% KOH statistically.

RESULTS

Total 409 samples either tissue, swab or both were received from 359 patients and subjected to 10% KOH mount. Results of which are shown in Table No. 1 and same presented graphically (Fig. 1). Results of tissue samples when compared with swab samples shows percent positivity of tissue (48.39%) on 10% KOH mount is significantly (P value <0.01) more than swab (10.53%).

Table – 1Results of Tissue And Swab On 10% KOH

Direct 10% KOH result			S	Swab		ue	Total	Ζs	core =
Positive				30)	90	8.49. P value	
Negative				255		ł	319		
Total			285 124		409	<0.01			
10	0.00%				89.479	%			
8	0.00%	78	3.009	.00%					
6	0.00%					48	51.61 39%	%	
4	0.00%								
2	0.00%	22.00%		10.539	6				
	0.00%								
		Tot	al	Sv	vabs		Tissue		
	Positive Negative								

Figure 1: Results of Tissue And Swab On 10% KOH

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While analyzing data, it was found that both swab and tissue samples from same site and on same day were received from 50 patients. When findings of 10% KOH and growth on SDA slant of these 50 swabs (Table No. 2) and 50 tissues (Table No. 3) were evaluated, it shows sensitivity and PPV of tissue (91.66% and 91.66% respectively) are more than swab (66.66% and 40% respectively).

Table - 2 Results Of Swabs On 10% KOH Against Culture

Result (n=50)		Culture				
		Positive	Negative	Total		
10%	Positive	02	03	05		
KOH	Negative	01	44	45		
	Total	03	47	50		
Sensitivity = 66.66%; Specificity = 93.61%						
Positive predictive value (PPV) = 40%						

Table – 3 Results of Tissues On 10% KOH Against Culture

Result (n=50	Culture						
		Positive	Negative	Total			
10% KOH	Positive	22	02	24			
	Negative	02	24	26			
	Total	24	26	50			
Sensitivity = 91.66%; Specificity = 92.30%							
Positive predictive value (PPV) = 91.66%							

DISCUSSION

Opportunistic IFIs remain an important cause of morbidity and mortality.[10] In the backdrop of this COVID-19 expression, rise in cases of opportunistic IFIs particularly Mucorales may be due to the COVID related hypoxia, preexisting diabetes, steroid therapy, increased serum ferritin or decreased phagocytic activity of white blood cells (WBCs).[1,7]

These fungi commonly present in an environment such as in the soil and in decaying organic matter, hence being considered as common laboratory contaminants. [11,12] But their isolation from clinical specimen of susceptible hosts should be regarded as potentially significant.[12] Among these opportunistic IFIs, mucormycosis gain attention due to its invasive and rapidly progressive nature across tissue which may be associated with fatal outcome.[5,13] Hence early diagnosis is crucial for timely implementation of antifungal therapy and decreasing the unnecessary use of toxic antifungal agents.[10]

Standard approaches to the laboratory diagnosis of IFIs include direct microscopic examination in freshly obtained samples, histopathological demonstration of fungi within tissue sections, culture of the causative fungus and its further identification. However time required for direct microscopic examination is less compared to other methods, so rapid and tentative diagnosis can be made before the growth of fungus in culture.[10]

Direct KOH mount, one of the oldest and principle methods is an easy, rapid and economical point of care test requiring minimal assistance for demonstration of fungal elements.[8,14] But to establish rapid diagnosis of suspected fungal infection it is essential to provide appropriate specimen to the laboratory.[8] It was advised that whenever possible, material for examination must be collected from most heavily infected area or active growing edge of the infection.[15] KOH helps to clear the tissue and cellular debris from clinical specimen without damaging fungal cells. So detecting the presence of yeast forms or hyphal form in the specimen becomes easy.[9]

In our study out of 409 samples, 124 tissue samples and 285 swab samples were subjected for direct microscopy by KOH mount. It was found that 48.39% of tissue samples showed fungal elements compared to 10.53% of swab samples. This difference in predicting fungal element was statistically significant (Z score=8.49; p value < 0.01). Roy P. et al.[12] also showed 45% biopsy tissue positivity but not studied swab samples.

We also analyzed and compare samples of 50 patients from which both tissue(n=50) and swabs(n=50) were received from same site. Considering culture as a gold standard, the sensitivity of tissue was 91.66% and positive predictive value (PPV) was 91.66% compared to 66.66% sensitivity and 40% PPV of swab. Similarly, 90.63% sensitivity of tissue in direct KOH mount was observed by Roy P. et al. (2017) [12]. Negative results on culture instead of being observed in KOH may be because either the filamentous fungi particularly zygomycetes are fragile and improper handling of tissue leads to disruption of hyphae or samples were collected after administration of antifungal agents.[16,17] But observing fungal cells in clinical specimen may be more valuable criterion for diagnosis than isolating in a culture. Also when microscopy is combined with culture it will lead to increase the diagnostic yield by 15-20%.[12]

Meyer et.al. (1979)[15] documented that the diagnosis of mucormycosis is best established by direct examination and culture of infected tissue but tissue exudates, nasal discharges and swabs were disappointing. Similarly Procop G.W. et.al.[16] mentioned that the use of swabs is inappropriate for the detection of infections caused by filamentous fungi due to inadequate sampling and an inability to disassociate the hyphal elements from the human tissues in which they are invading.

After extensive literature search study comparing tissue sample against swab sample was not found for fungal diagnosis. But similar studies comparing tissue against swab for bacterial identification in diabetic foot and burn patients by Huang Ying et.al.(2016)[18] and Sjöberg T. et.al.[8] respectively concluded that tissue biopsy sample was better correlated for identifying infection than swab.

Hence it is important to collect proper clinical specimen to improve the diagnosis. However, whatever method is used ability to find the fungi on direct examination of samples is largely a matter of practice.[15]

CONCLUSION

Observing fungal elements in tissue samples was better correlated with clinical scenario. Hence, whenever possible tissue biopsy sample should be preferred.

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