



Clinicopathological Study of Mucormycosis in Post Covid Patients, an Epidemic in Pandemic

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Abstract: Mucormycosis is an angio-invasive disease caused by saprophytic fungi of the order Mucorales. Mucormycosis has been established and recognized as a complication of the SARS-CoV-2 infection. Mucormycosis, well known as "black fungus" is creating an epidemic within a global pandemic. The use of different dose regimens, prominent steroids, and multiple comorbidities like Diabetes Mellitus and post-COVID could predispose to mucormycosis. The aim of the study was to study the increase in cases and morphological features of mucormycosis infection in hospitalized post-COVID patients. This prospective study was done for four months, from May 1st, 2021, to August 31st, 2021. All the suspected cases of mucormycosis specimens sent to the Department of Pathology, J.J.M. Medical College, Davangere, for histopathological diagnosis were considered. For microscopic examination, the collected samples were fixed in 10% formalin solution, processed, and stained with Haematoxylin and Eosin. Periodic Acid-Schiff special staining was done. Thirty cases of mucormycosis were collected from patients who had recovered from COVID-19. Mean age 63 ± 12 (37-80) years with Male:Female ratio being 1.3:1. The mean duration between diagnosis of COVID-19 and development of symptoms of mucor was 23.5 ± 10 (7-60) days. Rhino-orbital mucormycosis was the most typical presentation. Out of 30 cases, 28 cases (93.3%) were Type 2 Diabetes Mellitus, and 2 cases (6.6%) were nondiabetic. All patients received corticosteroids for the treatment of COVID-19. Trinity of COVID -19, Diabetes Mellitus, and Corticosteroid treatment was significant for the rise in mucormycosis cases. Mucormycosis is an invasive fungal infection and had seen a sudden surge during the second wave of COVID-19. In our study, most patients had a history of diabetes, and corticosteroid therapy with rhino-orbital mucormycosis was the most typical presentation. As the severity of the necrosis increases, the presence of fungal hyphae also increases, which leads to extensive destruction of tissue. Extra vigilance in immunosuppressed patients helps in reducing the morbidity and mortality due to mucormycosis in Post COVID era.

Keywords: Diabetes mellitus; corticosteroids; mucormycosis; post covid patients; prevalence.

INTRODUCTION

An ongoing global pandemic of coronavirus disease 2019 (COVID-19) caused by (SARS-CoV-2) was first identified in December 2019 in Wuhan, China. The WHO declared a public health emergency of international concern regarding COVID-19 on 30 January 2020 and declared a pandemic on 11 March 2020 (V Jagtap et al., 2021).

Mucormycosis is rare, accounting for 1.8 cases per 1 million per year worldwide; however, during the COVID-19 pandemic, more cases have been reported. With the

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appearance of the second COVID-19 wave in India, a remarkable rise in mucormycosis cases has been reported, with over 40,854 reported cases and 3,129 deaths so far (Singh A.K et al, Mehta S, 2021,2020). Mucormycosis, known as “black fungus” in India, is a fungal infection caused by the mucormycetes family of molds, widespread organic matter decomposers found in soil and dust. This can be through inhalation, ingestion, direct contact, or traumatic inoculation (Reid G et al., 2020).

Mucormycosis occurs commonly in patients with immunosuppression like diabetic Mellitus and those on corticosteroids. Steroids are known to decrease immunity and raise blood sugar levels and also tend to increase clotting factors and fibrinogen concentrations in patients. This increases the opportunity for pathogens to evade the human immune system and infect the host. Rhino-orbital cerebral mucormycosis almost always occurs in immunocompromised hosts, including patients with uncontrolled diabetes mellitus, hematologic cancers, and solid organ or hematopoietic stem cell transplants (Singh A.K et al., 2021). Mucormycosis invades sinus tissues within 3 to 4 weeks (Bhatt K et al., 2021), and rhino cerebral mucormycosis can present with unusual signs and symptoms such as nasal blockage, crusting, proptosis, facial edema, ptosis, chemosis, ophthalmoplegia, in addition to headache, fever, and other neurological symptoms (Chavda V, 2021).

The current survival of patients without brain involvement can be up to 50%-80%, but the survival drops to 20% with brain involvement. It is thus vital to be aware that COVID-19 patients, particularly those with immunocompromised and diabetic patients, may acquire secondary fungal infections, and early detection is critical (Gangneux J.P et al., 2020). Other contributing factors which increase susceptibility to secondary infections are increased interleukin (IL)-1 and IL-6 with tumor necrosis factor-alpha and decreased levels of CD4 and CD8 T cells; persistent lymphopenia is also a contributor (Bhatt K et al., 2021). The high suspicion or diagnosis of mucormycosis triggers a medical and surgical emergency with the delay in treatment and increasing morbidity and mortality.

Mucormycosis were seen previously in immunocompromised patients. COVID19 is a recent onset pandemic and studying mucormycosis among patients who are post COVID and who have received corticosteroid therapy. Not many studies have been done on mucormycosis in Post COVID patients, and in our research, we looked into the severity of necrosis to the presence of fungal hyphae, which indicates the intensity of tissue destruction is the novelty in our study. The aim of the study was to study the increase in cases and morphological features of mucormycosis infection in hospitalized post-COVID patients.

MATERIALS AND METHODS

This prospective study was done for four months, from May 1st, 2021, to August 31st, 2021, in J.J.M Medical college, Davangere, Karnataka, India. All the suspected cases of mucormycosis from Rhino-Orbital-tissue specimens were sent to the Department of Pathology in a tertiary care hospital for histopathological diagnosis. History was collected, including the complaints, Post Covid status, comorbidities, and treatment. For microscopic examination, the collected specimens were fixed in 10% formalin solution, processed, and stained with Haematoxylin and Eosin. Periodic Acid-Schiff special staining was done on all the samples.

This study was reviewed and approved by the ethics review committee at the study site Jagadguru Jayadeva Murugarajendra Medical College, India. All patients provided informed consent before participating in the study. Inclusion criteria all cases of mucormycosis in post-COVID patients. Exclusion criteria Non-COVID patients.

Sample size 30 cases of mucormycosis in post-COVID patients. Data was entered in the excel spreadsheet. Descriptive statistics of the explanatory and outcome variables were calculated by the mean, standard deviation for quantitative variables, frequency, and proportions for qualitative variables. SPSS (Statistical Package for Social Sciences) version 20. IBM SPSS statistics was used to perform the statistical analysis.

RESULTS AND DISCUSSION

Table 1. Age Distribution

Age Group (Years)	Frequency/Number of Cases	Percent (%)
31-40	6	20.0
41-50	16	53.3
51-60	5	16.7
> 60	3	10.0
Total	30	100.0

We identified 30 cases of mucormycosis. The age of patients ranged from 31 to 70 years. Majority in the age group (41-50years) followed by 31-40 years, 51-60 years and >60years (Table:1). Out of 30 cases, Male:Female ratio 1.3:1 (Table:2).

Table 2. Sex Distribution

Sex	Frequency	Percent(%)
Female	13	43.3
Male	17	56.7
Total	30	100.0

Table 3. Mean Duration Between Diagnosis of Covid-19 & Development of Mucor Symptoms

	Number of cases	Minimum	Maximum	Mean	Std. Deviation
Post-COVID (days)	30	7	60	24.97	13.612

The mean duration between diagnosis of COVID-19 and development of symptoms of mucor is a minimum of 7 days to a maximum of 60 days post covid (Table 3).

Table 4. Site Predilection

Site	Frequency	Percent(%)
Orbito facial	1	3.3
Rhino orbital	28	93.3
Rhino sinusoidal	1	3.3
Total	30	100.0

Among 30 cases, the most common site involved in the Rhino orbital region, seen in 28 cases (93.3%) and one case (3.3%), each orbito-facial and sinusoidal rhino region is involved (Table 4).

Table 5. Association of Diabetes Mellitus and Mucormycosis

Diabetes Mellitus	Mucormycosis	
	Frequency	Percent (%)
No	2	6.7
Yes	28	93.3
Total	30	100.0

Of the 30 patients, 28 patients(93.3%) had Diabetes mellitus and 2 patients(6.7%) were non diabetic. All of these 30 patients(100%) received corticosteroid therapy during their COVID treatment (Table 5).

Table 6. Association of Inflammation and Necrosis with Mucormycosis

Inflammation/Necrosis	Mucormycosis	
	Frequency	Percent (%)
Mild	16	53.3
Moderate	7	23.3
Severe	7	23.3
Total	30	100.0

Among 30 cases, 16 cases (53.3%) had mild inflammation and necrosis, and 7 cases (23.3%) each had moderate and severe inflammation and necrosis (Table 6). The grading was done by looking at the amount of necrosis present under low power light microscopy in 10 low power fields. Mild<30% necrosis/10 low power field, Moderate 30%-50% necrosis/10 low power field and Severe >50% necrosis/10 low power field.

Table 7. Association of Necrosis with Fungal Hyphae

Necrosis	Fungal Hyphae			Total
	Mild	Moderate	Severe	
Mild	14 46.7%	2 6.7%	0 0.0%	16 53.3%
Moderate	0 0.0%	7 23.3%	0 0.0%	7 23.3%
Severe	0 0.0%	0 0.0%	7 23.3%	7 23.3%
Total	14 46.7%	9 30.0%	7 23.3%	30 100.0%

The above Table 7 shows that 14 cases (46.7%) had mild necrosis and presence of fungal hyphae, 2 cases (6.7%) had soft necrosis and moderate fungal hyphae accounting for a total of 16 cases (53.3%). Seven cases (23.3%) each had moderate and severe necrosis with moderate and severe fungal hyphae. The classification is based on the number of fungal hyphae present in the necrosis. Mild necrosis is associated with occasional fungal hyphae, moderate necrosis is associated with few fungal hyphae, and severe necrosis is associated with extensive infiltrates and balls of fungal hyphae. The literature review yielded no such studies done prior, and we tried grading necrosis in our institution. For all the 30 patients, a diagnosis of

mucormycosis was obtained on biopsy and confirmed by Per-iodic Schiff special staining.

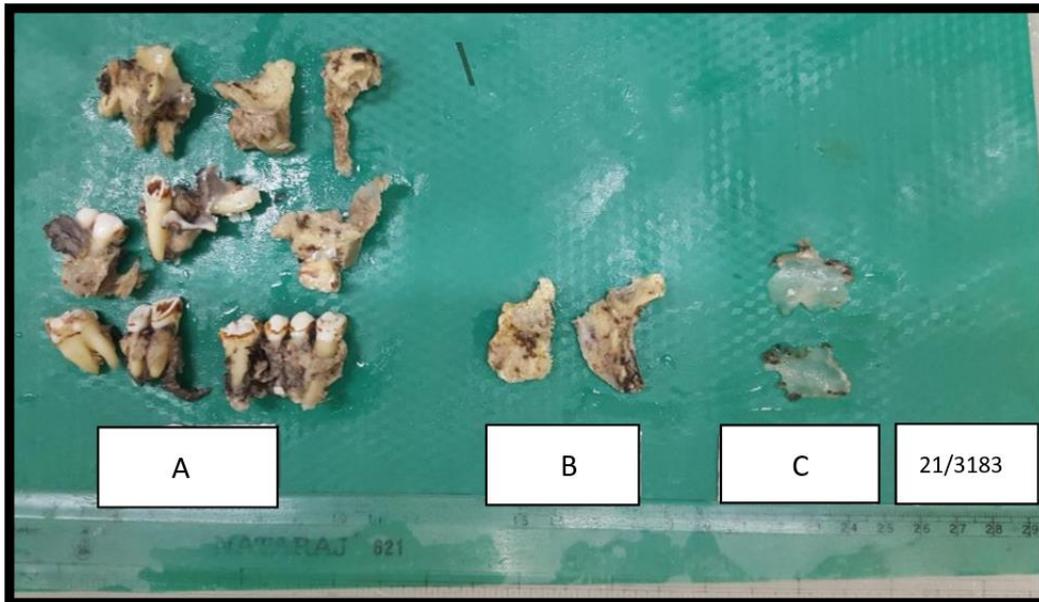


Figure 1. Gross image showing Necrotic tissue, Label A: From Maxilla showing teeth in multiple bits with attached soft tissue. Label B: From Zygoma. Label C: From Orbital floor showing grey white translucent quiet tissue bit

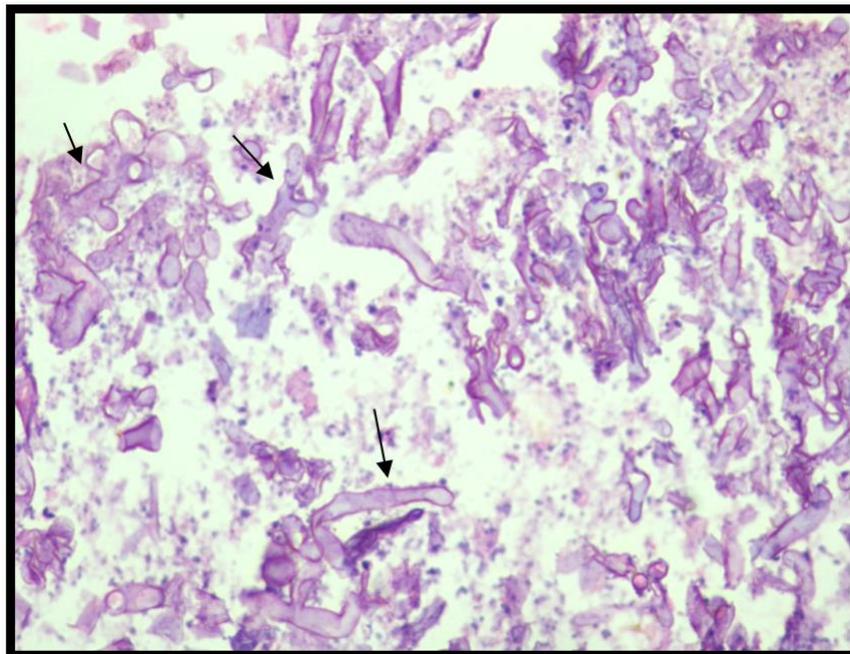


Figure 2. Hematoxylin and Eosin Stain of Mucor Showing Broad Nonseptate Hyphae with 90-degree Branching (Arrow) (40X).

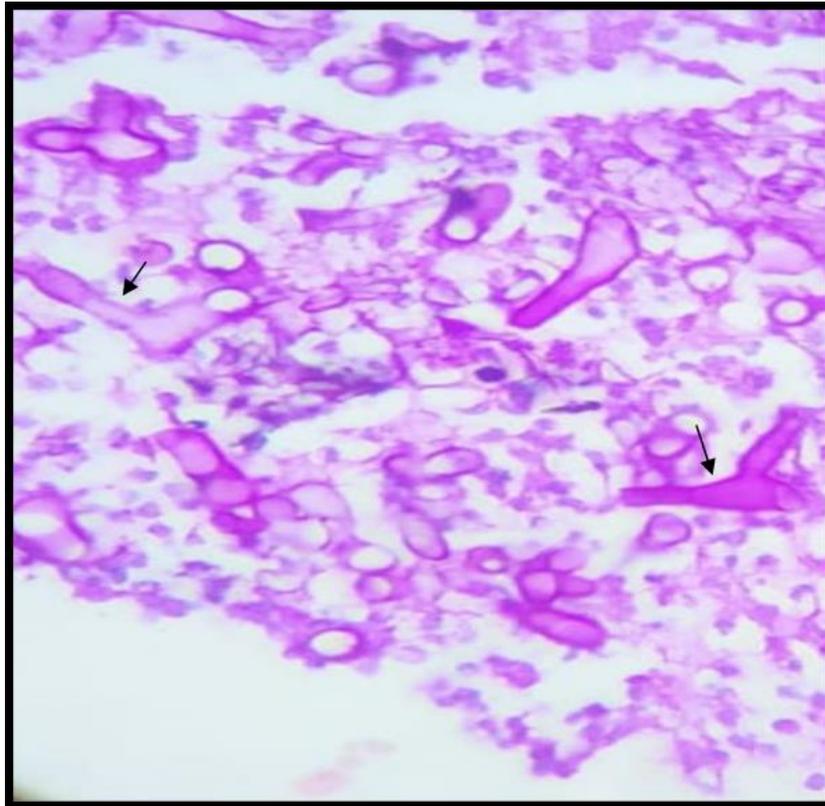


Figure 3. Periodic Acid Schiff Stain of Mucor Showing Broad Non Septate Hyphae with 90-degree branching (Arrow) (40X).

The majority of cases, i.e., 71%, are reported in India, and India has the highest burden of mucormycosis globally (Karadeniz Uğurlu, Ş, et al., 2015). Incidence varies from 0.005 to 1.7 per million population, and the global case fatality rate is as high as 46% (Werthman-Ehrenreich A, 2021). A study done by N. Colovic et al. showed that the number of cases of mucormycosis (black fungus) has increased in COVID-19 patients, both in hospitalized and those who have recovered (Čolović N et al., 2016).

The factors predisposing to the development of the infection are uncontrolled diabetes mellitus, neutropenia, elevated free iron levels, deferoxamine, hematological malignancies, stem cell transplants, and organ transplant patients on immunosuppressants (Papadogeorgakis N et al, 2010). Diabetes mellitus is the single most common risk factor for mucormycosis in India, being reported in over 50% of cases of mucormycosis (Prakash H, 2021). Goel A et al. did a study on 33 patients and showed that the fungal load in the tissue was categorized as mild, moderate, and marked with the survival rate of 75, 63, and 57%, respectively. They concluded that as the fungal load in the tissue increases, the survival rate decreases (Goel A, 2010).

In present study, the age range from 31-75 years, 17 cases were males, and 13 cases were females. Diabetes mellitus is the most common co-morbidity. The mean duration for the development of mucormycosis after the recovery from COVID-19 ranged from a minimum of 7 days to a maximum of 60 days. All 30 cases received corticosteroid therapy for COVID-19. The most common site of preference was the Rhino-orbital region (Table 8). A case report by Mehta S et al. showed that the patient was a male aged about 60 years, with Diabetes mellitus being the risk factor. The duration to develop mucormycosis symptoms after COVID-19 was three days, and the rhino-orbital region is the most common site to develop mucormycosis infection (WHO, 2019).

Table 8. Comparison with Other Studies

	Mehta S, et al	Sen et al	Karadeniz Uğurlu, Şeyda et al	Present Study
Age (Years)	60	46-74	55-77	31-45
Sex	Male	Males	3 Male 1 Female	17 Males 13 Female
Co morbidities	DM (10years)	DM	DM,CRF	DM
Post COVID-19 cases with mucormycosis	1	6	4	30
Corticosteroidal-therapy	Yes	Yes	Yes	Yes
Duration	3 days	3–42days.	9days-1 month	1-70 days
Site	Rhino -orbital	Rhino -orbital	Rhino- orbital	Rhino- orbital

Similar findings were seen in Sen et al.'s case series on six male patients aged 46-74 years, with Diabetes mellitus being the risk factor, and all 6 cases received corticosteroid therapy for COVID-19. The duration of developing mucormycosis after COVID-19 ranged from 3-42 days, with the rhino-orbital region being the most common site involved (Sen M et al., 2021). Karadeniz ugurlu seyda et al. did a similar study involving three male cases and one female case whose age ranged from 55-77 years with Diabetes mellitus and Chronic renal failure as risk factors (Karadeniz Uğurlu, Ş et al, 2015). All 4 cases received corticosteroid therapy with the mean duration to develop mucormycosis symptoms after COVID-19 was nine days to 1 month and the rhino-orbital region being the most common site of predilection. In another study by A.K.Singh et al. analyzed 101 cases with COVID-19, 80% were diabetic, and 78.9% were male, and corticosteroid therapy was taken in 76.3% of cases (Singh A K et al., 2021). All the above studies showed similar findings as in the present research done.

In addition, a case report done by A. Werthman Ehrenreich described a 33-year-old woman with mucormycosis and orbital compartment syndrome who had recovered from COVID-19 and had a history of asthma and hypertension and had very high blood glucose levels (Werthman-Ehrenreich A, 2021).

The World Health Organization strongly recommends systemic corticosteroids (oral or intravenous) rather than no corticosteroids for treating patients with severe and critical COVID-19 (WHO, 2019). They recommend not to use systemic corticosteroids therapy for non-severe cases. Based on the available literature, systemic corticosteroids are recommended for moderate to severe COVID-19 cases and have decreased mortality. But the treating physicians should handle the usage of doses for specific severe cases with strict monitoring of blood glucose levels. It is necessary to keep in mind the development of drug resistance while using prophylactic antifungal therapy. Patients should be made aware of the risks involved with the treatment and, therefore, the need for strict glycaemic control (Sen M et al., 2021).

Follow-up of the patient and small-sized samples was the drawback, but the prime aim was to report the increasing cases of mucormycosis following COVID-19 infection.

CONCLUSION

Mucormycosis is an invasive fungal infection that has seen a sudden surge during the second wave of COVID-19. In our study, most patients had diabetes and a history of corticosteroid therapy, with rhino-orbital mucormycosis being the most typical presentation. As the severity of the necrosis increases, the presence of fungal components also increases, which leads to extensive destruction of tissue. Extra vigilance in immunosuppressed patients helps in reducing the morbidity and mortality due to COVID. Therefore early diagnosis by the study of histo-morphological features of mucormycosis helps in the management with suitable and aggressive antifungal medication along with surgical debridement can improve survival. And a treating physician to be careful about the need and doses for specific severe cases with strict monitoring of blood sugar levels.

CONFLICT OF INTEREST

All of the authors have no conflict of interest

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