

The Clinicopathological Profile of COVID-19 Associated Mucormycosis in Patients of a Tertiary Care Hospital in Jammu Region

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Abstract

Background: With the ongoing serious COVID-19 pandemic in India, mucormycosis, colloquially called as black fungus, has emerged as a serious fungal infection in patients who were earlier infected with COVID 19 virus. The prevalence of mucormycosis in India is approximately 140 per million population which is roughly 80 times more than that in the developed countries. The aim of our study was to analyse the clinicopathological profile of COVID19 positive patients presenting with mucormycosis to the Department of Pathology, Government Medical College, Jammu. **Methods:** We, here present 60 COVID positive patients, who had later on presented clinically as mucormycosis, along with radiological evidence of the same which was then confirmed on histopathology. **Results:** Mucormycosis was more common in male patients with male: female ratio of 4:1. All the 60 cases presented as rhino-orbital-cerebral mucormycosis as their clinical presentation. Among various predisposing factors, diabetes mellitus came out to be the most common contributing factor. **Conclusion:** COVID 19 has set a fertile ground for development of life threatening infection like mucormycosis because of the various predisposing factors involved. Keeping in mind the fatality of this infection the diagnostic study for this opportunistic pathogen should not be ignored in case the patient is COVID-19 positive and immunosuppressed

Key Words

COVID-19, Diabetes mellitus, Immunosuppression, Mucormycosis, Mucorales

Introduction

The pandemic of coronavirus disease 2019 (COVID-19) had remained on an upsurge trend in the initial half of this year. This second wave of COVID-19 pandemic, has not only led to panic in our country but also invited some unwanted lethal opportunistic infections like Mucormycosis. Mucormycosis (also called zygomycosis) is a serious angioinvasive fungal infection caused by a group of molds called mucormycetes. ^[1] Mucormycosis,

colloquially known as the deadly black fungus, is a life-threatening fungal infection caused by fungi that belongs to the subphylum Mucoromycotina and order Mucorales. The most common causative agent of mucormycosis is *Rhizopus* which is responsible for 70% of all cases followed by *Mucor* and *Lichtheimia*. *Cunninghamella bertholletiae* has emerged as the most virulent Mucorales

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species in humans and has been associated with the highest mortality rates. ^[2] The prevalence of mucormycosis in India is approximately 140 per million population which is roughly 80 times more than that in the developed countries. The prevalence of mucormycosis in India is approximately 140 per million population which is roughly 80 times more than that in the developed countries. ^[3] *Mucor* is a ubiquitous fungi which is present all around us in India as well as in other developing countries. It is most likely that a distinct constellation of factors, few controllable and others uncontrollable ones are there in our settings that may be held responsible for the emerging epidemic of mucormycosis within this COVID 19 pandemic. Uncontrolled diabetes is so common in our country and a significant majority of patients do not have regular testing of their blood sugar levels. ^[4] Further, due to a tremendous overload of COVID-19 patients in the hospitals, the regular blood sugar testing had taken a back seat at one point of time. Moreover, it has been seen that many of these patients were irrationally using or self medicating with high doses of corticosteroids, which added further to the impending doom. ^[5] As few patients who developed diabetes during their COVID-19 illness were previously unaware of their blood sugar levels, this may have resulted from the direct damage to pancreatic beta cells by the SARS-CoV-2 due to its affinity for the ACE2 receptor and indirectly by damaging smaller blood vessels supplying the beta cells. Due to tropical and humid climate in India, the probability of having mucor spores indoor as well as in outdoor air is higher than in developed countries. Mucorales are usually found in environmental niches including soil, manure, decaying fruit, vegetable matter, bread, and dust. The different modes of acquiring infection can be from inhalation of spores into the respiratory tract, ingestion of contaminated foods, or from inoculation into disrupted skin or wounds. ^[6] The major predisposing factors for mucormycosis include uncontrolled diabetes mellitus resulting in diabetic ketoacidosis, other forms of metabolic acidosis, treatment with high dose corticosteroids, chronic, kidney disease, organ or bone marrow transplantation, febrile neutropenia, trauma and severe burns, various malignant hematologic disorders, and deferoxamine therapy in patients undergoing hemodialysis.

Despite the use of antifungal therapy and extensive dissection of the sinuses and exenteration of the orbit, the mortality rates are reportedly very high and it is even higher in cases of disseminated disease reaching upto 100% in some cases. ^[7]

Material and Methods

We, here present an observational study conducted from

1st February 2021 to 31st August 2021 comprising of COVID-19 positive patients, who presented to the Department of Pathology, Government Medical College, Jammu after getting permission from the institutional ethics committee vide no.IEC/GMC/Cat C/2021/227. This study comprises of 60 COVID-19 positive patients, who later on presented clinically as mucormycosis along with radiological evidence which was then confirmed on histopathology and culture. All the hematoxylin and eosin (H&E) and periodic acid schiff (PAS) stained slides from all cases were retrieved and reexamined for fungal morphology, branching angle and septation of the hyphae. Demographic details including age, gender, all other relevant clinical details like anatomical site of involvement, underlying illness, mode of diagnosis and culture reports were carefully analysed.

Results

Demographic profile: In this study, males constituted 80% (48 cases) and females constituted 20% (12 cases) with a male: female ratio of 4:1. The age of the patients ranged from 21 to 79 years with the mean age of 53.55 years. Most of the patients presenting with mucormycosis were in the age group of 51-60 years and constituted 46.6% (28 cases).

Clinical profile : Nasal blockage was the most common presenting symptom which was seen in 18 cases (30%) followed by nasal discharge (15 cases, 25%), anosmia, facial pain and ocular symptoms accounted for 9 cases (15%) each. Based on the anatomical site of infection, cases were categorized into different subtypes of mucormycosis like rhino-orbito-cerebral (ROCM), pulmonary, gastrointestinal, cutaneous, isolated renal and disseminated mucormycosis. In our study, all 60 patients presented with the clinical spectrum of rhino-orbito-cerebral type of mucormycosis. In the ROCM spectrum, limited sino-nasal subtype was the most common presentation and was seen in 36 cases (60%) followed by rhino-orbital disease in 15 cases (25%) and rhino-orbito-cerebral disease in 9 cases (15%). Most common predisposing factor in our study was diabetes mellitus, which was seen in 42 cases and constituted 70%. Out of 42 cases of diabetic mellitus, 1 case had diabetic ketoacidosis during its presentation to the hospital and 2 cases developed it after the initiation of corticosteroid therapy for treatment of COVID-19. Other common predisposing factors in our study were high dose corticosteroids in 12 cases (20%) and chronic kidney disease in 6 cases (10%). Reverse transcriptase-polymerase chain reaction (RT-PCR) tests for COVID-19 were positive in all the patients included in our study. Potassium hydroxide (KOH) wet mount was done from

Table 1. Age wise Distribution

Age (years)	No. of patients (%)
21 - 30	3 (5%)
31 - 40	6 (10%)
41 - 50	15 (25%)
51 - 60	24 (40%)
61 - 70	3 (5%)
71 - 80	9 (15%)

Table 3. Relation of ROCM to initial presenting symptoms

Initial presenting symptom	No. of patients (%)
Nasal blockage	18 (30%)
Nasal discharge	15 (25%)
Anosmia	9 (15%)
Ocular symptoms	9 (15%)
Facial pain	9 (15%)

the sinus biopsies obtained during debridement or from nasal swab obtained during diagnostic nasal endoscopy for all cases and was positive in 49 cases, rest 11 cases were diagnosed on culture.

Pathological profile: All the 60 cases were confirmed on histopathology and culture. The histopathological examination was done to distinguish mucormycosis from

Table 2. Relation of underlying risk factors - mucormycosis

Risk factors	No. of patients (%)
Diabetes Mellitus	42 (70%)
High Dose Corticosteroids	12 (20%)
Chronic Kidney Disease	6 (10%)
Soild malignant tumor	0
Immunosuppressive therapy	0
Hypertension	0
Nasal polyposis	0
Implants	0

Fig. 1 T2 weighted FLAIR image showing white matter edema in temporal lobe in a case of rhino-orbito-cerebral mucormycosis.

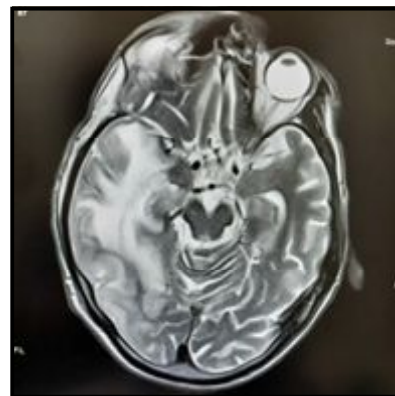
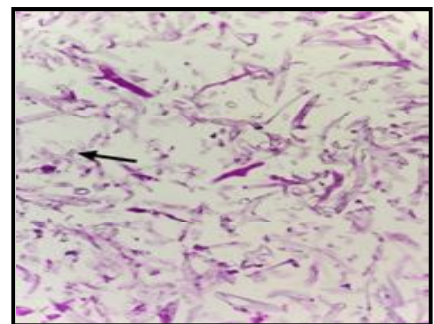
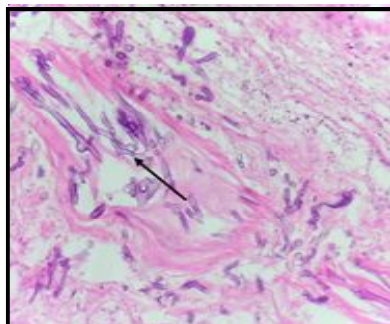
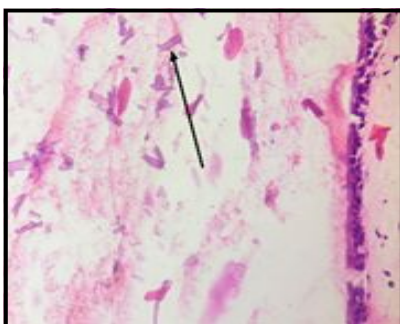


Fig II & Fig III: H&E stained sections showing broad aseptate (black arrows) fungal hyphae with irregular branching at right angles suggestive of mucor (H&E, 400X). Fig IV: PAS stained section showing fungal hyphae along with clear branching at right angles suggestive of mucor (black arrow) (PAS, 400X).



aspergillosis. The microscopic examination was done for each case to evaluate the morphology, branching angle and septation of the fungal hyphae. The hematoxylin and eosin slides showed fungal hyphae which were seen as broad aseptate, ribbon like hyphae with irregular

branching at right angles. These hyphae were interspersed in areas of necrosis along with inflammatory infiltrate and inflammatory granulation tissue. Special stain like Periodic Acid Schiff (PAS) stain was used to highlight the organism and to make a clearer assessment of the

morphology. 14 cases were reported to have shown angioinvasion while 36 cases showed no focus of angioinvasion. In cases of angioinvasion, fungal hyphae were clearly seen in the walls of blood vessels or were identified forming thrombi at foci of necrosis. The fungal hyphae were identified in the blood vessel wall or were seen forming thrombi at foci with necrosis of adjacent tissue. Radiological investigations like MRI and CT scan were done to support the clinical diagnosis of mucormycosis.

Discussion

Mucormycosis is very uncommon in immunocompetent individuals but several immunocompromised conditions are there which predispose an individual to this fatal infection.^[8] In our case series, diabetes mellitus (42 cases, 70%) was the most common predisposing factor. This is similar to study carried out by Prakash H *et al*^[9] and Patel A *et al*.^[10] Males (48 cases, 80%) were more commonly involved than females in this study, which was similar to other studies conducted by Rao VUS *et al*.^[11] Nasal blockage was the most common clinical presentation in 18 cases (30%), which coincided with the study carried out by Goh HC *et al*.^[12] In our study, ROCM emerged as the most common type of mucormycosis based on the anatomical area of involvement. This was similar to other study carried out by Mehta S *et al*.^[14] The spectrum of clinical manifestations of ROCM refers to the complete spectrum ranging from limited sino-nasal disease, limited rhino-orbital disease (progression to orbits) to rhino-orbital-cerebral disease, which is the most aggressive end of the spectrum.^[15] Limited sinonasal disease was the most common component of ROCM spectrum in our study, which was similar to the study carried out by Mehta S *et al*. Ethmoid sinus was the most commonly involved sinus in our study in all 60 cases (100%), which coincided with the study conducted was Sharma *et al*.^[16] It is important to understand the interaction of these predisposing factors with dysfunctional immune system as how these factors predispose to mucormycosis in COVID-19 patients. Infection with the SARS-COV-19 virus leads to a dysfunctional innate immune system. Since the immune system becomes crippled, the phagocytic capability of macrophages present in the airways also becomes impaired, which allows fungal spores to germinate into hyphae.^[17] In an immunocompetent individual, these hyphae are destroyed by mature neutrophils due to the action of free radicals, unlike in severe COVID-19 infection where there is neutrophilia, immature neutrophils are released from the bone marrow which by themselves are incapable of destroying the fungal hyphae.

Uncontrolled diabetes mellitus has proved to be a major risk factor responsible for upsurge of mucormycosis in COVID-19 patients.^[18] Hyperglycemic state leads to increased adhesion and penetration of Mucorales due to induction of endothelial receptor glucose regulated protein (GRP 78). The interaction of fungus with the vascular endothelial cells decides the extent of invasion and rapidity of spread. The thrombosis and tissue necrosis caused by this angioinvasive fungi leads to tissue gangrene which gives black colour to the tissue. Uncontrolled diabetes with ketoacidosis increases the adhesion of mucor hyphae to the extracellular matrix which helps in invasion of the endothelial cell lining of blood vessels.^[19] Excessive and injudicious use of corticosteroids in COVID-19 patients is believed to be responsible the upsurge of ROCM by most. Glucocorticoids inhibit dendritic cell (DC) maturation leading to decreased DC activity, which ultimately interferes T cell signaling resulting in suppressed T cell activity. Corticosteroids reduce the inflammatory cytokines, chemokines and the inflammatory enzymes (iNOS, and COX2). Inducible nitric oxide synthase plays a significant role in the formation of free radicals and its reduced expression may contribute to a crippled immune system which is incapable of inhibiting the fungal hyphae. Even with associated neutrophilia, there is inhibition of neutrophil adhesion to endothelial cells and hence decreased trafficking. Glucocorticoids impair macrophage phagocytic activity and also cause decreased expression of MHC Class II molecules. All these factors lead to increased susceptibility to infection.^[20] Thus, the use of corticosteroids in this pandemic has been like a double-edged sword which calls for their highly rational use in order to prevent this fatal complication of Mucormycosis.

Conclusion

Despite the rise in awareness of the disease, the early diagnosis of mucormycosis remains elusive due to difficulty in sample collection from deep tissues and absence of a biomarker. Increase in mucormycosis cases in our setting appears to be an unholy intersection of trinity of uncontrolled diabetes, irrational use of corticosteroids (increases blood glucose level and opportunistic fungal infection) and COVID-19 (cytokine storm, lymphopenia, endothelial damage). All efforts should be made to maintain optimal blood sugar levels and evidence based use of corticosteroids in patients with COVID-19 is recommended in order to reduce the burden of fatal mucormycosis epidemic within this COVID-19 pandemic. So, keeping in mind the fatality of this infection the diagnostic study for this opportunistic pathogen should not be ignored in case the patient is COVID-19 positive and immunosuppressed.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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