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Histomorphological Spectrum of Mucormycosis in Post Covid Patients

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A B S T R A C T

Introduction*:** The world faced deadly Covid-19 virus which was declared as Pandemic by WHO in March 2020. Covid-19 is known to be associated with immune dysregulation, which is further worsened by comorbidities & by widespread use of immunosuppressive agents and antibiotics.In our study, we studied the morphological spectrum of mucormycosis in Covid-19 patients and the causal relationship of mucormycosis with various clinicopathological factors. ***Material and Methods*:** An observational study conducted at tertiary care center from January 2021 to July 2021, 156 patients with histologically proven cases of mucormycosis were included in this study and concurrent or prior history of Covid-19 then obtained along with treatment given. Special stains were performed wherever necessary. Statistical analysis was done using SPSS statistics version 20. ***Results*:** Out of 156 cases 73% (n=114) were male and 26% (n=42) were female. Most of the patients were in the age group of 51-60 years. Out of 156 cases, 68% (n=106) were diabetic, 20.5% (n=32) had hypertension, 9% (n=14) had diabetes with hypertension and 11.5% (n=18) had no comorbidity. Steroid therapy was received by 64.1% (n=100) patients, of which 52% (n=81) were on oral steroids, 12.2% (n=19) were on iv steroids, 14.8% (n=23) were not on steroid therapy and conclusive history about steroid intake was not available in 21.1% (n=33). Other important findings in our study include, history of receiving O_2 therapy was given by 62.1% (n=97) patients, lymphopenia was found in 48% (n=75) patients. Statistically significant association of mucormycosis was found with diabetes mellitus and steroid use. Morphologically, 92.9% (n=145) cases showed mucormycosis alone and 7% (n=11) cases had additional infection by aspergillus. Rare morphological features like conidial heads, bony invasion, perineural invasion and granuloma formation were also seen.Conclusion*:** Early diagnosis and treatment of secondary fungal infections is essential to reduce the morbidity and mortality in patients with underlying comorbidities such as diabetes mellitus. Role of special stains is vital in highlighting the fungal hyphae with atypical morphology and in tiny necrotic biopsies.
Keywords: Covid, Mucormycosis, Aspergillosis, Fungal infection, Steroid use.

INTRODUCTION

Coronavirus disease 2019 (Covid-19) attributed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a global pandemic by the World Health Organization (WHO) in March 2020 [1-3].

The coronavirus is an enveloped, positive- sense single stranded virus that utilizes the human angiotensin-converting enzyme 2(ACE2) receptors located on cells in many organs/tissues, to gain entry and trigger a range of clinical manifestations [4].

Mucormycosis (black fungus) is an opportunistic infection which commonly affects nose, eyes & brain and has potentially fatal course specially in immunodeficient individuals like type 2 diabetes mellitus, autoimmune disorders, iatrogenic immune suppression or hematological cancers and organ transplant recipients [5].

There are several hypotheses as to what else may contribute to mucormycosis infections. The Covid 19 treatment protocol stated the use of steam inhalation for mild/asymptomatic patients in home quarantine [6]. Steam inhalation may play a role by impacting the mucosa, in addition to zinc supplementation being a fungal growth promoter [7].

Without population-based estimates, it is difficult to determine the exact incidence and prevalence of mucormycosis in Indian population. A computational-model-based method estimated a prevalence of 14 cases per 1,00,0000 individuals in India [8].

Covid 19 infection may induce significant and persistent lymphopenia which in turn increases the risk of opportunistic infections which means patients with severe cases of Covid 19 have markedly reduced absolute CD4+ and CD8+ cells [9]. It is also associated with increased pro-inflammatory markers, such as IL-1, IL-6, and TNF-α and decreased CD4 interferon-γ expression, therefore increases the susceptibility to bacterial and fungal infections [10].

In addition, use of corticosteroids during Covid-19 treatment may further reduce immune responses, allowing opportunistic fungal infections. It is therefore vital to be aware that Covid-19 patients, particularly those who are critically ill, may acquire secondary fungal infections and early detection of which is critical [11].

We received many cases of mucormycosis in second wave which prompted us to do this study. In our study, we studied the morphological spectrum of mucormycosis in Covid-19 patients and the causal relationship of mucormycosis with various clinicopathological factors.

Aim and Objectives

1. To study the causal relationship of mucormycosis with various clinicopathological factors.
2. To study the morphological spectrum of mucormycosis in Covid-19 patients.

Material and Methods

An observational, cross-sectional study was conducted at a tertiary care center from Jan 2021 to July 2021.

Histologicallydiagnosed cases of mucormycosis and or aspergillosis from Jan 2021 to July 2021 with prior or concurrent history of covid 19 infection were included in our study.

Mucormycosis and or aspergillosis cases without past or current history of covid-19 infection were excluded from our study. Also, covid-19 patients without mucormycosis and or aspergillus infection were also excluded from our study.

156 patients with histologically proven mucormycosis were included in this study and concurrent or prior history of covid-19 was then obtained. History of home isolation/hospital admission, comorbidities and treatment given including steroids, O2 therapy, antivirals and antibiotics was also obtained.

Surgical debridement material and tiny biopsies received from medicine, surgery and ENT departments were processed routinely and stained with H&E stain. Special stains such as periodic acid-Schiff (PAS) and Gomori'smethanamine silver (SM) were performed wherever necessary.

Statistical analysis was done using SPSS statics version 20 and Chi square test was used. P value of <0.05 was considered significant and a value of <0.01 was considered as highly significant.

RESULTS

Our study was conducted at a tertiary care center from January 2021 to July 2021. A total of 156 patients, who were Covid-19 positive and diagnosed with mucormycosis and aspergillosis on histopathological examination were included in this study.

Table 1: Age group Distribution

Age group	No of cases n (%)
<20	4 (2.5)
21-30	3 (1.9)
31-40	26 (16.6)
41-50	38 (24.3)
51-60	46 (29.4)
61-70	22 (14.1)
71-80	15 (9.6)
>80	2 (1.2)

Table 2: Sex Distribution

Male	Female
114 (73%)	42 (26.9%)

Majority of patients were in the age group of 51-60 years (Table 1) and were males (Table 2).

Table 3: Clinical Presentation of Patients

Clinical Presentation	No of cases n (%)
Facial Pain	104 (66.7)
Nasal Blockage	94 (60.2)
Orbital Cellulitis	64 (41.0)
Watering Eyes	31 (19.8)
Toothache	11 (7.0)
Headache	10 (6.4)
Fever	2 (1.2)

Most common clinical presentation in our patients was facial pain in 66.7% (n=104) followed by nasal blockage in 60.2% (n=94) followed by orbital cellulitis in 41% (n=64) cases. Most of the patients presented with more than one symptom (Table 3). We also received 4 cases of eyeball enucleation in patients presented with orbital cellulitis.

Table 4: Comorbidity Status

Comorbidity	No of cases n (%)
Diabetes Mellitus	106 (68)
Hypertension	32 (20.5)
Diabetes Mellitus and Hypertension	14 (9)
No comorbidity	18 (11.5)

Out of 156 cases, 68% (n=106) were diabetic, 20.5% (n=32) had hypertension, 9% (n=14) had diabetes with hypertension and 11.5% (n=18) had no comorbidity (Table 4).

Table 5: Steroid Use in study population

Steroid use	Oral	IV	No use of steroid	Not Available
No. of cases n (%)	81 (51.9)	19 (12.1)	23 (14.8)	33 (21.1)

Steroid therapy was received by 64.1% (n=100) patients, of which 52% (n=81) were on oral steroids, 12.2% (n=19) were on iv steroids, 14.8% (n=23) were not on steroid therapy and conclusive history about steroid intake was not available in 21.1% (n=33) (Table 5).

Statistically significant association of mucormycosis was found with diabetes mellitus and steroid use with P value 0.007 (highly significant) and 0.039 respectively.

Other important findings in our study were as follows-

Oxygen (O_2) therapy was given to 62.1%(n=97) patients, of which only 4%(n=6) patients required ventilatory support, lymphopenia was found in 48% (n=75) patients, history of steam inhalation in 35.8%(n=56) patients and

34.0%(n=53) patients received broad spectrum antibiotics. Fully vaccinated patients were 15% (n=24), while 8% (n=14) received a single dose of vaccine.

Statistically significant association of mucormycosis was not found with any of these findings.

Morphologically, 145 cases showed only mucor& 11 cases have mixed infection (mucor with aspergillus). Histologically mucormycosis is usually identified as broad, aseptate, ribbonlike hyphae with irregular branching and aspergillosis as septate hyphae with acute angled branching.

In our study rare morphological features like conidia (sporangia) (Fig1), perineural invasion (Fig 2), granuloma formation (Fig 3) and bony invasion (Fig 4) were also seen.

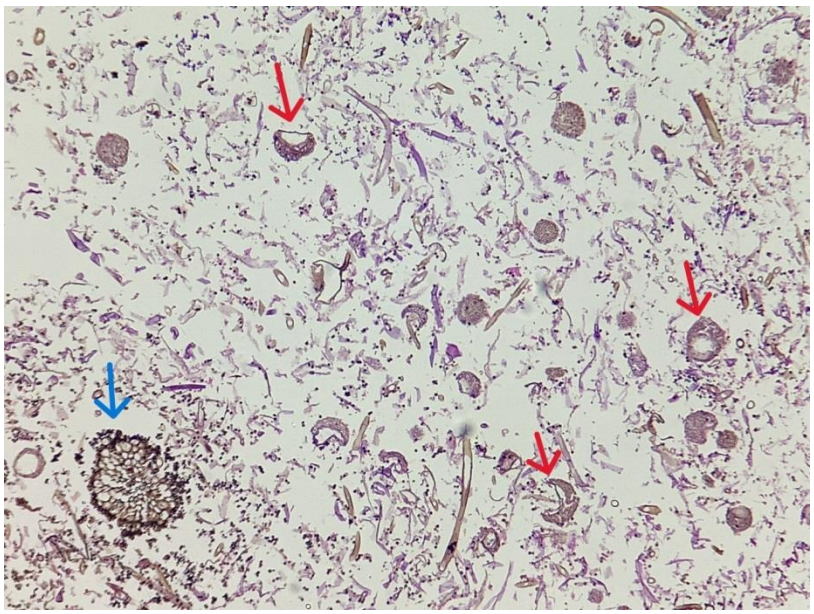

Figure 1: Conidial heads of Mucor (Red arrows) and Aspergillus (Blue arrow) (H&E 100X)

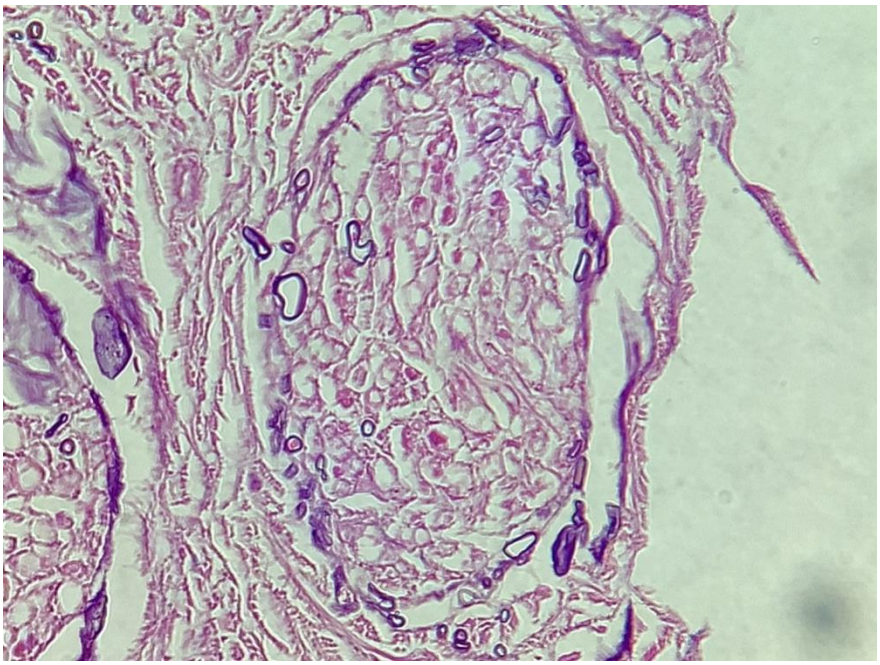

Figure 2: Perineural invasion by fungal hyphae (H&E 400X)

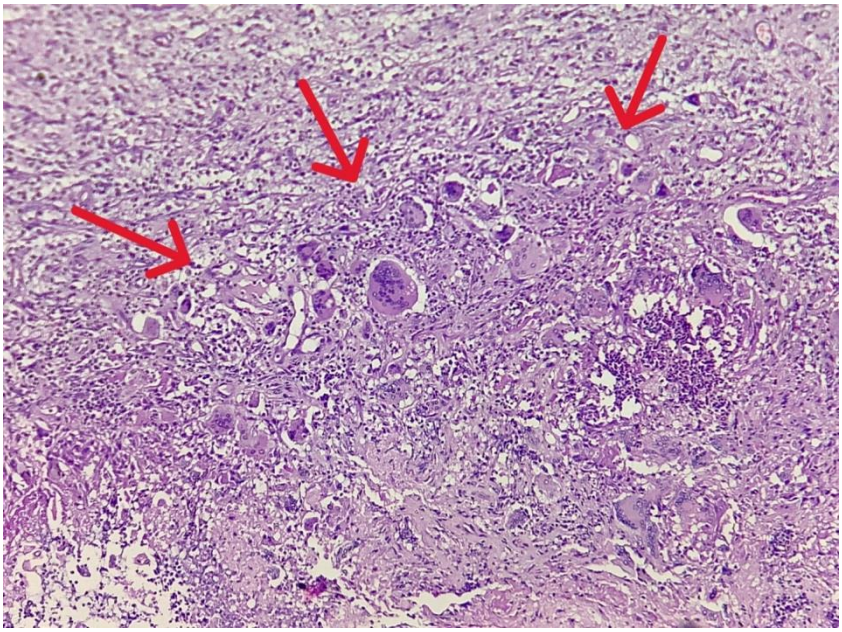

Figure 3: Granulomatous reaction (H&E100X)

In all tiny biopsies and biopsies mainly showing necrotic material special stains like PAS (Fig 4) and SM (Fig 5) were done.

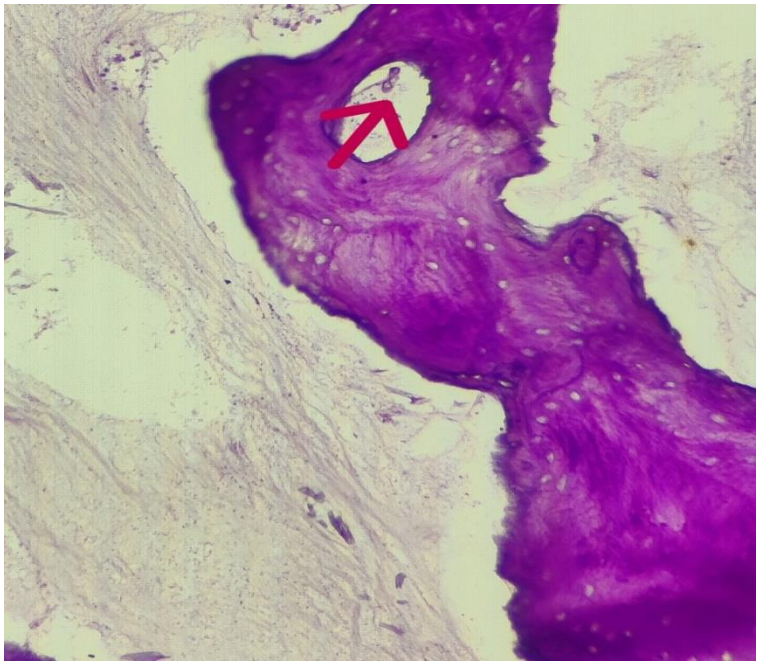

Figure 4: Bony Invasion, PAS stain (100X)

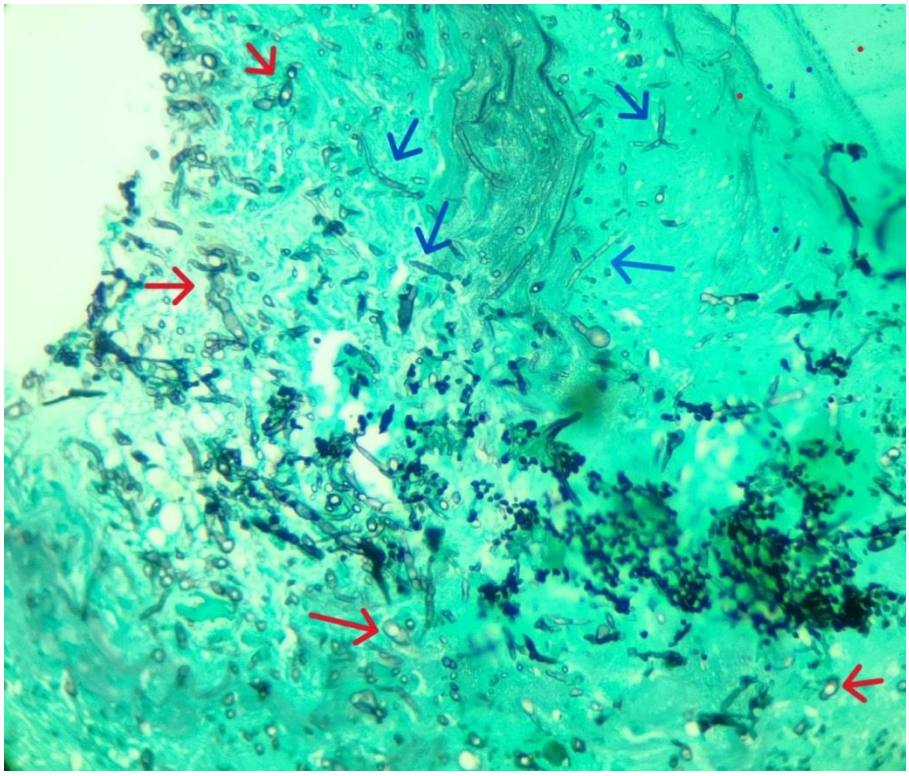

Figure 5: Mixed Infection showing Mucor (Red arrow) &Aspergillus (Blue arrow), SM stain (100X)

DISCUSSION

We have compared clinical and histopathological features of mucormycosis in Covid 19 patients based on comorbities, therapy given and vaccination status.

Table-6: Comparison of clinical presentation with other studies

Study	Clinical presentation
Selarka L *et al*., [12]	Nasal congestion n=47(100%) f/b headache n=35(74.5%)
El-Kholy NA *et al*., [13]	Headache n=27 (75%) f/b facial numbness n=24(66.7%)
Mitra S *et al*., [14]	Blurring of vision n=28 (87.5%) f/b headache n=21(65.62%)
Our study	**Facial pain n=104(66.7%) f/b nasal blockage n=94(60.25%)**

Clinical presentations of patient in our study were not in accordance with Selarka*et al*., [12], El Kholy NA *et al*., [13] and Mitra A *et al*., [14] studies (Table 6).

Table-7: Comparison of comorbidity status and therapy received with other studies

Study	Diabetes Mellitus	Steroid use	O2 therapy	Steam Inhalation
Selarka L *et al*., [12]	n =36(76.6%)	n=45(95.7%)	n=38(80.9%)	
El-Kholy NA *et al*., [13]	n=10(27.8%)	-	-	
Mitra S *et al*., [14]	n=32(100%)	n=25(78.1%)	n=9(28.1%)	
Sharma S *et al*., [15]	n= 21(91.3%)	n=23(100%)	-	
Rao VUS *et al*., [16]	n=27(96%)	n=13(46%)	n=17(61%)	
Varshney M *et al*., [17]	n= 29 (65.9)	n= 25 (56.8)		
Our study	**n=106(67.9%)**	**n=100(64.1%)**	**n=97(62.1%)**	**n=56(35.8%)**

Our findings regarding diabetes mellitus and steroid intake are in accordance with Selarka L *et al*., [12], El-Kholy NA *et al*., [13], Mitra S *et al*., [14], Sharma S *et al*., [15] , Rao VUS *et al*., [16] and Varshney M *et al*., [17] studies (Table 7).

And our findings for O_2 therapy are in accordance with Selarka L *et al*., [12], Mitra S *et al*., [14] and Rao VUS *et al*., [16] studies (Table 7).

In our study, 62.1% (n= 97) patients had history of receiving O_2 therapy and 25.6% (n=40) patients were not on O_2 therapy are in accordance with Selarka L *et al*., [12], Mitra S *et al*., [14] and Rao VUS *et al*., [16] studies (Table 7).

Table-8: Comparison of fungal morphology with other studies

Study	**Fungal morphology**		
	Mucor Only	**Aspergillus Only**	**Mucor with Aspergillus**
Selarka L *et al*., [12]	n=31(66.0%)	-	n=10(21.3%)
El-Kholy NA *et al*., [13]	n=28(77.8%)	n=11(30.6%)	-
Our study	**n=145(92.9%)**	**-**	**n=11(7.05%)**

Selarka L *et al*., [12] and El Kholy NA *et al*., [13] also had a greater number of mucor only cases same as our study (Table 8).

Our study was limited by non-availability of culture results in most of our cases and by recall bias of patients while retrieving history specially in case of steroid and antibiotic therapy.

CONCLUSION

Early diagnosis and treatment of secondary fungal infections is essential to reduce the morbidity and mortality. Role of special stains is vital in highlighting the fungal hyphae with atypical morphology and in tiny necrotic biopsies.

Impairment of barrier defense, lymphopenia, and use of immunosuppressive medications like steroids increases the susceptibility to mucormycosis in Covid 19 patients. Caution should be exercised in treating patients with underlying comorbidities such as DM. Thus, corticosteroids and broad-spectrum antibiotics should be judiciously used. These should only be administered in severe Covid 19 pneumonia and to reduce super-infections.

Ethics committee approval:
The study was approved by ByramajeeJeejeebhoy Government Medical College ethics committee.
Approval letter No. 1021274-274/ND Dept. Pathology.

List of Abbreviations:

1) Coronavirus disease 2019 – Covid 19
2) Severe acute respiratory syndrome coronavirus 2- SARS-CoV-2
3) World Health Organization – WHO
4) Angiotensin-converting enzyme 2 – ACE2
5) Periodic acid-Schiff - PAS and
6) Gomori'smethanamine silver -SM.

Conflict of Interest: None

Funding Statement: None

Author's Contributions:
AC performed the histological examination of all the cases and was a major contributor in writing the manuscript.
JK & SP helped in reporting complicated cases of mucormycosis.
DD performed the histological examination of all the cases, as all cases were reported by 2 pathologists independently.
LN was involved in overlooking the entire study.

All authors read and approved the final manuscript."

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