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# **Research Article**

# AN OVERVIEW OF CLINICAL AND MICROBIOLOGICAL CORRELATION OF MUCORMYCOSIS AS A POST COVID FUNGAL INFECTION

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#### **ABSTRACT**

Introduction: Mucormycosis is an invasive fungal infection caused by saprophytic fungi belonging to the genera Mucor, Rhizopus and Absidia which belong to the order Mucorales and class Zygomycetes. COVID-19 is a life-threatening, infectious disease in which there is dysregulation of immune system occur due to extensive use of steroids in management or associated with diabetes mellitus which suppress immunity, increasing susceptibility to opportunistic fungal infections to colonize. Materials and Methods: A retrospective study of 500 patients with invasive fungal infection who presented to the ENT department and who were either COVID-19 positive or had recovered from COVID-19 infection, were included in the study. Tissue samples from all suspected site were received in sterile container containing normal saline, were used for KOH smear and culture. Result: A total of 500 patients presented. Out of these patients 226(45.2%) cases were found to be positive for fungal elements based on direct microscopy-KOH. Out of these 226 cases 49(21.68%) cases shows morphology resembling to mucormycosis and 177 (78.32%) positive for other fungi. Out of these 49, 17(34.70%) also shows growth for mucormycosis on fungal culture media. Conclusion: COVID-19 associated with invasive mucormycosis is dangerous. Uncontrolled diabetes and impulsive use of steroids are two of the main factors aggravating the illness. If in infected patient, early surgical intervention and intravenous anti-fungal treatment should be started for management, good prognosis and less fulminant disease course can be achieved.

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# INTRODUCTION

Mucormycosis is a life-threatening infection caused by saprophytic fungi belonging to the order Mucorales and class Zygomycetes, it is also known as Black fungus [1]. It is a reemerging fungal infection and has been seen in immune compromised patients. Even though mucormycosis has been associated with high morbidity and mortality, its prevalence has been enormous. This disease has been in limelight in current scenario since the outbreak of COVID-19. The immune dysregulation caused by the virus along with use of immune modulatory drugs has increased the risk of these fungal infections. [2] Extensive use of steroids in COVID-19 management or associated with diabetes mellitus can also suppress immunity, allowing opportunistic fungal infections to colonize. [1] Among all the fungal infections, COVID-19 Associated Mucormycosis (CAM) has led to most deaths and caused a lot of illness worldwide. As India has the second largest population with diabetes mellitus in the world, mucormycosis has seen mostly in patients with uncontrolled diabetes mellitus with an estimated prevalence being 140 cases

per million population.<sup>[3]</sup> It has always been a challenge to diagnose this condition, and the prerequisites for an accurate diagnosis are a high index of suspicion and recognition of host factors along with a prompt assessment of clinical manifestations. [4,5] Microscopy along with culture and histopathology demonstration of Mucorales have been the mainstay of diagnosis. Various specimens can be collected depending upon the clinical manifestations and the site of infection, but tissue biopsy is the sample of choice. Direct microscopy of a tissue sample with a 20% of potassium hydroxide (KOH) helps us in identifying the typical pauciseptate/aseptate ribbon-like hyphae and gives us a presumptive diagnosis. It is of utmost importance as early treatment with specific antifungal drugs can be initiated to prevent a fatal outcome. [3] Furthermore, complete speciation can be done with the help of fungal culture, which is the standard for diagnosing this disease. The combination of direct microscopy and culture results assists in correctly identifying Mucorales causing mucormycosis. This study will be conducted with the objective of evaluating the role of

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microscopy along with culture in providing early diagnosis of mucormycosis in clinically diagnosed patients.

# AIMS AND OBJECTIVES

Evaluating the role of microscopy along with culture in providing early diagnosis of mucormycosis in clinically suspected patients, with these patients being, or having previously been, COVID-19 positive.

# MATERIAL AND METHODS

A retrospective study was undertaken by department of microbiology at Shri M. P. Shah Government Medical College and Guru Gobind sinh Government Hospital, Jamnagar, Gujarat, over a period of six months, from May 2021 to October 2021. All patients who presented to ENT department and who were either COVID-19 positive or had recovered from COVID-19 infection, with a high index of clinical suspicion for mucormycosis were included in this study. The specimens from the patients were received in sterile containers containing normal saline, and processed in the department of microbiology for KOH smear examination and fungal culture. Tissue was examined in 20% KOH. Culture was done on Sabouraud Dextrose Agar (SDA) with Gentamycin and incubated at 25°C and 37°C respectively. The cultures were examined for growth daily for the first week and twice a week for the subsequent period. The fungal isolates were finally identified by conventional techniques such as lactophenol cotton blue (LCB) mount. The diagnosis of mucormycosis was confirmed when broad aseptate/sparsely septate, ribbon-like hyphae with right (wide) angle branching were seen in the specimen with or without isolation of mucormycetes. After that we correlate the how many clinically diagnose patients of mucormycosis give positive result on KOH and Fungal culture.

# **RESULT**

Total of 500 samples of clinically suspected cases of mucormycosis received at department of microbiology during period of six months from May 2021 to October 2021.

**Table 1** Results of 500 clinically suspected cases of mucormycosis by direct microscopy-KOH preparation for fungal elements.

KOH RESULT	TOTAL NO.OF CASES	PERCENTAGE
KOH Positive	226	45.2%
KOH Negative	274	54.8%
TOTAL	500	100%

Table 1 shows that out of 500 samples of suspected mucormycosis 226 (45.2%) were positive by KOH preparation for fungal elements whereas 274 (54.8%) were negative, which is seen graphically in figure 1.

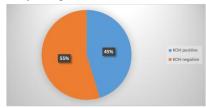


Figure 1 Results of 500 clinically suspected cases of mucormycosis by KOHpreparation.

**Table 2** Result of KOH preparation positive for mucormycosis

KOH RESULT	TOTAL NO.OF CASES	PERCENTAGE
KOH Positive for mucormycosis	49	21.68%
KOH Positive for other mycosis	177	78.32%

TOTAL	226	1000/
TOTAL	226	100%

Table 2 shows that out of 226 samples positive by KOH preparation for fungal elements,49 (21.68%) were shows morphology resembling to mucormycosis and 177(78.32%) shows other fungal morphology.

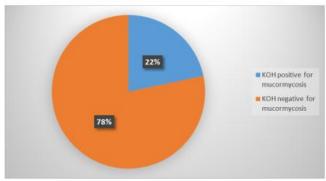


Figure 2 KOH result for mucormycosis

Table 3 Fungal culture result of samples positive by KOH preparation for mucormycosis

CULTURE	TOTAL NO.OF CASES	PERCENTAGE
Positive	17	34.70%
Negative	32	65.30%
TOTAL	49	100%

Table 3 shows that out 49 samples of KOH positive for mucormycosis 17 (34.70%) also shows growth for mucormycosis on fungal culture media, and 32 (65.30%) negative for mucormycosis growth on culture, which is also seen figure 3.

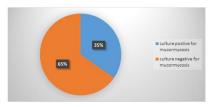


Figure 3 Fungal culture result of samples positive by KOH preparation for mucormycosis

**Table 4** Age wise distribution of samples positive by KOH preparation for mucormycosis

AGE	TOTAL NO.OF CASES	PERCENTAGE
1-25	1	2.04%
26-50	17	34.70%
51-75	28	57.14%
76-100	3	6.12%
Total	49	100%

Table 4 shows that maximum number of mucormycosis seen in age group (51-75yr) 57.14%.

**Table 5** Sex wise distribution of samples positive by KOH preparation for mucormycosis

SEX	TOTAL NO.OF CASES	PERCENTAGE
Male	30	61.22%
Female	19	38.78%
TOTAL	49	100%

Above table shows that males are more infected with mucormycosis i.e. 30 (61.22%)

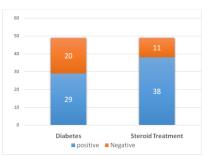


Figure 4 History of Diabetes and Steroid use in treatment in patients of mucormycosis

Figure shows that out 49 mucormycosis patients, 29(59.18%) had history of Diabetes and 38(77.55%) had history of Steroid treatment during Covid-19.

# **DISCUSSION**

During the second wave of the COVID-19 pandemic in india, a sudden and rapid rise in mucormycosis incidence was observed. Covid associated mucormycosis (CAM) has been identified as a deadly complication of this viral infection. Nearly 70% of CAM cases have been reported from India<sup>[7,8]</sup>. It is a lethal fungal disease, with rhino-orbito presentation being its most common form and is associated with significant morbidity and mortality<sup>[9]</sup>. Although it has a low incidence in pre pandemic era, varying from 0.005 to 1.5 per million population, with special high incidence in India, which is about 0.14 per 1000 population, [10] but with the surge of COVID-19, it has risen up to 26.7%, India being the most affected country. [11] In this study out of 226 (45.2%) KOH positive samples, 49 (21.68%) shows aseptate hyphae suggestive of mucormycosis which is in concordance with study conducted by Mohanty A et al<sup>[3]</sup> which reported 17.2%. Early diagnosis by KOH microscopy may help clinician to initiate antifungal treatment early in diseases to prevent further complication as culture report take time in week and also chance of contamination are there. In this study, 61.22% were male and 38.78% were female with mean age of 54 years, This was in concordance with the study conducted by Bidkar V et al [12] where the incidence was common in males 42 (66.6%) with mean age of 54.9 years, and in another study done by Gupta R  $et~al^{[13]}$  have 81 (70.4%) males and 34 (29.6%) females, and the median age of the patients was 54.2 years. This fungal infection occurred in COVID-19 infection because of ability of the fungal spore to germinate in the ideal environment set up by respiratory condition of COVID-19 patients, it is due to repeated use of steroid, antibiotics and oxygen masks during management. We have found that COVID-19 patients who were treated with steroids and have diabetes mellitus were prone to mucormycosis. In present study, 59.18 % of the patients have diabetes; similar finding have been observed in a study conducted by Yohai RA, et al [14] in which 60% of cases had diabetes and study by Bidkar V et al reported 61.4% of patients had diabetes. In this study, 77.5% of patients had history of steroid treatment during covid-19 infection, compared to study done by Aranjani JM et al<sup>[15]</sup> & Jadhav B et al<sup>[16]</sup> found that 71.4% & 75% of their patients were on corticosteroids during the treatment of COVID-19 respectively.

# **CONCLUSION**

For the diagnosis of mucromycosis, we employed KOH preparation microscopy and culture on SDA methods. We can

say that to make a rapid presumptive diagnosis of mucormycosis, KOH microscopy is a reasonably priced and irreplaceable method. It has been used since ages as the best means to diagnose this fatal condition and provide a presumptive guide to the clinicians in their management, as the fungal culture will take at least week to be positive & chances of contamination are more. So KOH microscopy helps them to initiate the appropriate treatment and therefore saving precious time. Uncontrolled diabetes and over-zealous use of steroids in COVID-19 management can also suppress immunity, allowing opportunistic fungal infections to colnize, which might aggravating the illness and both of these must be properly checked and awareness should be risen when dealing with patients with multiple co-morbidities. Multidisciplinary approach is essential in diagnosis as well as treatment with antifungals within few hours, as the timing of mucormycosis management is critical to the subject. Surgical intervention is also needed to control the spread of infection to other patient body parts. Finally, a regular follow-up procedure is recommended to the COVID-19 patient with a prior history of co-morbidities who has recently recovered mucormycosis.

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