



## Incidence and Screening for Rhino-Cerebral Mucormycosis and other Fungal Infections in Patients with COVID-19

<sup>1</sup>Dr. Kanchan Chaudhari, <sup>2\*</sup>Dr. Anahita V Bhesania Hodiwala, <sup>3</sup>Dr. Ujjwala Maheshwari, <sup>4</sup>Dr. Srivalli Natarajan, <sup>5</sup>Shrutika Sawant

<sup>1</sup>Assistant Professor; Department of Microbiology

MGM Medical College & Hospital; Kamothe, Navi Mumbai

<sup>2\*</sup>(Corresponding Author) Professor & HOD; Department of Microbiology

MGM Medical College & Hospital; Kamothe, Navi Mumbai

<sup>3</sup>Professor & Laboratory Director; Department of Pathology

MGM Medical College & Hospital; Kamothe, Navi Mumbai

<sup>4</sup>Dean. Professor & HOD; Department of Oral & Maxillofacial Surgery

MGM Dental College & Hospital, Kamothe, Navi Mumbai

<sup>5</sup>MSc Student

MGM Medical College and Hospital, Kamothe, Navi Mumbai

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### KEYWORDS

COVID-19;  
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KOH; SDA Culture;  
Mucormycosis

### ABSTRACT:

**Background & Objectives:** Mucormycosis is a fungal infection which mainly affects immunocompromised patients and the cases showed an upward trend coinciding with the second wave of COVID-19 pandemic in India. Considering the poor prognosis of established mucormycosis cases, it is worthwhile to identify them at earliest within COVID-19 patients. The objective of this study was to monitor COVID-19 patients prospectively for mucor spp. and other fungal infections via detailed microbiological analysis of their nasal wash samples. **Materials and Methods:** COVID-19 patients were called upon at every week interval for one month after their COVID-19 infection and their nasal wash samples were subjected to KOH mount, Gram staining, Calcofluor white staining, SDA culture and LPCB mount. **Results:** A total of 297 samples were derived from 90 patients. 16(5.3%) were positive for fungal elements in KOH and calcofluor staining. Culture of samples on SDA agar showed that 13(4.3%) samples had Mucor spp. All 13 patients were hyperglycemic during their course of COVID-19. **Conclusions:** Around 4.3% nasal wash samples of COVID-19 immunocompromised patients taken within two weeks of COVID-19 infection demonstrated definite growth for mucor spp.. These patients could be closely monitored for mucormycosis symptoms/complications and timely interventions could be provided therein.

### Introduction

Coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) relentlessly has created havoc across the globe until nearly 2.5 years after its first detection in December 2019. The outbreak was declared by the World Health Organization (WHO)

to be a public health emergency of international concern on 30 January 2020, and a pandemic on 11 March 2020.<sup>[1]</sup> As of 2<sup>nd</sup> Feb 2023, India reported a total of 4,46,83,023 confirmed cases, with 524,777 deaths. Currently, the number of cases per million is 30,428.<sup>[2]</sup> The second wave of led to dire consequences in the form of spiralling cases,



reduced supplies of essential treatments, and increased deaths particularly in the young population.<sup>[3]</sup>

Although vaccination contributed immensely to curtail the magnitude of third wave of COVID-19 in India, it was the second wave which spelt out of proportion consequences in India like high volume case load, reduction in supply of essential treatments and high mortality specifically in young population.<sup>[3]</sup> While tackling this scenario another complication of COVID-19 i.e. Mucormycosis became a serious issue in India due to its unprecedented surge and high mortality.<sup>[4]</sup> The number of COVID-19 associated mucormycosis (CAM) exceeded 41000 by mid-July in India with higher caseload than global average.<sup>[5]</sup>

Mucormycosis is a fungal infection caused by the mucormycetes family of moulds, which are widespread decomposers of organic matter found in soil and dust. Naturally, Mucorales occur in soil, their spores spread by air often contaminate foods, water, and clinical specimens. The *Mucor* sp., *Rhizopus* sp., *Absidia* and *Cunninghamella* are the main causative agent for MC in humans.<sup>[4,6]</sup>

Mucormycosis mainly affects people who are immunocompromised, or patients already infected with other diseases. High risk groups include people with diabetes (especially diabetic ketoacidosis), solid organ transplantation, neutropenia (low neutrophils, a type of white blood cells), long-term systemic corticosteroid use, and iron overload (hemochromatosis).<sup>[7]</sup> Various key mechanisms linking COVID-19 with mucormycosis include:<sup>[8]</sup>

- Prolonged uncontrolled hyper-glycaemic state
- Steroid induced immunosuppression, hyperglycaemia and lymphopenia
- Viral-induced lymphopenia and endothelitis
- Increased serum levels of iron (High ferritin levels)
- Ketonemia and ketoacidosis, in absence of diabetes mellitus

To ensure good prognosis and disease resolution, early recognition, diagnosis and prompt administration of appropriate antifungal treatment and surgical debridement (as needed) are important before angioinvasion occurs and necrosis becomes extensive, leading to dissemination. Early detection in such cases is necessary to reduce mortality and prevent end-stage organ damage. This also saves the patients from a disfiguring surgery in order to remove the necrosed tissue as curative and antifungal therapy alone is rarely successful. Availability of limited tools lead to early detection in 50% cases which is suspicious and is only diagnosed after post-mortem.<sup>[8,9,10,11]</sup>

The diagnosis of mucormycosis relies mainly on identification of morphological characteristics from cultural, histopathological, or radiological analysis.<sup>[11]</sup> (Microscopy (direct and on histopathology) and culture are the cornerstones of diagnosis.<sup>[12]</sup> For diagnosis, specimens are usually obtained from nasal and paranasal sinuses and include nasal wash, scrapings, or nasal crust biopsies. The usual diagnostic modalities used to examine these specimens include potassium hydroxide (KOH) mount, fungal culture and histopathology.<sup>[13]</sup>

Previous studies have established that mucormycosis was diagnosed at a median of 10 days (range 0–90 days) after diagnosis of COVID-19. Patients with uncontrolled diabetes developed mucormycosis (87% had rhino-orbital cerebral) a median of 2–3 weeks earlier after diagnosis of COVID-19 than patients with well-controlled diabetes.<sup>[14]</sup>

In view of this, it was thought essential to consider early laboratory detection of mucormycosis in COVID-19 patients with immunocompromised state. There exists limited data evaluating the same in published literature. This study was planned with an objective to assess the incidence of mucormycosis in immune-compromised COVID-19 patients within 1 month of their diagnosis through established microscopy and culture techniques.



### Study Methodology:

This was a prospective, interventional, single arm study conducted on 297 nasal wash samples of COVID-19 patients with immunocompromised state (Pre-existing or iatrogenic). The study was conducted by Department of Microbiology in collaboration with Pathology; Oral and Maxillofacial surgery departments from Mahatma Gandhi Mission Medical College & Hospital, Kamothe, Navi Mumbai between June 2021 to September 2021. The study was approved by Institutional Ethics Committee from Mahatma Gandhi Mission Medical College & Hospital, Kamothe, Navi Mumbai and was conducted in accordance with Helsinki Declaration (Revised 2000). (MGMIHS/RES/02/2020-21/502 dated 8<sup>th</sup> June 2021)

#### Inclusion Criteria:

1. Age -18 years of age and above with or without Diabetes Mellitus.
2. Participants diagnosed with Covid-19 and being hospitalized and undergoing treatment for the same.

#### Exclusion Criteria:

Participants who have succumbed to Covid -19 during the period of study.

The study was conducted after obtaining permission from the Institutional Ethics Committee. After obtaining written informed consent, demographic details were noted.

All samples were processed for KOH, Calcofluor staining, Gram staining and were cultured on SDA agar.

Samples received in Department of Microbiology were first stained with 10% KOH and observed under 40X. Simultaneously they were stained with Calcofluor white and observed under UV light in a fluorescent microscope. Gram staining was also performed on each sample. For tissue samples additional histopathological stainings like Haematoxylin and Eosin (H and E), Per-iodic acid Schiff stain(PAS) and Grocotte's Methanamine Stain (GMS). Further, they were cultured on Sabouraud Dextrose Agar (SDA) and growth was monitored for next 7 days. Yeast colonies obtained on SDA were subjected to gram stain while for mould colonies LPCB mount and slide culture was performed.

Data were analysed using descriptive statistics for incidence of mucormycosis in COVID-19 patients.  $P < 0.05$  was considered to be significant. Descriptive statistics was used for analysis.

### Results:

A total of 297 samples were received (nasal wash and tissue biopsy) for screening of various fungi including mucor species. Out of 297 samples derived from 90 patients, 68 were males while 22 were females. Nearly 32 patients were  $> 60$  years age and 26 patients had pre-existing diabetes.

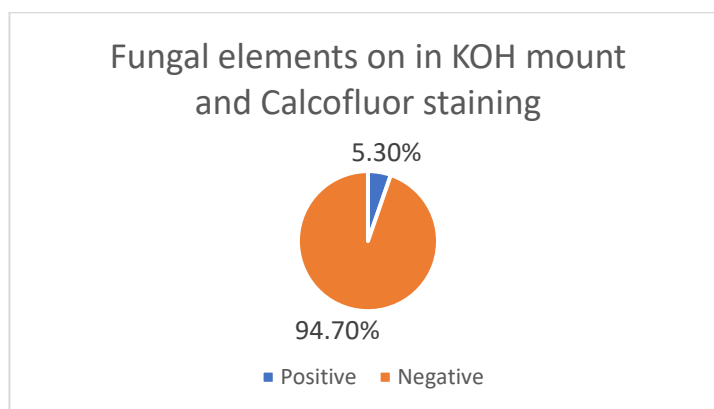
**Table 1: Baseline Demographic Details of Study participants (n=90)**

Parameter	Mean±SD
<b>Age</b>	
11-40 (Number and Percentage)	16
40-60	42
>60 years	32
<b>Gender</b>	
Males (Number and Percentage)	68
Females (Number and Percentage)	22



<b>Immunocompromised (Number and percentage)</b>	
Diabetes mellitus	26
Steroids used (>7 days)	77
<b>Established Mucormycosis with tissue involvement</b>	2

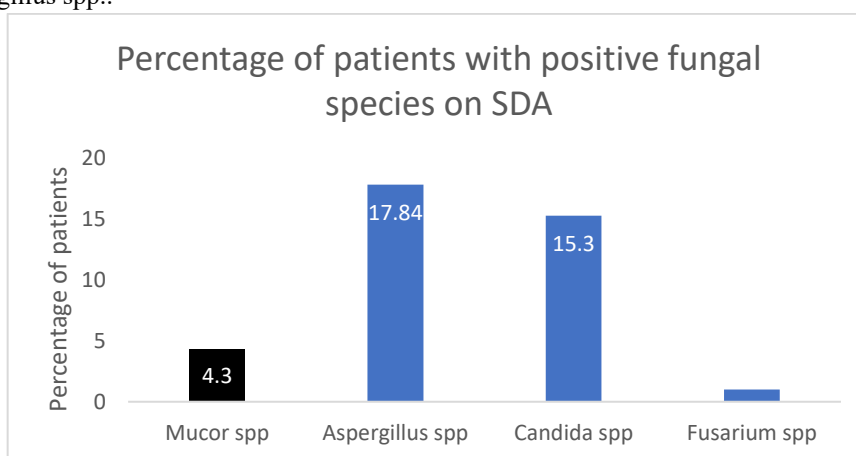
Out of 297 samples, 16(5.3%) were positive for fungal elements in KOH and calcofluor staining as shown in Figure 1



**Figure 1: Positivity of fungal elements in KOH mount and Calcofluor staining**

The culture of samples on SDA agar as shown in Figure 2 demonstrated that:

- 13(4.3%) samples had Mucor spp.
- 53(17.84%) samples had shown growth of Aspergillus spp..
- 42(15.3%) samples have shown Candida spp..
- 3(1%) samples have shown Fusarium spp..

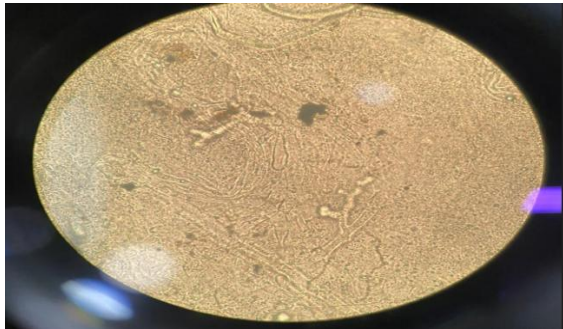


**Figure 2: Positivity of fungal species on SDA**

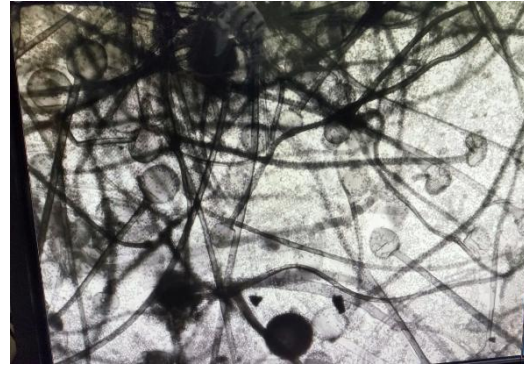


This shows that nearly 4.3% samples had definite growth of mucor spp.. These 13 patients having positive mucor spp.. were all hyperglycemic and

their mean age was 50 years. Microscopy and culture images of few representative isolates are as shown in Figure 3



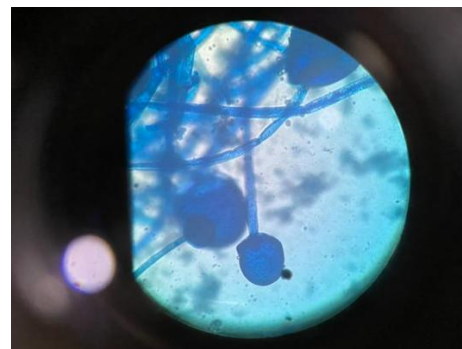
a.



b.



c.



d.

Figure 3

**a. KOH staining showing broad, ribbon shaped non-septate hyphae**

**b. Calcofluor white staining showing broad, ribbon shaped non-septate hyphae with sporangia**

**c. SDA culture showing greyish white cottony growth**

**d. LPCB mount showing sporangium with sporangiophore**

Few patients were also reported with multiple fungal isolates in their cultures as shown in Table 2. Four patients had both *Aspergillus* spp.. and *Candida* spp.. detected in their cultures

Table 2: Instances with two fungal isolates in one patient

Number of patients	Fungal Isolates
4	<i>Aspergillus</i> spp.. and <i>Candida</i> spp..
2	<i>Mucor</i> spp.. and <i>Candida</i> spp..
2	<i>Aspergillus</i> spp.. and <i>Mucor</i> spp..



## Discussion

This study shows that nasal wash samples of COVID-19 immunocompromised patients within one month of COVID-19 infection could be useful for early detection of mucor spp.. and it was found within 4.3% samples therein.

The early diagnosis of mycosis and pursuant treatment is crucial for a propitious outcome and prognosis. The mean day of diagnosis was  $25.6 \pm 21$ . In another systematic review 34 studies and 275 cases, the mean diagnosis time was 19.5 days from COVID-19 onset.<sup>[15]</sup> In this study we could detect 13 patients with positive mucor spp.. cultures on SDA within 3 weeks of COVID-19 infection.

It was found in one study conducted in three tertiary hospitals in Gujarat, Delhi and Raipur amongst 2547 COVID-19 patients, 1.8% were diagnosed with mucormycosis. Mean age was  $55 \pm 12.8$  years, and majority suffered from diabetes mellitus ( $n = 36$ , 76.6%). Most were not COVID-19 vaccinated ( $n = 31$ , 66.0%) and majority ( $n = 43$ , 91.5%) had developed moderate-to-severe pneumonia, while 20 (42.6%) required invasive ventilation. All patients had received corticosteroids and broad-spectrum antibiotics while most ( $n = 37$ , 78.7%) received at least one anti-viral medication. Mean time elapsed from COVID-19 diagnosis to mucormycosis was  $12.1 \pm 4.6$  days.<sup>[16]</sup> Findings of positive mucor spp.. samples were  $>2$  times higher in our study. Earlier study cited above investigated for mucormycosis in clinically suspected patients by direct examination in 10% KOH from scrapping, biopsy and proven based on micro-biological culture or specific histological features from biopsy specimen which has nearly same modalities as our study.

While we had diagnosed 4.3% of samples for mucor spp.. on SDA culture and 1% on KOH mount, another cross-sectional observational study was conducted in a microbiology laboratory of a tertiary care hospital of central India, in which out of 35 suspected clinical specimens from 27 post-COVID-19 patients, 8 specimens from 5 patients were positive for MC by gram and KOH smear method and they saw filamentous fungi by

conventional microscopic method.<sup>[17]</sup> In our study, we considered that KOH mount sensitivity and specificity would be low and hence we confirmed microscopy findings with SDA cultures.

Culture of specimen is also important method as mucorales are able to grow rapidly at temperature of  $24-37$  °C within a time frame of 24-48 h.

## Significance of the Study

*What's Already known about the topic?*

The diagnosis of mucormycosis relies upon the identification of fungal species grown on laboratory culture media for mucormycosis. A multidisciplinary approach in diagnosis is essential as well as treatment with antifungals within few hours, as the timing of mucormycosis management is critical to the subject.

*What does this study add?*

Early detailed microbiological examination of nasal wash samples from immunocompromised post COVID-19 patients can help to diagnose presence of Mucor spp.. and appropriate clinical monitoring and interventions can be planned accordingly

## Conclusions

This study concluded that 4.3% nasal wash samples of COVID-19 immunocompromised patients taken within one month of COVID-19 infection were positive for mucor spp.. Such follow-up testing of COVID-19 patients could be useful for early detection of mucor spp.. to provide timely interventions and prevent future complications of mucormycosis especially in diabetic patients and those who had been on steroid treatment during COVID-19 management.

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