

Mucormycosis and COVID 19: Demography, associated co-morbidities and laboratory diagnosis in a Tertiary Care Hospital

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Abstract

Purpose: The pathogenesis and treatment of severe COVID-19 infection in a patient makes him susceptible to many co-infections/super-infections, out of which the highest surge was of Post COVID mucormycosis. Prevention, early detection and management of Post COVID mucormycosis is essential due to its devastating and fatal outcome. The present study was performed to describe the clinico-demographic risk factors and role of various diagnostic modalities available for diagnosing mucormycosis in COVID-19 patients admitted in a super-speciality hospital.

Method: A retrospective observational study was performed on 15 patients admitted with a clinical diagnosis of mucormycosis associated with COVID-19 infections, in a tertiary care centre between May 2021 to July 2021. Demographic details such as age, gender, Diabetes mellitus (DM), hypertension, glycosylated haemoglobin status, existing immunocompromised conditions, other co-morbidities, time duration for reporting diseases after COVID-19 treatment, use of oxygen and/or ventilator support, steroid microbiological test results as well as pathological test results were collected from the patient records retrospectively. The data was compiled and analysed for association with development for mucormycosis.

Results: The most common fungal species isolated in the study was *Rhizopus* species (53.33%). The most common clinical presentation was Nasal mucormycosis (53.33%) followed by Rhino –cerebral mucormycosis (13.3%) and Sino-nasal mucormycosis (13.3%). Diabetes mellitus was the most common associated co-morbidity (66.7%). Microscopy for fungal elements positivity was 66.67% while culture positivity was 85.7%. Culture and Histopathology findings correlated well clinically.

Conclusion: Mucormycosis in COVID -19 was associated with deranged blood sugar levels either due to poorly controlled diabetes or steroid therapy. Fungal microscopy helped in early diagnosis while culture and histopathology could be employed together reliably for confirmatory diagnosis.

Keywords: Mucormycosis; COVID 19; Steroid; Diabetes mellitus; Fluorescent microscopy

1. Introduction

Mucormycosis is an infection caused by fungi belonging to the order Mucorales. The most common organism isolated from patients with mucormycosis is *Rhizopus oryzae*, responsible for ~70% of all cases of mucormycosis. Mucormycosis can be classified into rhino-orbitocerebral, cutaneous, disseminated, gastrointestinal, and pulmonary types. Uncontrolled Diabetes mellitus in ketoacidosis, other forms of metabolic acidosis, treatment with corticosteroids, organ

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or bone marrow transplantation, neutropenia, trauma and burns, malignant hematologic disorders, and deferoxamine therapy in patients receiving hemodialysis are the major risk factors for mucormycosis [1].

The prevalence of mucormycosis varies from 0.005 to 1.7 per million population, while it is nearly 80 times higher (0.14 per 1000) prevalent in India compared to developed countries [2]. Coupled with the fact that India is the second largest pool of diabetics worldwide [3], the highest number of cases of COVID -19 patients with mucormycosis have been reported in India.[4].

Other theories have been proposed for attributing to flaring up of COVID -19 patients with mucormycosis in India like prolonged use of unhygienic masks, new construction sites, overcrowding and repeated traumatic sampling[3]. The recent pandemic of COVID-19 has not only left the world to succumb due to its half understood direct pathogenesis but also left many scars for humanity in form of its after effects out of which mucormycosis is a major one.

Few crucial factors in COVID-19 related pathogenesis are said to be responsible for developing mucormycosis like an ideal hypoxic environment(caused by COVID-19) for the spores to germinate, high glucose (Diabetes mellitus, new-onset hyperglycemia, steroid-induced hyperglycemia), acidic medium (metabolic acidosis, diabetic ketoAcidosis), high iron levels (increased ferritin) and decreased phagocytic activity of white blood cells (WBC) due to immune-suppression (COVID-19 mediated, steroid-mediated or background co-morbidities). The acidic environment in results in increased production of free iron by reducing its binding to transferrin and low level of dialyzable inhibitory factor in patients with uncontrolled blood sugar levels provide suitable conditions for fungal duplication [5]. Several other risk factors including prolonged hospitalization with or without mechanical ventilators, injudicious use of antibiotics also contribute [2]. COVID-19 pathogenesis induced endothelial damage, endothelitis and thrombosis and prolonged lymphopenia are also important predisposing factor for developing mucormycosis. [3]. Thrombosis and tissue necrosis are major symptoms in mucormycosis and may require antifungal treatment with or without debridement of the infected tissue [6].

The early detection and management of mucormycosis is crucial as the infection has a high fatality rate due to its angio-invasive nature. Understanding of the patient characteristics, associated risk factors and timeline for a COVID-19 patient to develop mucormycosis post recovery should be laid emphasis upon.

The present article aims to describe the clinico-demographic risk factors and various laboratory tests/assays available for diagnosing mucormycosis in COVID-19 patients admitted in a super-specialty hospital.

2. Material and methods

This is a retrospective observational study performed on fifteen COVID -19 patients with mucormycosis admitted in a tertiary care Centre from May 2021 to July 2021.

The study was approved for waiver off patient consent by the Institutional Ethical Committee of the hospital.

We collected detailed clinical and demographic data such as age, gender, DM status, hypertension, any existing immunocompromised conditions, associated co-morbidities, glycosylated hemoglobin (HbA1c) status, time duration for reporting diseases after COVID-19 treatment, and use of oxygen and/or ventilator support, steroid, medications, treatment received for COVID-19, nasal endoscopy findings, microbiological, pathological and radiological details, retrospectively from the available medical records. The data was tabulated for the presence of any associated risk factors and the various laboratory methods were also compared with each available method and the gold standard i.e., histopathological examination.

- **Inclusion criteria:** All patients admitted for suspected mucormycosis after a recent or with present infection with COVID-19.
- **Exclusion criteria:** Patients whose disease had not been established histopathologically and with incomplete records were excluded from the study.

For the fifteen samples included results of the various tests performed like microscopy by 40% KOH(Potassium Hydroxide) mount, Calcoflour stain, fungal culture on Sabouraud Dextrose Agar (SDA), SDA with Gentamicin and SDA with Cycloheximide and Hematoxylin and Eosin (Hand E) stain in Histopathological examination were tabulated.

3. Results

In this study, out of 15 patients admitted with suspected mucormycosis 14 (93.33%) were male. Average age of presentation was 55.25 years. Twenty days was the average duration of presentation with symptoms of mucormycosis in the COVID 19 patients. **Table 1** shows 10 out of 15 (66.7%) patients had pre-existing co-morbidity and DM was the most common comorbidity in all patients with /without other co-existing diseases (66.67%) followed by history of steroid administration during COVID-19 treatment (53.33%)

Table 1 Associated Co-morbidities and risk factors for Mucormycosis

Co -morbidity	Number of patients (%)
Diabetes mellitus only	3(20%)
Diabetes mellitus with other co-morbidities	7(46.67%)
Diabetes mellitus with raised HbA1c	7(46.67%)
Pulmonary hypertension, Chronic liver disease	1(6.67)
Diabetes mellitus with steroid administration	6(40%)
Only steroid administered	2(13.33%)
Elevated Serum Ferritin levels	6(40%)
Oxygen administration	5(33.33%)
Total	15

The most common microbiological sample received was from nasal and para nasal tissue (scraping/tissue)[80%(12/15)]. On performing 40% KOH mount and Calcoflour white staining, both septate and aseptate branching fungal hyphae were observed in 10 samples (66.67%) out of 15 (**Figure 1**) and(**Figure 2**).

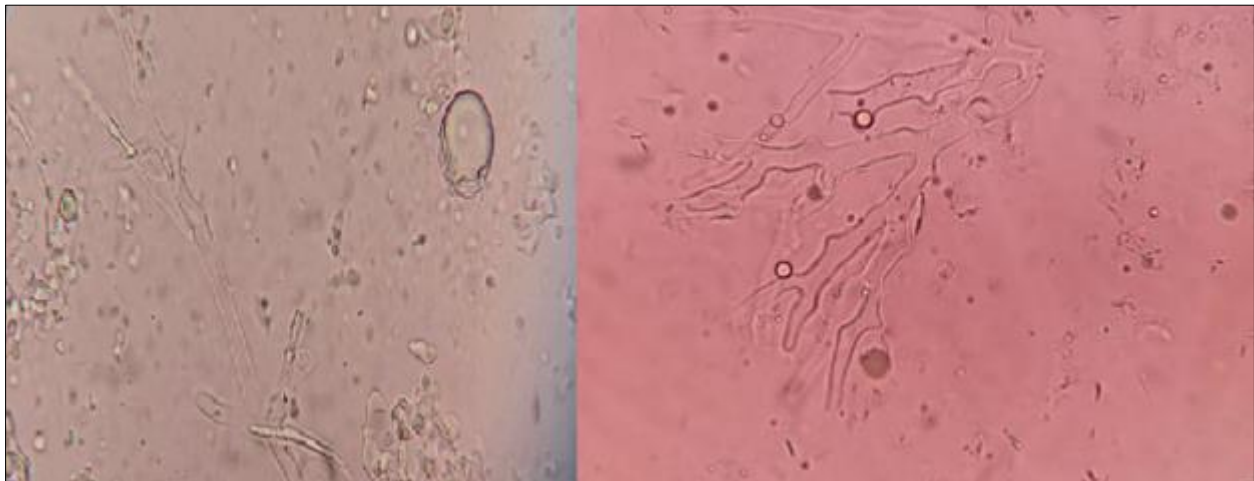


Figure 1 Broad aseptate branching hyphae seen in 40% KOH mount(Magnification:40X)



Figure 2 Broad aseptate branching hyphae seen in Calcofluor (Magnification:40X)

Fungal culture was requested for 14 samples, out of which molds were isolated in 12 (85.7%) samples. **(Figure 3)**



Figure 3 Salt and Pepper appearance of Fungal colony on SDA after 48-72 hours of incubation(24 Celcius and 37 Celcius)

Table 2 Correlation of Diagnostic modalities

Test#	Number of Samples positive for KOH/Calcofluor	Number of samples positive for Fungal Culture*	Number of sample positive HPE**
Number of samples positive for KOH/Calcofluor	10	7	5
Number of samples positive for Fungal Culture*	7	12	7
Number of sample positive HPE**	4#	7	7

*In 1 case ,culture was not requested therefore not done; **In 5 cases histopathological examination was not requested. Of the 7 Histopathology positive for mucormycosis, 3 were reported as invasive mucormycosis, 1 as Aspergilloma and 3 as Mycotic infection due to Mucor species ; # 4 cases were diagnosed by all the three methods i.e., KOH, Culture and histopathology.

The most common isolate in fungal culture was *Rhizopus* species (53.33%) and the other species isolated were *Aspergillus flavus* (8.3%), *Aspergillus fumigatus*(8.3%),*Rhizomucor* spp.(8.3%) and *Mucor* spp. (8.3%). Histopathological examination was done in 8 samples and 7 were positive for mucormycosis and 1 sample had shown multiple

aspergilloma. Out of 8 samples which showed invasive fungal infection in Histopathological examination, 7 samples (87.5%) yielded fungal growth (**Table 3**)

Table 3 Correlation of different diagnostic modalities with each other taking and HPE and associated risk patient factors

Clinical diagnosis (number of cases)	KOH /calcoflour findings (number of cases)	Fungus isolated in Culture (number of cases)	Histopathological Diagnosis (number of cases)
Nasal mucormycosis(8)	Aseptate fungal hyphae(5) Branching septate fungal hyphae seen(1)	Rhizopus species(7) **	Invasive Mycotic infection (Mucormycosis) (4)*
Rhino cerebral mucormycosis(2)	Aseptate fungal hyphae(2)	Rhizopus species(2)	Invasive Mycotic infection (Mucormycosis). (1)***
Sino-nasal mucormycosis(2)	Aseptate fungal hyphae(2)	Rhizomucor S(1) Sterile(1)	Invasive Mycotic infection (Mucormycosis).(2)
Multiorgan mucormycosis (1)	Aseptate hyphae seen	sterile	Not done
Clinical diagnosis (number of cases)	KOH /calcoflour findings (number of cases)	Fungus isolated in Culture (number of cases)	Histopathological Diagnosis (number of cases)
Persistent Post COVID lung disease (1)	No fungal element seen	Aspergillus flavus	Not done
Left sided pulmonary thromboembolism with invasive aspergillosis (1)	No fungal element seen.	<i>Aspergillus flavus</i>	Multiple aspergilloma

*Histopathology was not done in 4 cases; **Fungal culture was not performed in 1 sample; *** Histopathology was not done in 1 case

As seen in Table 3, the most common clinical presentation was Nasal mucormycosis (53.33%) followed by Rhino – cerebral mucormycosis (13.3%) and Sino-nasal mucormycosis(13.3%). Fungal culture results correlated with Histopathology as out of 8 samples for which HPE was performed 7(87.5%) yielded fungal growth consistent with HPE. The results for culture versus Histopathology was 100 % for cases clinically diagnosed as Nasal mucormycosis and Sino-nasal mucormycosis. Out of 15 patients, 4 patients succumbed to mucormycosis during the hospital stay and the mortality rate was 20%.

4. Discussion

In this study, the predominance of male patient was similar to other studies[2,4,7]. Average age of presentation for mucormycosis was 55 years similar to John TM, et al[7]. The average duration of presentation with mucormycosis after COVID infection was 20 days in this study. Rao VUS et al, reported an average duration of presentation of 10-15 days post COVID infection [8], while Skiada A. et al reported as short as 10 days[9].

Amongst the predisposing factors for developing mucormycosis, pre-existing DM was found in 66.87% patients, previous studies have reported 80% subjects had pre-existing DM as risk factor for developing mucormycosis in COVID -19 patients [2]. Diabetes mellitus has been reported to be the major risk factor associated with mucormycosis in Mexico (72%), Iran (75%), and the USA (52%) whereas the prevalence of diabetes in mucormycosis is much lower in European countries (17–23%) [10]. HbA1c is an important indicator to monitor the glucose levels over past 3 months. In this study HbA1c was deranged in 46.67% patients with DM at the time of presentation. Diabetes mellitus is known to dysregulate immune response of the body and thus making such individuals prone to many types of infections easily. Hyperglycemia leads to decrease in chemotaxis and phagocytic efficiency and makes its conclusive for opportunist organisms like Zygomycetes to thrive in an acid rich environment. Hyperglycemia also helps stimulates the proliferation

of fungi is the tissue. Enzyme ketoreductase produced by *Rhizopus oryzae* gets a suitable substrate in the diabetic ketoacidosis patient as the ketone bodies are high and helps the fungi thrive well[11].

History of steroid administration amongst the patients infected with COVID-19 and developing mucormycosis was also frequent in this study, similar to previous reports[12 -16]. Steroid is the major culprit in drug-induced hyperglycemia. Steroid administration in patients with pre-existing DM has been reported to be increasingly associated in mucormycosis after Influenza infection [15]. Though in India 3–26% of mucormycosis cases have been reported from the immunocompetent host, compared to 18–19% globally [10]. In this study, corticosteroid were administered to 31.25% cases, whereas a systematic review has reported 76.3% intake of corticosteroid for the treatment of COVID-19 in patient who developed mucormycosis[2].

Considering diagnostic modality to be the most reliable for COVID-19 mucormycosis, results of this study were similar to fungal pathology in general, where fungal culture and Histopathology are considered the gold standard. Histopathology is a very important diagnostic tool to distinguish between colonization, superficial infection, culture contamination and to establish angio- invasion. Fungal staining methods prove indeed useful for early initiation of antifungal therapy in a highly suspected case, as results in this study have shown two cases with fungal hyphae on microscopy did not yield any growth in fungal culture, however both of these cases were positive for invasive fungal disease in histopathological examination. In this study the use of fluorescent Calcofluor white together with KOH mount enhanced the visualization of the characteristic fungal hyphae, similar to a previous study[17]. In this study fungal culture was positive for 87.5% with positive histopathological analysis, however a lower positivity has been reported in previous study [9].

Rhizopus species is the most common species causing mucormycosis, especially in patients with Diabetes mellitus. Results of this study also showed highest prevalence of *Rhizopus* species, similar to previous studies[2,8]. Also in this study *Aspergillus* species were also found in few case (*Aspergillus flavus* and *Aspergillus fumigatus* 8.3%, each). This could be due to less number of patients included in this study. Culture though was sterile in few cases compared to microscopy and histopathology but was important for speciation of the fungi.

The mortality rate in this study (20%) was comparable to the mortality rate of 30.7% reported by Singh AK, et al.² The mortality rate in mucormycosis in India varies between 18.8% to 61%[10].

Nasal mucormycosis was the most common diagnosis (66.67%) in this study, similar to a previous study(88.9%) [2]. Rao VUS, et al in his study reported mucormycosis pansinusitis and maxillary mucormycosis as the most common [8], while a review article by Skiada A et al, sinusitis was the most common presentation[9].

5. Conclusion

Mucormycosis in COVID 19 was associated with deranged blood sugar levels either due to poorly controlled diabetes or steroid therapy. Therefore close monitoring of blood sugar levels and glycemic control can prevent mucormycosis in most of the cases. For diagnosis, fungal fluorescent microscopy plays an important role in early and preliminary diagnosis which is helpful in timely antifungal treatment initiation. Culture correlates well with Histopathology for confirmation of mucormycosis.

Limitations

Due to paucity of data no statistical tool could be used in this study but publishing even a small data would help gather information for further understanding of mucormycosis in COVID cases. All tests could not be performed uniformly on all patients as it was a retrospective descriptive study.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Ethical Committee of the Institute. (LetterNumber:IEC/JH/IM/O2/2022/DR000111,dated:18/10/2022)

Statement of informed consent

Not applicable as study was a retrospective study

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