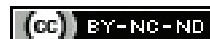


Sensitivity of KOH Mount in the Early Diagnosis of Fungal Infections of Nose and Paranasal Sinuses in COVID-19 Infected Patients Suspected with Mucormycosis

KS GANGADHARA¹, M NAGARAJ², P NANDINI³, SHUBHAM MALAWADI⁴

ABSTRACT

Introduction: Mucormycosis represents a group of life threatening infections caused by fungi of the order Mucorales especially in Coronavirus Disease-2019 (COVID-19). It is a highly invasive and rapidly progressive disease resulting in high grades of morbidity and increased mortality.

Aim: To study the sensitivity of potassium hydroxide (KOH) mount in the early diagnosis of mucormycosis in COVID-19 infected patients.

Materials and Methods: A prospective, longitudinal (single group) study was done in McGANN Teaching District Hospital, Shimoga Institute of Medical Sciences, Shivamogga, Karnataka, India, among the patients with high index of clinical suspicion of mucormycosis from May to August 2021. Specimen from the nasal cavity or the suspected lesion on hard palate, gums, skin was sent for KOH mount and fungal culture and also biopsy of the nasal mucosa sent for histopathological examination. The sample was sent both preoperatively and intraoperatively. The positive KOH report and its role in early diagnosis and management of mucormycosis suspected cases were assessed.

Results: Out of 26 patients, 6 (23.1%) were females and 20 (76.9%) were males. COVID-19 positive suspected mucormycosis were

5 (19.2%) and post COVID-19 suspected mucormycosis were 21 (80.8%). Species isolated after processing the sample were *Rhizopus* in 10 (38.5%), *Candida* in 1 (3.8%), *Aspergillus* in 1 (3.8%); while in 14 (53.8%) no organisms were isolated. Out of 26 patients, KOH positive sample on the day of admission confirming with positive intraoperative histopathological examination were 10 (41.7%). Admission day fungal culture positivity was seen in 12 (46.2%) and negativity in 14 (53.8%) cases. Intraoperative fungal culture was positive in 24 (92.3%) cases. There was 100% specificity for both fungal culture and KOH mount and sensitivity for KOH was 41.7% and 50% for fungal culture. False negativity rate for KOH was 58.3% and for fungal culture was 50%.

Conclusion: The results of KOH mount can be obtained within an hour of sending nasal scrapping samples. Hence, it helps in making the early diagnosis and early initiation of treatment for suspected cases. But in the current study, even in patients presenting with high clinical suspicion of mucormycosis, the sensitivity of KOH mount on the day of admission to the hospital was 41.7%. In view of severe morbidity and mortality of mucormycosis, this sensitivity though less is of paramount importance in these patients because treatment can be started early.

Keywords: Coronavirus disease-2019, Fungal culture, Microscopy, Potassium hydroxide, Sensitivity

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) remains the main culprit in causing Coronavirus Disease-2019 (COVID-19). It has been known to be associated with broad range of bacterial and fungal opportunistic infections. The main organisms responsible for co-infection in people with COVID-19 are both *Aspergillus* and *Candida* species and increasing number of mucormycosis cases have been reported worldwide, especially from India [1]. Mucormycosis is previously rare but now is a serious fungal infection caused by a group of molds called mucormycetes. Several risk factors for mucorales spores to germinate in COVID-19 patients are hypoxia, hyperglycemia, acidic medium (metabolic acidosis, diabetic ketoacidosis), immunocompromised status leading to decreased phagocytic activity of white blood cells and longer duration of hospital stay with or without mechanical ventilators [1].

Increased incidence of COVID-19 is due to its inherent angioinvasive nature. Corticosteroids with microangiopathy of diabetes and possible peripheral microthrombi in COVID-19 acts as favourable host factors for mucormycosis due to their immunocompromising effects [2]. As far as the number of diabetics is concerned, India stands at the second position in the world. The prevalence before the pandemic has been reported to be 0.005-1.7 per million population worldwide.

The prevalence in India is nearly 80 times more than in other parts of the world i.e., 0.14 per 1000 which is always been much higher. The incidence of secondary bacterial or fungal infection is 8% with COVID-19 and aspergillosis and *Candida* being the most common fungal organisms reported [3].

The recent surge of COVID-19 Associated Mucormycosis (CAM) in India is alarming due to its high mortality, the sheer number of cases and limited availability of antifungal drugs. India has reported more than 47,000 cases of mucormycosis in three months (May to July 2021) [4]. Detecting the sensitivity of KOH mount is of utmost importance as early diagnosis helps in initiating the appropriate treatment with specific antifungal drugs and thus saves precious time which could help in preventing the fatal outcome in nose and paranasal sinus fungal disease in COVID-19 patients. The aim of this study was to detect the sensitivity of KOH mount in patients suspected to have mucormycosis.

MATERIALS AND METHODS

This was a prospective, longitudinal (single group) study conducted in the Department of Otorhinolaryngology, McGANN Teaching District Hospital, Shimoga Institute of Medical Sciences, Shivamogga, Karnataka, India, from May to August 2021 on COVID-19 positive patients. Total 26 cases who presented to the department were

included in the study after obtaining informed consent from them. The Institutional Ethical Committee had approved the study (SIMS/IEC/594/2021-22 dated 30/06/2021).

Inclusion criteria: All COVID-19 positive patients with high index of clinical suspicion of mucormycosis of nose and paranasal sinuses were included in the study.

Exclusion criteria: All COVID-19 positive patients with a clinical suspicion of mucormycosis but unwilling to get admitted for evaluation and mucormycosis but unwilling to get admitted for evaluation and treatment and patients who were unfit for surgery due to presence of other serious co-morbid conditions were excluded from the study.

Sample Processing and Procedure

Digital nasal endoscopy was done to look for the presence of black crusts, status of turbinates, nasal mucosa and deviated nasal septum. Specimen from the nasal cavity or the suspected lesion on hard palate, gums, skin was sent for KOH mount and fungal culture on the day of admission.

The samples were collected in sterile containers and processed in the Department of Microbiology. Testing of samples included direct KOH, fungal culture {Sabouraud Dextrose Agar (SDA) culture medium}. The KOH wet mount specimen was examined under microscope to look for the presence of fungal hyphae with features of broad, septate, ribbon like hyphae with wide or right angle branching pattern [Table/Fig-1]. The second sample was sent during surgery in the intraoperative time for KOH mount, fungal culture, and Histopathology Examination (HPE). Number of cases positive for KOH mount (report received within an hour) was compared with the fungal culture positivity (report received on day 3-4), and the same was confirmed with histopathological examination report.



[Table/Fig-1]: Pottasium Hydroxide (KOH) wet mount preparation showing evidence of fungal hyphae (40X magnification).

The KOH report and its role in early diagnosis and management of mucormycosis suspected cases were assessed. Histopathological assessment was considered as gold standard in the diagnosis of mucormycosis. Both reports of KOH mount taken at the time of admission and intraoperatively, were compared with the HPE findings to assess the sensitivity of KOH mount. In patients where KOH mount was positive in first sample, antifungal therapy was initiated on admission day itself. In the rest, initiation of antifungal therapy had to be delayed till a confirmative diagnosis was made by HPE.

STATISTICAL ANALYSIS

All the data was entered into the Microsoft excel spread sheet and percentages, proportions, sensitivity, specificity and false negativity values were calculated.

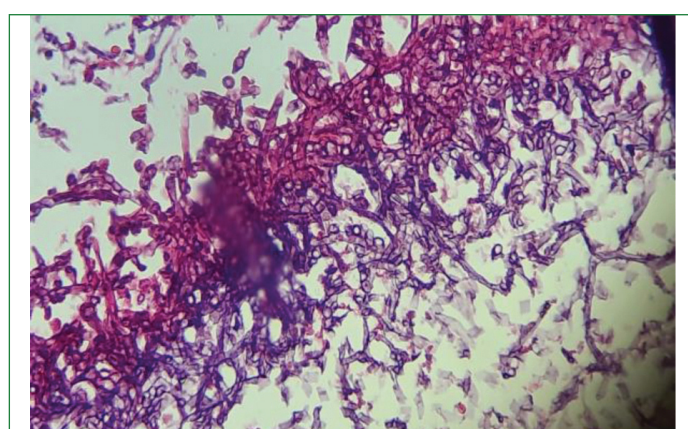
RESULTS

A total of 26 COVID-19 patients presented with suspected fungal infections of nose and paranasal sinuses within the four month study period. There were 6 (23.1%) females and 20 (76.9%) males in the

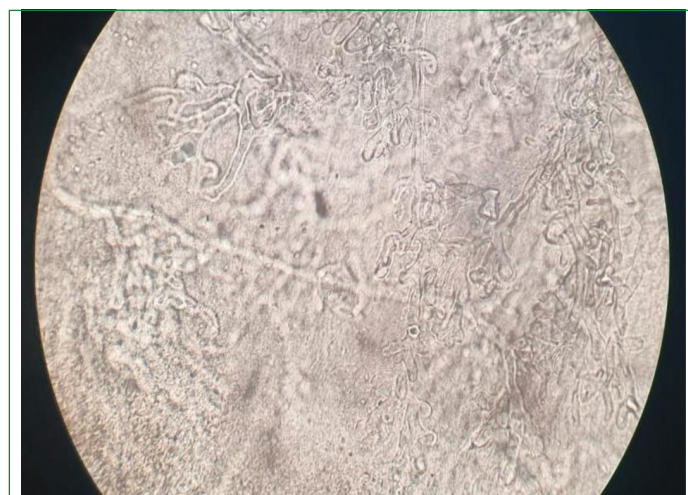
study. Five (19.2%) patients were COVID-19 positive suspected mucormycosis and 21 (80.8%) were post COVID-19 suspected mucormycosis. On the first day culture, out of 26, fungal organisms were isolated in 12 patients {10 (38.5%) *Rhizopus*, 1 (3.8%) *Candida*, 1 (3.8%) *Aspergillus*}. Out of 26 patients, KOH positive on admission confirming with positive intraoperative HPE in 10 (41.7%) patients KOH negative and HPE positive were 14 (58.3%) and both KOH and HPE negative were 2 (100%). Admission day KOH positive were 10 (38.5%) and negative 16 (61.5%) patients [Table/Fig-2-4].

KOH admission day	Intraoperative HPE		Total
	Positive	Negative	
Positive	10 (41.7%)	0	10 (38.5%)
	True positive	False positive	
Negative	14 (58.3%)	2 (100)	16 (61.5%)
	False negative	True negative	
Total	24 (100)	2 (100)	26 (100)

[Table/Fig-2]: Admission day Potassium Hydroxide (KOH) and intraoperative histopathological examination results cross tabulation.



[Table/Fig-3]: Broad, septate hyphae suggestive of mucormycosis in tissue (H&E, 40X).



[Table/Fig-4]: Potassium Hydroxide (KOH) wet mount preparation showing direct evidence of fungal hyphae suggestive of mucormycosis (40X).

On admission day, fungal culture positivity was seen in 12 (46.2%) and negativity in 14 (53.8%) patients [Table/Fig-5]. Intraoperatively, fungal culture was positive in 24 (92.3%) patients and negative in 2 (7.7%) patients [Table/Fig-6]. Number of species isolated respectively on admission and intraoperatively were *Rhizopus* {10 (38.5%) and 17 (65.3%)}, *Candida* {1 (3.8%) and 3 (11.5%) and

Fungal culture admission day	n (%)
Positive	12 (46.2)
Negative	14 (53.8)
Total	26 (100)

[Table/Fig-5]: Admission day fungal culture results.

Aspergillus [1 (3.8%) and 4 (15.3%)]. In 14 (53.8%) cases species were not isolated on admission day and 2 (7.7%) cases species were not isolated in the intraoperative sample [Table/Fig-7].

Fungal culture (Intraoperative)	Frequency n (%)
Positive	24 (92.3)
Negative	2 (7.7)
Total	26 (100)

[Table/Fig-6]: Intraoperative fungal culture results.

Microorganisms	Admission day (n)	Admission day (%)	Intraoperative (n)	Intraoperative (%)
<i>Aspergillus</i>	1	3.8	4	15.3
<i>Candida</i>	1	3.8	3	11.5
<i>Rhizopus</i>	10	38.5	17	65.3
Nil	14	53.8	2	7.7
Total	26	100	26	100

[Table/Fig-7]: Number of fungal culture isolates on day of admission and intraoperative sample.

Out of 26 patients, intraoperative KOH and HPE positive were 24 (92.3%) and both negative were 2 (7.7%). Intraoperative KOH positive were 24 (92.3%) and negative were 2 (7.7%). HPE positive were 24 (92.3%), negative were 2 (7.7%) [Table/Fig-8].

Intraoperative KOH	Intraoperative HPE		Total n (%)
	Positive	Negative	
Positive	24 (100)	0	24 (92.3%)
	True positive	False positive	
Negative	0	2 (100)	2 (7.7%)
	False negative	True negative	
Total	24 (100)	2 (100)	26 (100%)

[Table/Fig-8]: Intraoperative Potassium Hydroxide (KOH) and histopathology results cross tabulation.

In present study, authors found 100% specificity for both fungal culture and KOH mount and sensitivity for KOH was 41.7% and 50% for fungal culture. False negativity rate for KOH was 58.3% and for fungal culture was 50%.

On the day of admission only, there will 100% true negatives and almost 50% of patients were started on antifungal therapy for remaining 50% of patients intraoperative KOH and HPE results were obtained to proceed further. Admission day fungal culture reports and intraoperative culture reports are varied probably because of less sterile conditions in Outpatient Department (OPD) compared to operation theatre, varied site of sampling and disease progression from the day of admission to the intraoperative time period.

DISCUSSION

The laboratory diagnosis of mucormycosis is challenging because of rapid and fulminating course of the disease and doubtful significance of organisms isolated. So, only samples from the infection site are necessary to establish the diagnosis based on the microscopic detection of typical hyphae and confirmation with culture and HPE. This study aimed at detecting the sensitivity of KOH mount in the early diagnosis of fungal infections of nose and paranasal sinuses in COVID-19 infected patients suspected to have mucormycosis. Authors found 41.7% sensitivity of KOH mount on the day of admission in patients presenting with high clinical suspicion of mucormycosis.

In a study by Meher R et al., on 131 patients, the most common species identified in histopathology was mucorales in 122 patients and *Aspergillus* species in six patients while both *Aspergillus* and *mucormycosis* species in three patients. KOH mount showed aseptate hyphae in 81 patients, septate hyphae in 26 patients while 24 patients reported no fungal elements [5].

In a study by Joshi PA et al., on 48 COVID-19 patients 35 (72.91%) were positive for fungal filaments in either KOH mount or fungal growth on SDA. KOH was found to be more sensitive in detecting the fungi as compared to culture. The most common fungi isolated in this study was *Rhizopus* followed by mucormycosis [6]. In present study, it was found that sensitivity for KOH was 41.7% and 50% for fungal culture. False negativity rate for KOH was 58.3% and for fungal culture was 50%.

In a study by Patil A et al, Out of 43 cases of mucormycosis, 24 (55.8%) cases were detected with KOH mount with sensitivity of and 39 (90.7%) cases with crush cytology smears with sensitivity of. All 43 cases were confirmed on histopathological examination [7].

In a study by Mohanty A et al., on 186 cases, on direct microscopic examination 33 were mucormycosis, 21 were fungal culture positive and only 11 cases showed aseptate hyphae in histopathological study confirmative of mucormycosis. In this study admission day KOH positive were 10 (38.5%) and intraoperative KOH positive were 24 (92.3%) out of 26 patients. Direct microscopy becomes more relevant and essential in the quick diagnosis of this deadly condition as isolating these organisms on routine culture media is difficult as they usually do not grow well and also histopathological reports are not confirmative [8].

In a study by Bala K et al., out of total 36 cases, the mucormycosis cases were positive by KOH (84%), histopathology (58%) and culture (61%). Out of 23 cultured organisms, *Rhizopus arrhizus* was the most (37.5%) dominant species, followed by *Apophysomyces variabilis* (29.2%), *Lichtheimia ramosa* (16.7%), *Rhizopus microsporus* (4.2%), *Rhizomucor pusillus* (4.2%), and *Apophysomyces elegans* (4.2%). *Rhizopus arrhizus* was most predominantly isolated from rhino-orbitocerebral mucormycosis and apophysomyces species were usually obtained from cutaneous mucormycosis [9]. Among mucormycetes, members of the genera *Rhizopus*, *Mucor*, *Lichtheimia*, *Rhizomucor*, and *Apophysomyces* are commonly cause infections in human beings. Patients with mucormycosis show *Rhizopus* species as the most predominant species when isolated on culture media [10]. It is important to rule out mucormycosis in diabetic patients, as early diagnosis and treatment can reduce the morbidity and mortality. Delay in management often leads to a fatal outcome [11].

Mucormycosis is most commonly encountered in patients with uncontrolled diabetic status, over usage of corticosteroids and use broad spectrum antibiotics. High risk individuals has to be investigated early and they need early attention to make early diagnosis and start required treatment on time as mucormycosis is associated with high mortality [12].

In a study by Shakir M et al., nasal and throat fungus smear/KOH preparation revealed few broad coenocytic aseptate hyphae, moderate budding yeast cells with pseudohyphae and culture grown *Rhizopus* species. Their study concluded that clinically suspected mucormycosis cases should be investigated radiologically and with accurate diagnostic modalities. Actions and interest from multidisciplinary teams including infectious diseases, ophthalmology, otorhinolaryngology, emergency medicine, neurosurgery, pathology and microbiology departments is much more needed. Delay of only six days in establishing diagnosis can double the 30 day mortality from 35-65%, so only accurate diagnosis and treatment on time is required especially in cases of invasive rhino-orbital mucormycosis [13]. In present study, COVID-19 patients presenting with high clinical suspicion of mucormycosis the sensitivity of KOH mount on the day of admission to hospital is 41.7%. Results are matching with few other studies and immensely helps in initiation of antifungal therapy which is of paramount importance in mucormycosis.

Limitation(s)

The sample size of the study was small, therefore a study with larger sample number is required to comment on sensitivity and specificity of KOH mount in the early diagnosis of fungal infections. This study

was a pilot study in this direction. Methods to improve the sensitivity of KOH mount are not addressed in this study. These issues should be addressed preferably by a microbiologist from their view points.

CONCLUSION(S)

In current study, even in patients presenting with high clinical suspicion of mucormycosis, the sensitivity of KOH mount on the day of admission to hospital found to be 41.7%, which cannot be ignored. The results of KOH mount comes within an hour of sending nasal scrapings samples. Hence, it helps in making the early diagnosis and early initiation of treatment for suspected cases to decrease the chance of further spread of disease, decreases the morbidity and mortality of the patient. The KOH sample can be sent from peripheral hospitals only and hence early diagnosis can be done at the peripheral hospitals itself. Patients can be referred to tertiary centre for further management. Positive KOH mounts even though less in number can help those patients by starting the antifungal therapy at an earliest phase of the disease. Remaining patients can be evaluated further by means of taking samples from deeper tissues, waiting for clinical progression of the disease, assessing through the radiological investigation etc, which will take more time causing more morbidity. Hence, KOH mount has to be considered as a primary diagnostic modality even though it is less sensitive.

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