Original research article

Expanding the Spectrum of Fatal Necrotizing Fungal Infections Presented as Sinonasal and Rhino Orbital Mucormycosis and Aspergillosis in Post Coronavirus Disease.

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Abstract
The present study is aimed to clinico pathologically characterize a subset of mucormycosis and aspergillosis cases presented as Sinonasal and rhino orbital fungal infection in post coronavirus disease. The study comprises a microscopic analysis of specific histopathologic variables on 35 cases of mucormycosis that were diagnosed and treated in a six month period. We reviewed Sino nasal and orbital biopsies of 35 patients whose specimens received in a tertiary care hospital histopathology lab. All patients were admitted for suspected fungal sinonasal and rhino orbital infections. Detailed microscopic examination and clinicopathological correlation study were done. Fungal load in the tissue (graded as mild, moderate and marked), degree of neutrophilic and granulomatous response, tissue invasion and necrosis were graded.
We noted that necrosis is seen in all cases. Co infections with aspergillosis are also commonly noted. Vascular changes are marked. Angioinvasion, bone marrow invasion and peri neural/neural invasion are frequently noted. Fungal culture can become negative in histologically proven mucormycosis. History of diabetes present in majority of cases. Use of glucocorticoids and hypoxemia is also noted in many cases. The prevalence of mucormycosis fungal infections is very high in post coronavirus patients especially in diabetic patients. Histopathologic examination remains one of the major diagnostic tools in diagnosis of mucormycosis. Early diagnosis with surgical excision, appropriate debridement, proper antifungal treatment and management of risk factors lead to subsequent reduction in mortality and morbidity.
Keywords: Aspergillosis, Coronavirus disease,Mucormycosis,Sinonasal,rhino orbital

Introduction

Coronavirus disease is caused by severe acute respiratory syndrome coronavirus 2.There have been many twirls in pathophysiology, diagnosis, treatment and fate of COVID-19 infections[1] Amid this pandemic in India, rhino-orbital-cerebral Mucormycosis (ROCM) has come to light as emerging new complications. It is present globally, but certain agents are prevalent in India.[2] Mucormycosis is a rare but aggressive, angioinvasive and opportunistic fungal disease caused by Mucorales. It mainly involves the patients with deranged immune systems among which uncontrolled diabetes mellitus associated with COVID-19 is the most common cause.[1]. In this scenario, histological examination can reveal optimal uses of nephrotoxic drugs and radical surgeries.

Materials and Methods

A Descriptive observational study was undertaken at Pathology Department over a period of 6 months, from December 2020 to May 2021.The study included all cases of Sino nasal and rhino orbital mucormycosis that were diagnosed in histopathology lab who had past history of coronavirus infection. The patients' presentation details, imaging findings, history of comorbidities, histopathology findings were obtained, recorded and analyzed. All patients were operated upon, keeping complete surgical debridement as the aim, along with intravenous amphotericin administration.

By computed tomography /magnetic resonance imaging and Histopathological examination according to site they are divided into 3 stages:Stage I: Infection of the nasal mucosa and sinuses.,Stage II: Orbital involvement and Stage III: Cerebral involvement.

All received biopsies were stained by Haematoxylene and Eosin stain, Periodic Acid Schiff (PAS)stain and Gomori's Methenamine silver stain (GMS)stain and Ziehl-Neelsen stain (in cases of granulomatous inflammation). Detailed microscopic examination and clinicopathological correlation study were done.

The sample preparations were studied for the following microscopic details

- Fungal load (graded as mild, moderate and marked).
- Fungal morphology delineated by H & E, GMS, and PAS as broad pauci/aseptate hyphae with irregular/right angle branching were identified as *Mucorales* species. Presence of mixed infection mucormycosis and aspergillosis were noted. Presence of Sporangiospores of mucor and fruiting bodies of aspergillosis were also noted.
- Culture reportswere collected.
- Composition of inflammatory infiltrate.
- The presence of neutrophilic response was graded (mild, moderate to marked) depending on the intensity of the infiltrate.
- Presence of granulomatous inflammation and giant cell reactions were noted.
- Degree of tissue necrosis Tissue necrosis was semi-quantitated as percentage of total area of the tissue sampled.
- Tissue invasion into soft tissues and bone, type of spread (angio/perineural) by fungus noted.
- Invasive ROCM was further categorized as acute when the duration of signs and symptoms was equal to or less than 4 weeks and chronic when the duration of signs and symptoms was more than 4 weeks.

Available clinical data including symptoms and signs, duration between coronavirus disease and development of mucormycosis, treatment for coronavirus disease, history of co-morbidity also collected and analyzed.

Results

A total of 35 patients of post covid mucormycosis were clinically diagnosed and confirmed at histology during the six months study period.

Males predominated (60%, *n* =21), with the male: female ratio being 1.5: 1. The patients' age ranged from 32 to 70 years with the larger incidence in the 61-70 years (50%) age group. Approximately 82% (*n* = 29) were immunocompromised with diabetes mellitus (80%, (*n* = 28) as the commonest factor among these patients. One patient with HIV infection developed mucor infection. 60% of patients(n=21) had history of corticosteroid taken for covid treatment.48 % patients(n=17) had history of hypoxemia.

Of the total 35 cases of invasive type, all cases are acute having a history of less than 4 weeks.

Of the total 35 cases, 30 cases were in stage 1(having only Sino nasal involvements) and 5 cases in stage 2(having Sino nasal and orbital involvement.)

Of the total 35 cases, only one case had received complete vaccination.

Duration between covid infection and development of mucormycosis was 14 days to 45 days found.

Of the total 35 cases, 8 cases had bilateral involvement.

[Table 1] shows frequency of signs and symptoms of rhino-orbital mucormycosis in 35 patients.

Table 1: Frequency, signs and symptoms of cases of rhino-orbital mucormycosis

Clinical features (non ophthalmic)	(n =35) (approximate % of total cases)
Facial pain	24(68%)
Facial numbness	5(14%)
headache	15(42%)
toothache	5 (14%)
Burning sensation of mouth	2 (6%)
Difficulty in swallowing	2(6%)
Nasal discharge	2(6%)
Gum bleeding	1(3%)
Nasal blockage	1(3%)
Epistaxis	1 (3%)
Clinical features (Ophthalmic)	
Swelling around eye	9 (26%)
Decreased vision	4(11%)
Eye pain	1(3%)
Watering from eye	1(3%)

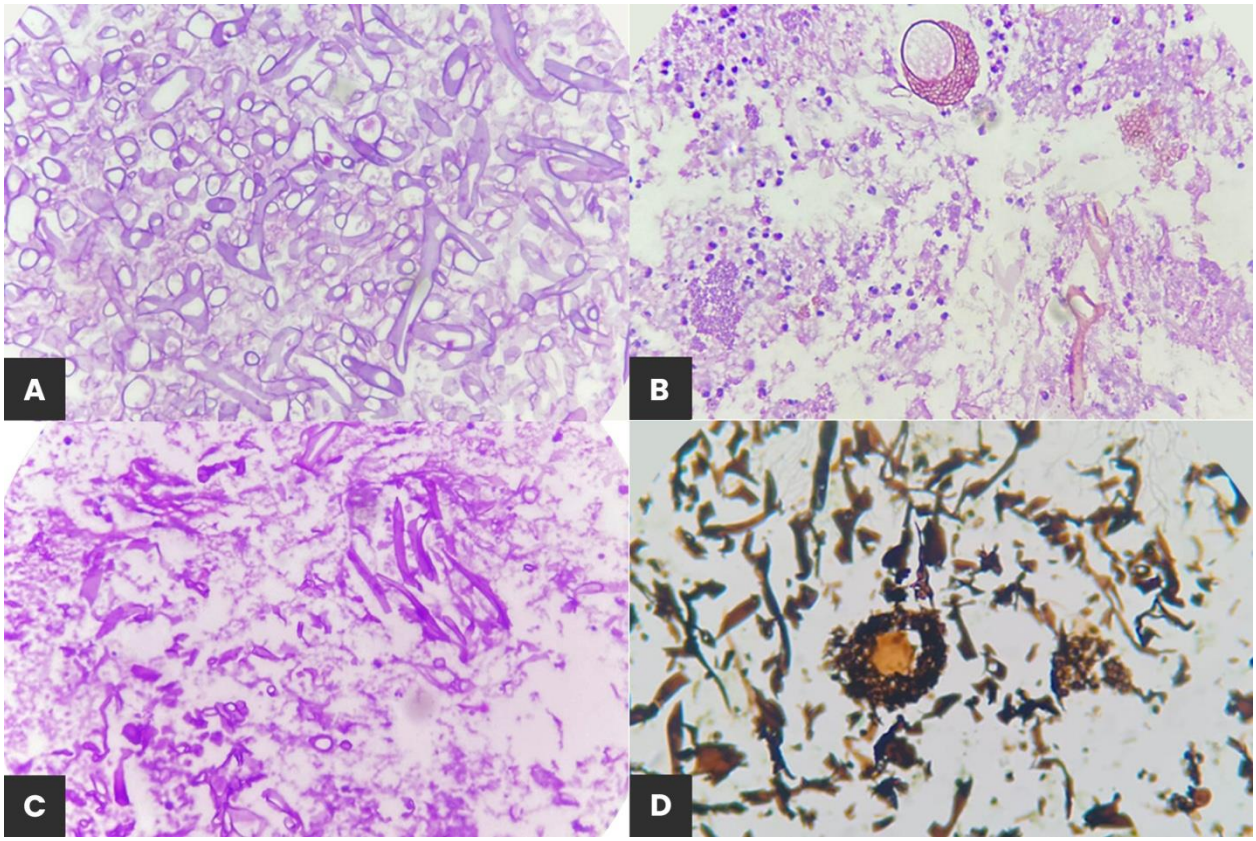
The tissue samples sent for paraffin sections were studied along with special stains. The tissue samples were chiefly mushy, friable and grey-white to black in color. Table 2 shows histological analysis of specimens received from nasal cavity, maxillary sinus, frontal sinus, ethmoid sinus, sphenoid sinus, hard palate /alveolus and orbit.

Table 2: shows histological variables in 69 specimens from various different sites of 35 patients.

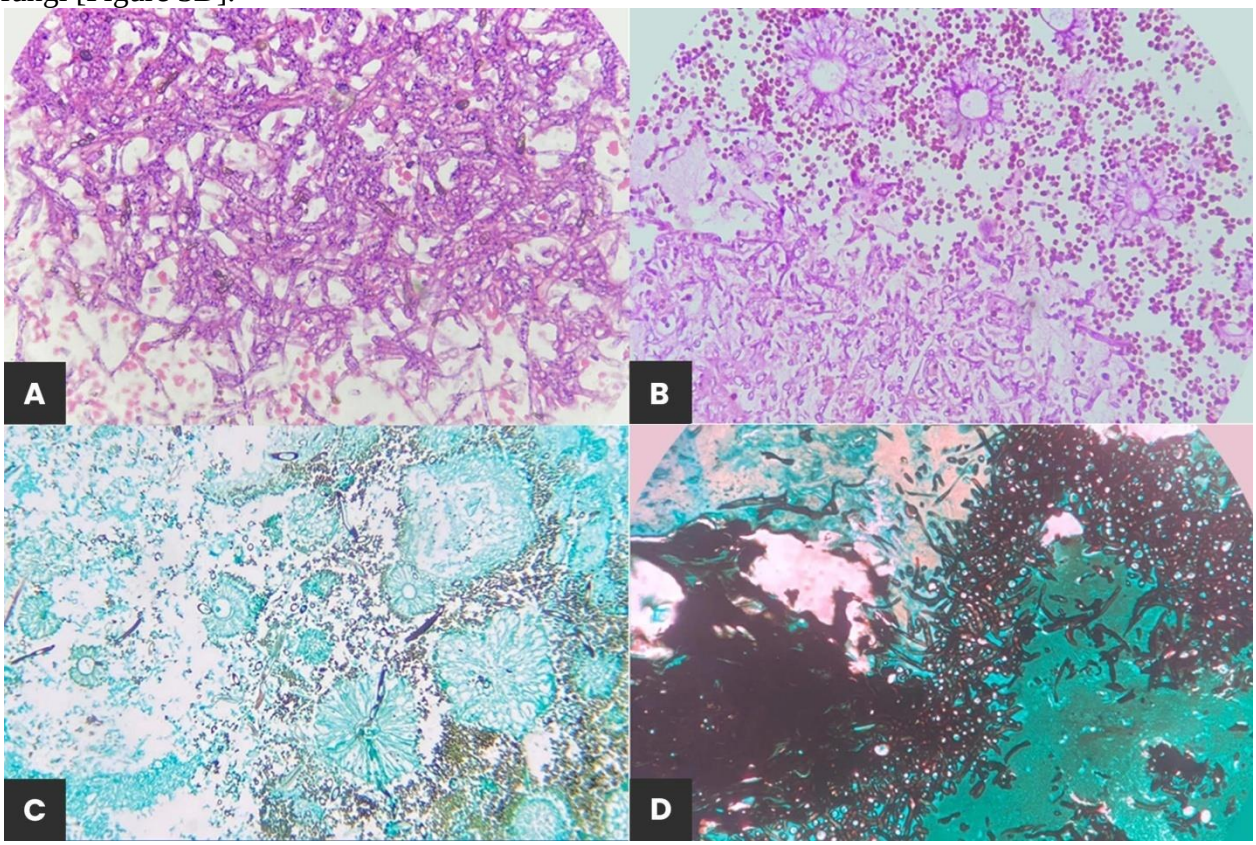
Types of specimen received	Fungal load of Mucor	Inflammation	Tissue Necrosis	Angioinvasion	Bone marrow invasion
Nasal cavity n=19	Dense n=2 Moderate n=6 Mild n=3 Absent n=8	Dense n=3 Moderate n=15 Mild n=2	<50%:15 ≥ 50%=04 Absent:0	n=5	n=0
Maxillary sinus n=22	Dense n=7 Moderate n=7 Mild n=4 Absent n=4	Dense n=8 Moderate n=8 Mild n=6 Absent n:0	<50%:11 ≥ 50%=10 Absent:0	n=5	n=11
Frontal sinus n=1	Absent:01	Dense n=1	<50%:01	n=0	n=0
Ethmoid sinus n=8	Dense n=1 Moderate n=2 Mild n=1 Absent n=4	Dense n=2 Moderate n=2 Mild n=4	<50% n=4 ≥ 50% n=2 Absent n=2	n=0	n=0
Sphenoid sinus n=7	Dense n=3 Moderate n=1 Mild n=1 Absent n=2	Dense n=1 Moderate n=0 Mild n=6 Absent n=0	<50% n=0 ≥ 50% n=5 Absent n=2	n=1	n=2
Hard palate /alveolus n=7	Dense n=3 Moderate n=3 Mild n=1 Absent n=0	Dense n=1 Moderate n=06 Mild=0 Absent:0	<50% n=5 ≥ 50% n=2	n=2	n=2
Orbit n=5	Dense n=0 Moderate n=3 Mild=2 Absent=0	Dense n=1 Moderate n=2 Mild=2	<50% n=2 ≥ 50% n=1 Absent: n=2	n=2	

The most commonly received specimens were from maxillary sinus and nasal cavity. Angioinvasion also commonly noted in specimens of maxillary sinus and nasal cavity. Bone marrow invasion the most commonly noted in specimens of maxillary sinus.

The characteristic hyphae of Zygomycetes, which were broad, ribbon-like and predominantly aseptate with wide-angle branching were better visualized with H and E stains than with special stains. [Figure 2]. Occasional cross-walls were observed in a few hyphae. The load was found to be more in the areas of necrosis. Sporangiospores of mucor were seen in two cases.[Figure 2B].Fungal elements were also well appreciated with PAS [Figure 2C] and GMS stains[Figure 2d].



Co infection with aspergillosis [Figure 3] were observed in 6 cases. Dichotomous (into two nearly equal branches, or 45 degrees) branching, hyphae with frequent septation, diameter ranges from 2.5 to 4.5 μm[Figure 3a] were seen.Many fruiting bodies of Aspergillus noted in all 6 cases of co infections [Figure 3B]. A GMS stain was carried out, which highlighted the fungi [Figure 3D].



We have noted that granulomatous inflammation, giant cell reaction,angioinvasion,perineural and bone marrow invasion are not seen in cases with co-infections of mucormycosis and aspergillosis.

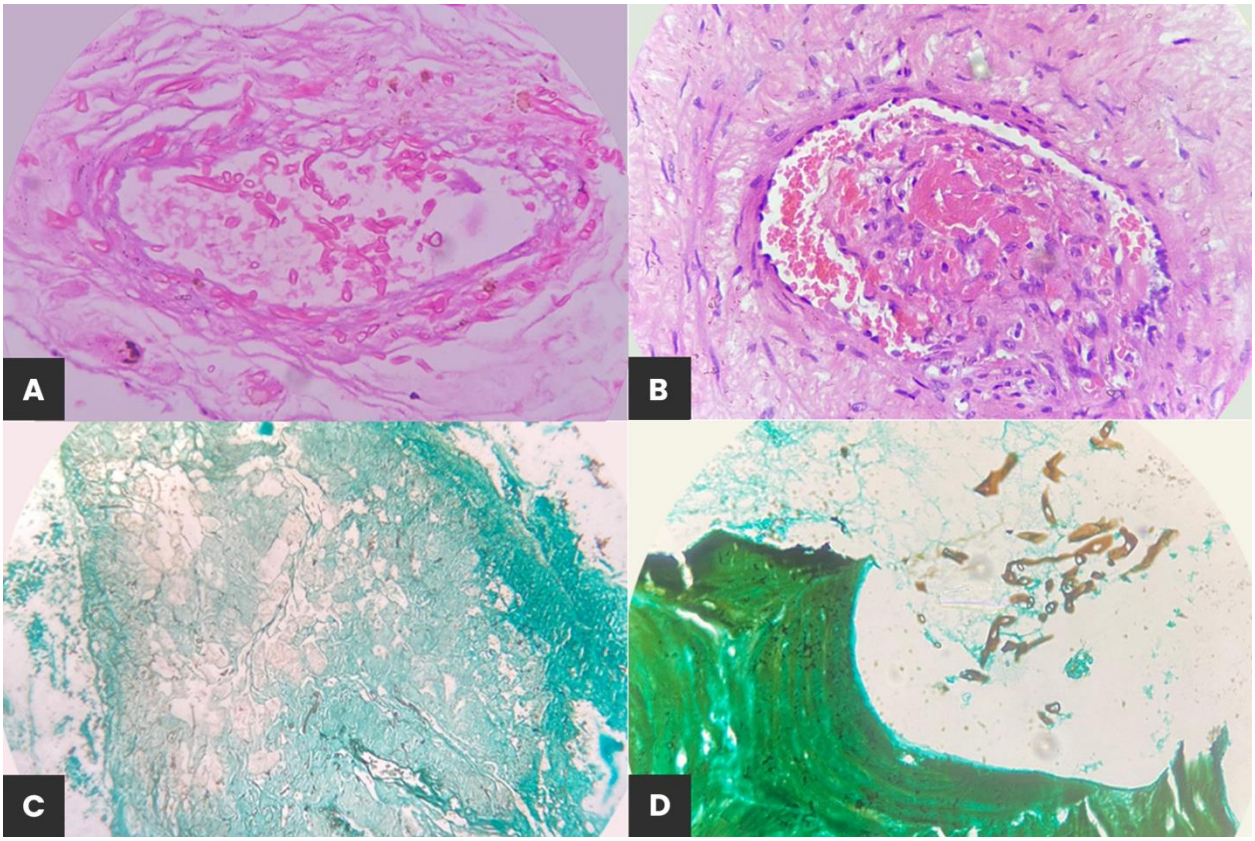
Granulomatous inflammation noted in 4/35 cases (11%) were composed of macrophages, multinucleated giant cells and lymphocytes with central necrosis. 3 cases with granulomatous inflammation were of stage 2 .The fungal hyphae were identified in the necrotic areas and within the multinucleated giant cells at foci. Giant cell reactions without definite granuloma formation were seen in 22/35 cases (63 %).

Neutrophilic infiltration was mild in 4 cases and moderate to marked in 31 cases. All the 35 (100%) cases of mucormycosis showed tissue necrosis. Necrosis was classically pale basophilic with minimal inflammatory response and fungal hyphae were distinctly seen in it. The percentage of necrosis varied from 3 to 95% and the density of fungal organisms was highest in necrotic tissues. Although necrosis was found at microscopy in all the patients of mucormycosis, no necrosis was found in two specimens of orbits.

Angioinvasion was seen in 15 (43%) of the 35 patients. Those blood vessels showed either viable or part of the necrotic focus. The fungal hyphae were identified in the wall of the blood vessels [Figure 4a] or forming thrombi [Figure 4b]observed. Both veins and arteries were involved by the process.

Perineural/neural invasion [Figure 4c] seen in 4 (11%) cases.

Bone marrow invasion [Figure 4d] was noted in 15 (43%) cases with hyphae localized in the bone marrow.



Fungal culture carried out in 35 cases, 30 (86 %) showed distinct growth for Mucorales, while 5 showed no growth. The Mucorales produced fluffy white/gray, or brownish colonies.

Discussion

The Covid-19 infection caused by the novel SARS-CoV-2 has been associated with a wide range of disease patterns, ranging from a mild cough to life-threatening pneumonia.[1] As the ongoing COVID-19 pandemic, more and more experts are aware of fungal co-infections. The

main fungal pathogens for fungal co-infections in severe COVID-19 patients are *Aspergillus*, Mucor, Cryptococcus and *Candida.* Hence, it is necessary for severely ill and co morbid patients to receive fungal pathogens surveillance .[4]

Mucormycosis is a very rapidly progressive disease and may prove fatal if timely diagnosis and treatment are not given. Several genera are associated with this disease, the most common forms are Rhizopus, Rhizomucor and Absida. Rhizopus is the predominant pathogen accounting for 90% of the cases of rhinocerebral Mucormycosis.[5]According to its clinical presentation and site, invasive Mucormycosis is classified in 6 major clinical forms: (1) rhinocerebral, (2) pulmonary, (3) cutaneous, (4) gastrointestinal, (5) disseminated, and (6) uncommon rare forms, such as endocarditis, osteomyelitis, peritonitis, and renal infection .[6]
Mucormycosis is caused by fungi belonging to the family mucormycetes, which are widely distributed in nature (soil, dust). They infect mainly immunocompromised patients. They are transmitted usually via inhalation of spores causing infection of nasal sinuses and lungs and rarely through ingestion causing involvement of gastrointestinal tract.Clinically Mucormycosis may present as rhinitis, pneumonia or cerebral infection. Pneumonia is usually seen in immunocompromised patients and cerebral infection involving brain and orbit is seen in uncontrolled diabetes.[4]

Upon germination, the invading fungus may spread inferiorly to invade the palate, posteriorly to invade the sphenoid sinus, laterally into the cavernous
sinus to involve the orbits, or cranially to invade the brain. The fungus invades the cranium through either the orbital apex or cribriform plate of the ethmoid bone and ultimately kills the host. Occasionally, cerebral vascular invasion can lead to hematogenous dissemination of the infection with or without development
of mycotic aneurysms .[6]
Occasionally, cerebral Mucormycosis can happen through hematogenous route from distinct organs.[7]

The common non-ophthalmic symptoms include facial pain, headache, toothache, facial numbness, burning sensation of mouth, falling of teeth, difficulty in swallowing, gumbleeding, nasal discharge, epistaxis, nasal blockage.
The ophthalmic signs and symptoms include eye pain, decreased vision, watering from eyes and swelling around eyes. Most of the ocular and orbital findings result from an intra-arterial spread of the disease that causes ischemic necrosis of the intra-orbital nerves. The disease may result in fungal invasion of the orbital blood vessels and subsequent thrombosis.[8]
On microscopy, Mucormycosis are non-septate, broad (6– 50 μm) hyphae of variable width with right angle branching. Folding of hyphae may be seen. Vascular invasion and tissue destruction can be present.

Aspergillosis is the second most common opportunistic mycoses. The commonest species is A. fumigatus. They are transmitted as airborne conidia. The most commonly involved organs are lungs, nasal cavity and sinuses. In healthy persons, it causes allergic bronchopulmonary aspergillosis while in immunocompromised patients it may cause serious sinusitis, aspergillus pneumonia, aspergilloma, invasive pulmonary aspergillosis, and disseminated aspergillosis.
Aspergillus on microscopy presents as hyphae with conidia. The hyphae are uniform, narrow (5-10 μm), non-pigmented (hyaline), regularly septated and branching at acute angle (40 degree). On H&E stain, viable hyphae appear basophilic and degenerated and necrotic hyphae appear eosinophilic. These hyphae are strongly positive with PAS and GMS. If there is fungal invasion of the vessels, infarct and necrosis of the tissue may be seen.[4] The hyphal

forms of Aspergillus can be confused with the hyphal forms of other fungi, but when fruiting bodies are present a definite diagnosis is possible at the histopathology level itself.Fruiting bodies (Conidia) of Aspergilli develop from mycelia in areas of high oxygen tension, or severe infections. They are not usually seen in histopathology sections, [9] but in this study all cases show presence of fruiting bodies of Aspergillosis.
The mucor and aspergillus fungi penetrate arterial walls producing ischemia, thrombosis and infarction.[6]

Zygomycetes are difficult to recognize when significant overlap can occur with septate hyphae with dichotomous branching seen in *Aspergillus* spp .[3]The fungal co-infections associated with global COVID-19 might be missed or misdiagnosed. [10]
Bone marrow invasion and necrosis of bone are common in Sino nasalmucormycosis. Perineural/neural invasion frequently seen in rhino orbitalmucormycosis.
Histopathologic examination remains one of the major diagnostic tools in mycology because it permits rapid, presumptive identification of fungal infections .[11]
The imaging studies may help to delineate the extent of tissue invasion. The computed tomography and magnetic resonance of paranasal sinus necrotizing fungal infection included mucosal thickening, osseous erosion, sinusitis with hypo/mild/hyper intense lesions, and destruction of bones in nasal septa, orbit, maxilla and mandible. In case of orbital and cerebral extension, the disease can be seen as orbital cellulitis, optic neuritis, soft tissue infiltration in the optical apex, bone rarefaction and erosion of the skull base, cavernous sinus and internal carotid artery thrombosis, infarcts and intracranial abscess in the brain .[6,12]
A complex coaction of various factors such as COVID-19 infection, preexisting co-morbidity, use of immunosuppressive drugs and risk of opportunistic infections lead to secondary necrotizing fungal infection.[1]
Chronic corticosteroid-based therapy is another primary risk factor that enhances a patient's susceptibility to mucormycosis by causing defects in macrophages and neutrophils and/or steroid-induced diabetes. [6]
In the pandemic of COVID-19, with the absence of an effective vaccine or antiviral therapy, supportive treatment with glucocorticoids play a vital role in COVID-19 management. Glucocorticoids are inexpensive, widely available, and have been shown to reduce mortality in hypoxemic patients with COVID-19.[13]
Glucocorticoids can cause drug induced hyperglycemia by making the liver resistant to insulin. [14]They not only exacerbate hyperglycemia in patients of diabetes mellitus, but also cause diabetes mellitus in patients without documented hyperglycemia before the initiation of glucocorticoids therapy.[15] Higher blood sugar levels and more acidic blood creates a fertile environment for Mucorales fungi to thrive.
Glucocorticoids increase the risk of secondary infections. The immune dysregulation caused by reduced numbers of T lymphocytes, CD4+T, and CD8+T cells by the virus and the use of concurrent immunomodulatory drugs such as tocilizumab could further increase the risk of infections in COVID-19 patients.[16,17]
Invasive Mucormycosis has been diagnosed in mild to moderate SARS-CoV-2 infections. In undiagnosed or uncontrolled diabetics, the strongest predisposing factor appears to be hyperglycemia which leads to increased expression of the endothelial receptor GRP78, resulting in polymorphonuclear dysfunction, impaired chemotaxis and defective intracellular killing .[18]
Aspergillus and Mucormycosis infections in COVID-19 patients will require early detection by a comprehensive diagnostic intervention (histopathology, direct microscopic examination, culture, β-D-glucan, galactomannan, and PCR-based assays) to ensure effective treatments.

There are many factors in developing necrotizing fungal infections in post covid patients.Further research required to know the etiopathogenesis of necrotizing fungal infections in post covid patients.

Conclusion:

COVID-19 is associated with a higher incidence of secondary infections, including fungal infection. Moreover, widespread use of glucocorticoids in COVID-19 causes deterioration of preexisting fungal diseases. So, the clinicians must be aware of possibility of invasive fungal infection in such COVID patients with history of diabetes and other comorbidities. Histopathologic examination remains one of the major diagnostic tools in diagnosis of mucormycosis. Clinical suspicion with early diagnosis of acute invasive fungal sinusitis among COVID-19 patients and early management with antifungal therapy and surgical debridement is essential for better outcome and higher survival.

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