Original Article

Rhinocerebral mucormycosis: A clinicopathological analysis of COVID-19-associated mucormycosis

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Abstract

Background: During the coronavirus disease 2019 (COVD-19) pandemic, the incidence of mucormycosis also increased, especially affecting individuals who have had the COVID-19 infection in the past. **Aims:** The aim of the study is to assess risk factors and clinical and histopathological features of mucormycosis in post-COVID-19 cases. **Methods:** This is a retrospective study conducted in a tertiary care COVID-19-dedicated hospital, Dehradun, Uttarakhand, India, over a period of 2 months during the COVID-19 pandemic. All surgical specimens submitted for histopathology with a suspected diagnosis of mucormycosis were included. Histopathology was considered the gold standard. All histopathologically confirmed cases were studied in detail with respect to histopathology, clinico-radiological features, and microbiological results. **Results:** Of 25 cases with clinical diagnosis of mucormycosis, nine were histopathologically confirmed as mucormycosis. Seven patients had diabetes, while two did not have any co-morbidity. The fungal load was heavy in 50% cases, and the proportion of necrosis was higher with diabetes mellitus, as compared to non-diabetic and non-co-morbidity patients. Angioinvasion (33.3% cases), soft-tissue invasion (44.4%), Splendor–Hoeppli phenomenon (44.4%), and neural invasion (11.1%) were also present. Mixed infection (Mucormycosis and Aspergillus species) was present in three of the cases who also had diabetes. The microbiological investigations were positive in only 55.5% cases. **Conclusion:** Post-COVID Mucormycosis has fatal outcomes. Early diagnosis and treatment are the key to successful treatment. Early and reliable diagnosis can be offered by histopathological examination.

Keywords: Aspergillus, histology, histopathology, mixed infection, mucor, mucorales, zygomycosis

Introduction

The coronavirus disease (COVID- 19), caused by severe acute respiratory syndrome 2 (SARS-CoV-2), had resulted in massive mortality in recent past during the first and second waves of the pandemic. The resulting post-COVID complications also resulted in significant morbidity in affected patients. Of these complications, the invasive fungal infections emerged as a life-threatening acute emergency condition. Although Aspergillus and Candida species have also been reported to infect the COVID-19 patients more frequently, mucormycosis is the most severe fungal co-infection in COVID-19 cases. Rhino-orbito-cerebral mucormycosis is the fungal infection caused by filamentous fungi primarily affecting nose, paranasal sinuses, orbit, and brain. It has a mortality rate of 50% despite aggressive therapy.[1]

However, this infection is not new to humanity. It was first described as Phycomycosis or Zygomycosis in 1885 and later in mid-19th century as Mucormycosis.[2] During the COVID-19 pandemic, the prevalence of mucormycosis in India has been reported to be 0.14 cases per 1000 population, which is 80 times of the prevalence in developed countries.[3] Predisposing factors for this fatal infection include uncontrolled diabetes mellitus,

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hematological malignancy, stem cell and solid organ transplant, prolonged neutropenia, acquired immunodeficiency syndrome, iron chelation therapy with desferoxamine, and corticosteroid therapy.[1,4] It has been observed that poorly controlled diabetes with superadded steroid therapy on hit and trial basis had led to a surge in the incidence of mucormycosis infection.

Considering the morbidity and mortality associated with this co-infection, studies have been conducted recently and in the past, focusing on the clinics, radiology, and treatment of the disease. Although histopathology has played a key role in the diagnosis and facilitating the management of the disease during the pandemic, not many studies are available highlighting the same.[4,5] This study is being conducted to review the clinical, radiological, and histological features of the disease.

Materials and Methods

This retrospective cross-sectional study was conducted in the histopathology laboratory of a government tertiary COVID-dedicated hospital, located in Dehradun, Uttarakhand, India, over a period of 2 months (May and June, 2021). A total of 25 histopathology specimens, including small biopsy and resection specimens, suspected of mucormycosis on clinicoradiological grounds received in the facility during the above-mentioned period, were included in the study. Histopathology was considered as the gold standard test for diagnosis of mucormycosis.[6] The study was performed in accordance to the Helsinki Declaration of 1975 and as revised in 2000. As this study was retrospective, consent could not be taken from patients.

The morphology of fungus was studied for width of hyphae (broad/narrow), septations (present/absent), and branching (present/absent/angle and type of branching). The presence or absence of fruiting bodies or sporangiospores was also recorded. The fungal load was analyzed as mild (+), moderate (++), and heavy (+++). Necrosis was quantified as percentage of the tissue studied. Besides detailed histomorphology, the clinical and radiological findings were recorded. These included onset of disease, symptoms and organ involvement, and type of medical and surgical treatment received. Since all the patients developed signs and symptoms of mucormycosis within a few days after recovering from COVID-19 infection, complete information pertaining to COVID disease like severity of disease and type of treatment received was also taken into account. All the details related to predisposing factors/co-morbidities were also noted. Information of other diagnostic modalities for mucormycosis like microbiological culture, KOH mount, and so on was also recorded [Tables 1 and 2].

Results

A total of 25 biopsy specimens of suspected mucormycosis in COVID-19-positive cases were received for histopathological examination. Of these, 9 cases (36%) were confirmed histologically to be mucormycosis. The remaining 16 cases were negative on KOH mount, culture, and histology. The details of these nine histologically confirmed cases were further studied [Tables 1 and 2].

The affected patients were in the age group of 39 to 67 years with the mean age being 50 years. The male-to-female ratio was 1:1.1. All the cases were COVID RT-PCR-positive either presently or in the recent past. The symptoms related to fungal infection were observed after 1 to 3 weeks after COVID illness. Most of these patients had severe COVID infection in the past with 66.7% of the cases requiring oxygen and steroid therapy. Mild COVID infection was observed only in (3/9, 33.3%) cases with neither requirement of any hospitalization nor any oxygen or steroid therapy. None of the patients required ventilatory support. Immunomodulator drugs were not used in any of the patients.

The majority of patients had the associated co-morbidities, the most common being diabetes mellitus (n = 7; 77.7%) and hypertension (n = 3; 33.3%). Of the diabetic patients, two had poorly controlled diabetes. Only two of the nine cases (i.e., 22.2%) were free of any co-morbidities. None of the patients had AIDS, malignancy, or history of any organ transplantation.

The most common symptoms in the present study were facial and ocular pain and swelling. The site of involvement was confirmed on radiological investigations: computed tomography or magnetic resonance imaging, or both. It was observed that the site(s) of involvement were variable but usually limited to the head and neck region only. The most common sites involved in decreasing order were paranasal sinuses (n = 8, 88.9%), nose (n = 7; 77.8%), orbital (n = 7; 77.8%), cerebral (n = 1; 11.1%), and pulmonary (n = 1; 11.1%). When the paranasal sinuses were involved, maxillary sinus was always found to be affected.

Histologically, all the cases had broad aseptate filamentous hyphae with irregular branching, morphologically consistent with mucormycosis. Fruiting bodies were seen in two cases. The fungal load was more frequently heavy (+++) in patients

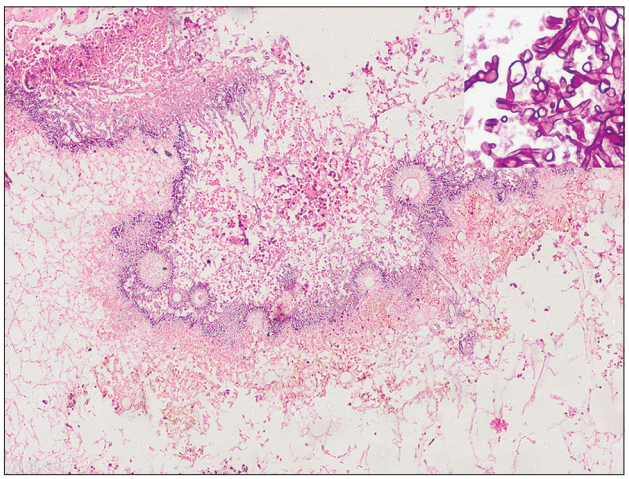

Figure 1: Mucormycosis with a heavy fungal load. The non-pigmented, irregular broad septate hyphae and the fruiting bodies are seen. [H and E, 20x] Inset shows PAS-positive Mucorales hyphae

Table 1: The clinical profile of the cases of mucormycosis (*n*=9)

Case	1	2	3	4	5	6	7	8	9
Age (years)	67	48	46	41	61	39	41	66	40
Gender	Female	Female	Female	Male	Male	Male	Male	Female	Male
Covid status	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Comorbidities present	DM, HTN	DM, HTN	DM, HTN	DM	DM	Absent	Absent	DM, HTN	DM
Duration of diabetes mellitus	Uncontrolled Diabetes for 10 years	Controlled DM for 5 yrs	Controlled DM for 15 yrs	Uncontrolled DM for 7 years	Controlled DM for 13 years	Not applicable	Not applicable	Controlled DM for 22 years	Controlled DM for 2 years
Severity of covid	Mild	Moderate	Moderate	Moderate	Moderate	Mild	Moderate	Moderate	Mild
Duration of hospital stay during covid	No hospitalization	7 days hospitalization	14 days hospitalization	19 days hospitalization	12 days hospitalization	No hospitalization	8 days hospitalization	15 days hospitalization	No hospitalization
Steroids administered	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Oxygen administered	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Ventilator support	No	No	No	No	No	No	No	No	No
Symptoms related to mucormycosis	Facial puffiness; facial puffiness, Eye pain	Eye pain, Nasal pain, facial pain	Eye pain, blurred vision, facial swelling, pain in sinuses	Eye swelling, proptosis, decreased vision	Dental caries, facial swelling, difficult chewing	Facial heaviness with tingling sensation over cheek	Facial puffiness and pain	Parasthesia with cheek pain	Facial swelling and pain Blurred vision
Involvement in mucormycosis (based on CT findings)	Sino-nasal, orbital (maxillary sinus and ethmoid sinus)	Sino-nasal, orbital (maxillary sinus); pulmonary	Sinonasal (maxillary and ethmoid sinus), orbital	rhinocerebral, orbital, pan sinusitis	Rhino-orbital, maxillary sinus	Rhino-orbital, maxillary sinus	Sinonasal (maxillary sinus)	pansinusitis,	Rhino-orbital
Surgical interventions for mucormycosis	Done	No	Done	Done	Done	Done	Done twice	Done twice	Done

DM - Diabetes mellitus; HTN - Hypertension; CT - Computerized Tomography

Table 2: Histopathological and microbiological results (*n*=9)

Case	1	2	3	4	5	6	7	8	9
Fungal Culture	Rhizopus species	Negative	Negative	Aspergillus and Rhizopus species	Positive for mucormycosis	Positive for mucormycosis	Positive for mucormycosis	Negative	Negative
KOH mount	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Negative
Type and degree of inflammation	Dense lymphoplasmacytic infiltrate	Mixed acute on chronic infiltrate	Dense lymphoplasmacytic infiltrate	Mild lymphoplasmacytic infiltrate	Dense neutrophilic	Dense lymphoplasmacytic	Mild mixed infiltrate	Dense polymorphic infiltrate	Mixed acute on chronic inflammation
Necrosis	10% Present	20% Present	40% Present	60% Present	70%	Absent	10% present	80% present	20% present
Granuloma	Epithelioid and foreign body granulomas	Foreign body granuloma present	Foreign body granuloma	Absent	present	absent	Absent	absent	present
Morphology of fungus on histopathology	Wide, aseptate hyphae ribbon; fruiting bodies	Wide aseptate l hyphae; septate hyphae having dichotomous branching	Wide aseptate hyphae, thin septate hyphae with dichotomous branching	Wide aseptate hyphae with irregular branching and fruiting bodies of aspergillus with thin septate hyphae	wide aseptate hyphae and fruiting bodies	Wide aseptate fungal hyphae with right angled branching	Wide aseptate fungal hyphae with right angled branching	Wide aseptate ribbon like hyphae with irregular branching	Wide aseptate hyphae
Fungal load	+	++	+++	+++	++	+	++	+++	+
Fungal angioinvasion	Absent	Absent	Present	Present	Absent	Absent	absent	Present	Absent
Tissue invasion	Present focally	Absent	Present	Present	Absent	Absent	absent	Present	Absent
Thrombosis	Absent	Absent	Present	Absent	Absent	Absent	Absent	Present	Absent
Splendor-Hoeppli phenomenon	absent	absent	present	present	absent	absent	Absent	present	present
Histopathology diagnosis	Mucormycosis	Mixed Infection (Aspergillus + Mucor)	Mucormycosis and Aspergillus positive	Mucormycosis and Aspergillus positive	Mucormycosis	Mucormycosis	Mucormycosis	Mucormycosis	Mucormycosis

with diabetes mellitus (n = 3/6; 50%) in comparison to mild-moderate fungal load in those without diabetes [Figure 1]. The amount of necrosis varied from 10 to 70% in patients with associated diabetes while nil to 10% in patients without any co-morbidity. Background inflammation was variable. However, granulomas (including epithelioid cell and foreign body granulomas) were present in 33.3% cases. Angio-invasion was present in three cases (33.3%), while soft tissue invasion was found in four cases (44.4%) [Figure 2a]. Intraneural invasion was noted in one case (11.1%) [Figure 2c]. Bone invasion was not seen in any case. Intravascular thrombi were present in two cases (22.2%) [Figure 2d]. Angio-invasion [Figure 2b] and tissue invasion were altogether absent in the patients without diabetes or any other co-morbidity. The Splendor–Hoeppli phenomenon, that is, presence of eosinophilic material surrounding the fungi, was noted in 4 cases (44.4%) [Figure 2d].

Mixed infection of mucormycosis and aspergillus was also observed in (3/9, 33.3%) of the cases in the present study. These three cases had mixed fungal infection with two types of hyphae and fruiting bodies. Besides, the broad aseptate hyphae of mucor, another fungus with thin septate hyphae with acute angled branching and fruiting bodies (morphologically consistent with Aspergillus sp.), was found. All the patients with mixed fungal co-infection were diabetic and had a moderate to severe grade of COVID illness in past.

Fungal culture and KOH mount correlated with the histopathology diagnosis in 55.5% (n = 5) cases. There were two cases in which either of the two were negative. The histopathological diagnosis was considered as a gold standard for positivity of fungal infection. All the patients were treated similarly with adequate local debridement of the infected and necrotic tissue along with intravenous amphotericin B in addition to treatment of COVID-19 disease.

Discussion

Mucormycosis is a highly aggressive and many a time fatal invasive fungal infection, which was very commonly encountered in India during the surge of COVID-19 pandemic. Among the Mucorales, the most common causative agent is Rhizopus species, followed by Mucor species and Lichtheimia crymbifera.[6] The mononuclear and polymorphonuclear phagocytes in humans, under normal conditions, kill mucorales by generation of oxidative metabolites and defensins. Hence, the patients having neutropenia with dysfunctional phagocytes are more prone to develop invasive mucormycosis. The non-segmented negative sense RNA virus in COVID-19 infection causes significant lymphopenia. As the infection progresses, viral replication increases, leading to increased inflammatory response with influx of monocytes and neutrophils. This results in endothelitis, endothelial barrier disruption, dysfunctional alveolar capillary oxygen transmission, and impaired oxygen diffusion capacity. This predisposes a COVID-19-infected individual to co-infection by fungi.[2]

Despite the pandemicity of the infection, the cases of mucormycosis were more frequently reported from India. This is explained by a higher prevelance of uncontrolled diabetes. In fact, administration of steroids also induces hyperglycemia in an individual. This explains the occurrence of mucormycosis in individuals without diabetes mellitus or any co-morbidity. In the present study, both the cases without any co-morbidity had received steroid therapy during the past COVID-19 infection. The presence of acetone reductase in mucor favors their growth in presence of high glucose levels and low pH.[7] Another mechanism involves hyperglycemia-induced and COVID-19-related cytokine (Interleukin-6)-induced increased availability of free iron in tissues. Thus, the combination of low pH, high glucose, and increased free iron allows mucor to thrive in the fertile grounds provided by the COVID-19 infection.[8]

In addition to steroid-induced hyperglycemia, steroids cause impaired migration of neutrophils to the inflammatory site because of the inhibitory effect of cytokines and chemokines. Steroids further compromised the immune status in patients infected with COVID-19, leading to the development of many opportunistic infections, of which invasive mucormycosis has a very high morbidity and mortality. Supporting this hypothesis, there are studies by Mehta and Pandey,[9] Werthman-Ehrenreich,[10] and Chowdhary *et al.*[11] These studies have shown the development of post-COVID fungal infection after the use of steroids as per the treatment protocol of COVID-19. Contradictory to this, development of post-COVID mucormycosis was observed in three cases in the present study, without the use of any steroids. This could be explained by the fact that the immune status of COVID-19 is already compromised, which is further augmented by the use of steroids.[2]

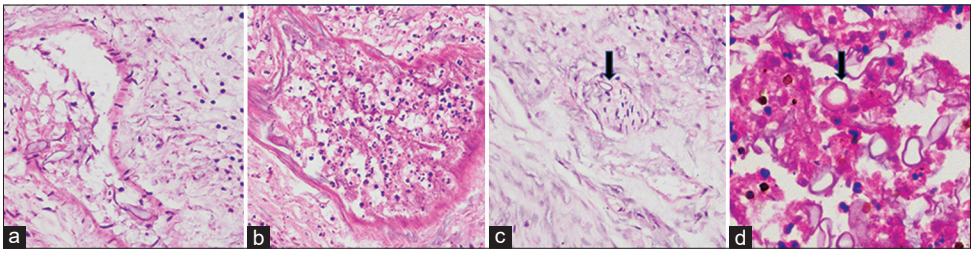

Figure 2: (a) Angioinvasion by Mucorales hyphae [H and E, 40x]. (b) Angioinvasion leading to thrombus formation [H and E,40x]. (c) Neural invasion by Mucor (black arrow) [H and E, 40x]. (d) Splender Hoeppli phenomenon (black arrow) [H and E, 100x]

The most common sites of occurrence of mucormycosis were paranasal sinuses, followed by nose and orbit, while the cerebral involvement was uncommon in present series. Similar findings were reported by previous reports.[2,5] Another study from pre-COVID era (2005–2007) reported pulmonary followed by rhinocerebral and soft tissues to be commonly afflicted sites.[6]

The success of the treatment of mucormycosis largely depends on how early the condition is diagnosed. The risk factors need to be reversed, antifungal therapy should be started, and surgical debridement should be performed as early as possible. The ancillary investigations done for the diagnosis include KOH mount and culture. The KOH mount may give false negative results, while fungal culture takes time.[12,13] The sensitivity of direct microscopy using KOH mount for diagnosis of mucormycosis has been reported to be 90%, and that of culture only 50%.[12] Currently, certain biochemical and molecular tests are also available, which can give rapid results, but these are still evolving. The sensitivity of molecular tests is 75%. In such scenario, histopathological examination gives promising results, with a reported sensitivity of 80%.[13] By utilizing faster processing techniques like microwave fixation, the turn-around time of report can be reduced to a day or two. The histopathology can reliably identify the organism by utilization of special stains: periodic acid Schiff (PAS) stain and methenamine silver stains. The identification of species, *per se*, is important more for epidemiological purposes rather than from treatment perspective.[13] In present study, histopathology was positive in 100% cases, while KOH mount and culture were positive in 55.5% cases.

The hyphae of Mucorales are broad, irregular, ribbon-like non-pigmented, and non-septate to pauciseptae with variable and often perpendicular (>45–90°) branching. The width of the hyphae varies between 6 and 25 μm. Although aseptate to pauciseptate, false septation may be seen due to folding of the hyphae.[12,13] The septations are rare and irregularly placed.[13,14] The hyphae of Aspergillus are narrower (3–5 μm wide), regularly septate, and more uniform and show acute angled or dichotomous branching.[12] The histopathological hallmarks included presence of characteristic PAS-positive non-pigmented broad hyphae with or without fruiting bodies (100%), necrosis, angioinvasion, and perineural invasion.[13-15] Sometimes, angioinvasion can lead to vascular thrombi formation and coagulative necrosis in surrounding tissue. Tissue response can be variable with acute or chronic inflammation including formation of granulomas and necrosis. The Splendor–Hoeppli phenomenon, first described by Splendor (1908) and later by Hoeppli (1932), can be seen in mucormycosis. It is the eosinophilic material deposition surrounding the fungus and represents the antigen–antibody complex formation.[13] In present study, this phenomenon was observed in 44.4% cases. In present study, the fungal load was moderate to heavy in diabetic patients and mild to moderate in non-diabetic and non-comorbid patients.

The histopathological features like heavy fungal load, extent of necrosis, angioinvasion, and tissue invasion were not seen in non-diabetic and non-comorbid patients. However, the sample size in the present study was low to obtain any significant impression. A multi-institutional study with a large number of cases is required in future.

Conclusion

In clinically suspected cases, early diagnosis and early administration of aggressive treatment can prevent the mortality and morbidity in cases of mucormycosis. Diagnosis of mucormycosis requires a high degree of clinical suspicion and should be supported by laboratory diagnosis. Histopathology is the most sensitive investigation which is widely available for the diagnosis of this fatal invasive fungal disease.

List of abbreviations

Abbreviation	Definition
COVID-19	Coronavirus disease 2019
RT-PCR	Reverse transcriptase polymerase chain reaction
AIDS	Acquired immunodeficiency syndrome
PAS	Periodic acid Schiff

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Conflicts of interest

There are no conflicts of interest.

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