



RARE INFECTION TO AN EPIDEMIC: A TALE OF MUCORMYCOSIS IN ERA OF COVID-19 PANDEMIC – A STUDY OF 150 CASES

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**ABSTRACT**

Mucormycosis is an invasive disease mainly occurs in immunocompromised patients and is associated with high mortality. There is a surge in cases of mucormycosis in post COVID-19 patients. The study comprises of histopathological evaluation of 150 cases of clinically and radiologically suggestive of mucormycosis concurrent with COVID-19 disease in a period of single month. A definitive diagnosis is based on presence of aseptate fungal hyphae of Mucor species in tissue sections. The features observed on microscopy include tissue invasion by fungal hyphae, necrosis, vascular invasion, neutrophilic infiltration and granulomatous reaction. Necrosis and angioinvasion are hallmarks of infection. As mucormycosis is a deadly disease, early diagnosis is imperative to institute surgical and antifungal treatment.

**KEYWORDS :** Mucormycosis, Histopathology, COVID-19

**INTRODUCTION**

Mucormycosis is a rare and potentially lethal opportunistic aggressive fungal infection usually associated with severe immune deficiency<sup>1,2</sup>. The disease originates in the nasal/sinus mucosae after inhalation of fungal spores and take a rapidly progressive course extending to neighbouring tissues, including the orbit, and sometimes to the brain<sup>3</sup>. Early diagnosis of mucormycosis is of utmost importance, since it may improve outcome and it may also reduce the need for or extent of surgical resection<sup>4,5</sup>. Diagnosis consist of recognition of risk factors, assessment of clinical manifestations, imaging modalities and mycologic examination based on histopathology, cultures and advanced molecular techniques<sup>4</sup>. A definitive diagnosis is based on the demonstration of fungal hyphae typical for mucormycetes in biopsies of affected tissues<sup>5</sup>.

Recently, we have noticed increased incidence of invasive mucormycosis and other opportunistic fungal infections in association with COVID-19 disease. Here we present a largest case series of histopathological evaluation of 150 cases of clinically and radiologically suggestive of mucormycosis concurrent with COVID-19 disease in west India zone.

**MATERIAL AND METHODS**

The present retrospective cross-sectional study has been conducted at the Department of Pathology, Medical College and SSG Hospital, Baroda during the month of May, 2021 in era of COVID-19 pandemic.

The study included histopathological evaluation of 150 tissue biopsies received in the Histopathology section of Department of Pathology, Mediacal colloge Baroda.

The tissue biopsies sent for histopathological evaluation were from those patient who were clinically and radiologically suggestive of mucormycosis and have been infected with SARS-COV 2. The patients were between the age group of 20 to 80 years.

The tissue biopsies sent for diagnosis were examined grossly and processed as for routine paraffin sections. Samples consisting of bony bits were first decalcified with nitric acid and then processed.

These tissue sections were stained with Hematoxylin and Eosin (H & E) stain. Sections were also stained with Periodic acid – Schiff (PAS) stain to highlight the fungal hyphae as and when required.

**RESULTS**

A total of 150 cases of clinically and radiologically suggestive of mucormycosis were examined histopathologically during the month of May, 2021.

The tissue biopsies sent for histopathological evaluation were taken from various sites such as nasal cavity, paranasal sinuses (maxillary, ethmoid, sphenoid), orbital region, hard & soft palate and cerebral region.

The patients were presented with various signs and symptoms as follow:

**Facial signs & symptoms:** swelling and pain over cheek, numbness, blackening and ulcer formation and toothache.

**Nasal signs & symptoms:** nasal crusting, pain, nasal obstruction, discharge, epistaxis and tissue protrusion from nasal cavity.

**Ophthalmic signs & symptoms:** pain, periorbital swelling, reduced or complete loss of vision, ptosis, redness and watering of eye.

**Other symptoms:** headache, fever, palatal crusting and ulcer formation.

Most common presentations among these were headache, periorbital & facial swelling and nasal crusting.

**Table 1: Frequency of signs and symptoms of mucormycosis**

Clinical features	Frequency (% of total cases)
Facial symptoms	60 cases (40%)
Nasal symptoms	30 cases (20%)
Ophthalmic symptoms	77 cases (51.3%)
Headache	30 cases (20%)
Palatal lesions	12 cases (8%)

Out of 150 cases 45 cases had association with Diabetes mellitus and 11 cases were associated with Hypertension.

Out of 150 cases in this study, 108 were male and 42 were female patients.

The age of the patients were ranging from 21 to 80 years. The peak incidence of the mucormycosis was in the age group of 41 to 50 years (43 cases, 27.3%), followed by 51 to 60 years (33 cases, 23.3%) and 61 to 70 years (31 cases, 20.7%).

All these patients were diagnosed and managed for COVID 19. Most of the patients were hospitalized and have been treated with steroids.

All the patients had imaging evidence in the form of MRI brain or CT PNS revealing mucosal thickening of sinuses or erosion of adjacent bone.

The tissue biopsies sent for histopathological evaluation were examined grossly and microscopically. These tissue biopsies were in the form of soft tissue and bony bits. We have also received teeth and eye ball in few cases.

Grossly, tissue samples were friable, necrotic and grey-white to black in colour.

Out of 150 cases, 106 cases were diagnosed as Mucormycosis, while 06 cases were diagnosed as mixed fungal infection i.e. Mucormycosis and Aspergillosis on histopathology.

Other 32 cases were negative for fungal infection on histopathological evaluation.

On microscopic examination, all the cases of mucormycosis showed tissue invasion by characteristic fungal hyphae of Mucormycetes. The fungal hyphae were typically aseptate or pauciseptate with right angle branching, nonpigmented, broad and ribbon-like [Fig 1]. The hyphae varied in width, appeared crinkled and were scanty to marked in load. Angioinvasion by fungal hyphae, the hallmark of mucormycosis was seen in most of the cases [Fig 2]. We have also observed the presence of sporangia and spores of Mucor species in tissue sections [Fig 3].

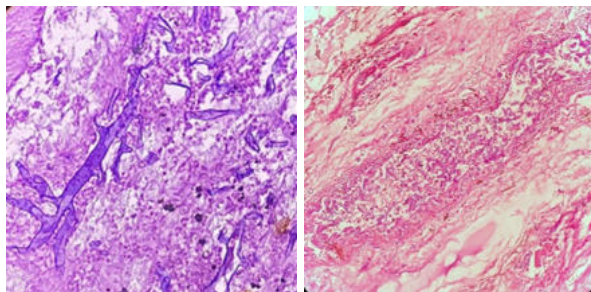


Fig 1

Fig 2

[Fig 1: Photomicrograph showing broad, aseptate, ribbon-like fungal hyphae with right angle branching; characteristic of Mucormycetes along with background necrosis (H & E stain, 40x); Fig 2: Angioinvasion by Mucormycete fungal hyphae (H & E stain, 40x)]

Other microscopic features were necrosis, inflammatory infiltration consisting of neutrophils, lymphocytes, histiocytes and presence of granulation tissue. Granulomatous reaction along with foreign body type of giant cells was also seen in few of the cases.

The decalcified sections of the hard tissue bit received showed bony trabeculae with absence of osteoblastic rimming, prominent reversal lines and presence of fungal hyphae in marrow spaces.

Cases of mixed fungal infection showed presence of fungal hyphae of both mucor and aspergillus species. They showed presence of septate fungal hyphae of aspergillus species with acute angle branching along with characteristic fruiting bodies [Fig 4].

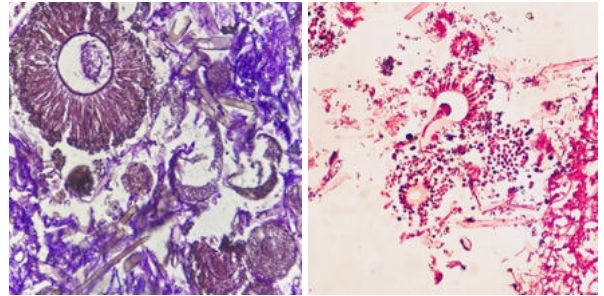


Fig 3

Fig 4

[Fig 3: Photomicrograph of mixed fungal infection showing sporangia, spores and fungal hyphae of Mucormycetes along with fruiting body of Aspergillosis (H & E stain, 40x); Fig 4: Characteristic Fruiting bodies of Aspergillosis (H & E stain, 40x)]

Tissue sections were stained with PAS stain in cases with scanty fungal hyphae and difficulty in identification of hyphae. PAS stain highlighted the wall of hyphae with magenta pink colour [Fig 5].

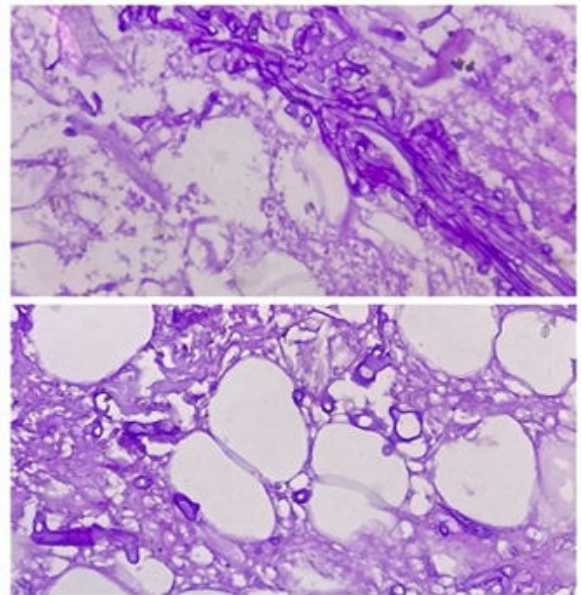


Fig 5

[Fig 5: PAS stain highlighting aseptate fungal hyphae (40x)]

## DISCUSSION

SARS COV-19 has affected millions worldwide and has presented as a global health crisis<sup>6</sup>. Now we are facing another devastating disease, Mucormycosis in patients infected with COVID-19. Mucormycosis is a rare fungal infection, that mainly affects immunocompromised persons<sup>1-5</sup>.<sup>7</sup>. The people are prone to infection because of suppression of inherent immunity with medications, high dose steroids, HIV, organ transplants, diabetes mellitus, cancer etc.<sup>7,9</sup>.

Mucormycosis is caused by proliferation of filamentous fungi of the zygomycete class of the Mucorales order. Mucorales genera are ubiquitous in the environment and can be found in soil and decomposing matter<sup>1-3,5</sup>.

Disease transmission seems to occur mainly via inhalation of spores from environmental sources. Inhaled spores cause disease in the upper and lower respiratory tracts of immunocompromised persons<sup>4,5,10</sup>.

Host defense against the Mucorales genera is primarily through macrophages that inhibit germination of spores and

neutrophils that use the oxidative burst to kill proliferating hyphal elements; thus patients who have disease affecting the function of these two cell types will be at risk for infection<sup>11</sup>.

COVID-19 patients with trauma, diabetes mellitus, glucocorticoid use, hematopoietic stem cell transplant and solid organ transplant are more likely to develop mucormycosis<sup>12</sup>. Also prolonged hospitalization, intubation/mechanical ventilation, cytokine storm lead to increased chances of fungal infection<sup>7</sup>.

In our study also, 45 out of 150 patients were having diabetes mellitus and most of these patients were hospitalized & treated with high doses of steroids.

Rhizopus arrhizus studies have demonstrated that the ketone bodies present in immunocompromised patients are metabolized by a ketone reductase, which allows them to survive in conditions with an acid medium; thus the fungi become hyphal forms in host tissues and then invade blood vessels<sup>13</sup>. Tissue necrosis is the hallmark of mucormycosis, resulting from angioinvasion and subsequent vascular thrombosis<sup>2,14</sup>.

The three most frequent primary clinical manifestations of mucormycosis are rhino-orbito-cerebral (ROCM), pulmonary and cutaneous<sup>5</sup>. Initial symptoms of ROCM are consistent with those of sinusitis and periorbital cellulitis that include eye and facial pain, facial numbness, blurring of vision. Other signs and symptoms suggestive of mucormycosis in susceptible individuals include, nasal discharge, nasal stuffiness, headache, acute ocular motility changes, acute vision loss and proptosis<sup>13,15-17</sup>.

As mucormycosis is a deadly disease, early detection at the primary site is imperative to institute surgical and antifungal treatment<sup>2,4,5</sup>. Radiological examination on CT or MRI finds an aspect of sinusitis and bone destructions, determines locoregional extension toward the orbit and brain & may identify cerebral thrombosis<sup>1,13</sup>. But whatever the location, definitive diagnosis is based on the demonstration of fungal hyphae typical of mucormycetes in biopsies of affected tissues<sup>5,18,19</sup>.

Mucorales genera typically produce non-pigmented, broad (5-20  $\mu$ m), thin walled, ribbon-like hyphae with no or few septations and right-angle branching, in contrast to *Aspergillus* species, which are typically 3-5  $\mu$ m wide, septate and form acute-angle branching<sup>4,5,13</sup>. In our study, routine H & E stain of tissue sections showed above mentioned typical features of fungal hyphae in both cases of mucormycosis and aspergillosis. In addition to fungal hyphae cases of aspergillosis showed the presence of characteristic fruiting bodies. On occasion hyphae were degenerated and very scanty. However, we were able to highlight these scanty or degenerated hyphae with the PAS stain.

Another microscopic feature observed in our study was presence of sporangia along with sporangiophore and spores of *Mucor* species on H & E stain of tissue sections in few of the cases. It is quite a rare feature which has not been mentioned in any of the published studies on Mucormycosis. Presence of sporangia of *Mucor* in tissue could have an association with severe immunocompromised state in patients of post COVID-19 mucormycosis.

Microscopically, we have observed varying degree of necrosis in all the cases of mucormycosis and fungal load was higher in necrotic tissue. So, necrotic tissue must be well sampled to identify the fungal elements. Similar features were also observed in a study by Goel A et al<sup>20</sup>.

Another microscopic feature, which has a prognostic

significance and should be mentioned in histopathological report is angioinvasion by fungal hyphae, as degree of vascular invasion has an association with survival rate<sup>20</sup>.

Spores of mucorales are easily airborne, which can cause contamination of laboratory media. Tissue identification of these molds is a very important diagnostic tool, since it distinguishes the presence of the fungus as a pathogen in the specimen from a culture contaminant and is indispensable to define whether there is blood vessel invasion<sup>4,5</sup>. It can furthermore reveal coinfection with other molds such as *aspergillus*, *candida* species<sup>4,13</sup>.

Though culture should be sent from tissue biopsy specimens for completeness, the slow growing nature of such fungi in culture will often preclude these tests from being clinically useful, as the rapid progressiveness of disease may result in fatal complications, long before the cultures have yielded results<sup>13</sup>. So, the histopathology would remain the rapid and definitive way for evaluation of fungal hyphae in tissues and should be preferred over culture. Certain authors even recommend frozen section biopsy, secondarily confirmed and completed on paraffin section<sup>1</sup>.

## CONCLUSION

Patients with COVID-19 infection have shown increased surge of mucormycosis because of immunocompromised state secondary to prolonged hospitalization, high doses of steroids, mechanical ventilation & associated comorbidities such as diabetes mellitus. To the best of our knowledge, this is the largest case series of histopathological evaluation of post COVID-19 Mucormycosis in west India zone, that too in a period of single month. Histological evaluation of mucormycosis is the mainstay of diagnosis as a definitive diagnosis is based on the demonstration of fungal hyphae typical of mucormycetes in tissue biopsies. It also allows to evaluate presence or absence of angioinvasion which is an important prognostic indicator and other fungal coinfection.

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