

# Association of COVID-19 with Mucormycosis- An Appalling and Enervating Disease in North-western India

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## ABSTRACT

**Introduction:** The pandemic Coronavirus Disease-2019 (COVID-19) continues to be a significant problem worldwide. The disease pattern ranges from mild to life threatening pneumonia. Association of COVID-19 with mucormycosis is rare but an increase in this association has been observed recently, particularly in a background of immunocompromised state with rhino-orbital-cerebral region being the most common site of involvement.

**Aim:** To study the site and presentation of mucormycosis in COVID-19 patients with histopathological association.

**Materials and Methods:** A descriptive study was conducted at American International Institute of Medical Sciences, Udaipur, Rajasthan, India, in which first 20 patients of COVID-19 with mucormycosis of rhino-orbital region were included in the study. The study was conducted for a period of two months (April-May 2021). Detailed clinical history and associated co-morbidities were noted for each patient. Histopathological examination of all the specimens resected for fungal infection was done along

with special stain. Statistical analysis was done using Chi-square test.

**Results:** COVID-19 association with mucormycosis was observed in all 20 cases. Maximum number of cases (11) were present in the age group of 20-40 years. Diabetes Mellitus (DM) had a strong association with mucormycosis. Maxillary sinus was most commonly associated site. Preoperative Potassium Hydroxide (KOH) was positive in all 20 cases, detailed histopathology study along with Periodic Acid Schiff (PAS) stain was performed in all cases. Additional microscopic findings like angioinvasion (03), granuloma (01) and giant cell reaction (13) were also recorded.

**Conclusion:** COVID-19 has emerged as a global threat to mankind. In the present scenario the entire medical fraternity should have a prompt and team approach towards the management of this pandemic, in terms of, early detection of the infection, meticulous use of corticosteroids and screening of co-morbidities to safeguard patients from such life threatening fungal infections.

**Keywords:** Co-morbidities, Coronavirus disease 2019, Diabetes mellitus, Opportunistic infections, Pneumonia

## INTRODUCTION

The pandemic COVID-19 continues to be a significant problem worldwide [1]. The patients of coronavirus have a high probability of developing severe opportunistic infections, this probability is even more in a background of associated co-morbidities e.g., DM, immunocompromised conditions and chronic obstructive pulmonary disease. The reports of oropharyngeal candidiasis, *Pneumocystis jirovecii* pneumonia, pulmonary aspergillosis, blood stream *Candida* infections in COVID-19 disease are widely recognised in literature [2-4]. However, there are only few cases of COVID-19 Associated Mucormycosis (CAM) available in literature so far, but a significant rise in cases of CAM has been observed in the recent times. Most of the times, mucormycosis is associated with immunocompromised clinical states, but on rare occasion, it can be encountered even in immunocompetent patients [5]. The most common site of involvement by mucormycosis is the rhino-orbital-cerebral region and inhalation of fungal spores is considered to be the common mode of acquiring the same [5].

Hence, present study aimed to study most common age group and gender affected and also to study association of mucormycosis with immunocompromised state.

## MATERIALS AND METHODS

The present study was a hospital based descriptive study conducted in the Department of Pathology, American International Institute of Medical Sciences, Udaipur, Rajasthan, India for period of two months (April-May 2021) in which 20 cases of COVID-19 associated with mucormycosis were included. Ethical clearance was taken for the present study (IEC no. AIIMSUDR/2021/IEC/05).

The age, gender, chief presenting complaints, status of COVID-19 infection, primary site of fungal involvement, history of DM (type I/ type II), preoperative investigations (Total leucocyte count, Differential leucocyte count and KOH wet mount) were taken into account and detailed histopathological study along with special stain comparison of all the specimens resected for fungal infection was done.

### Inclusion criteria:

- Patients presenting with pus discharge from teeth, facial pain, eye swelling and oral ulcers with history of COVID-19 infection.
- Lesions of nose, maxillary sinus, palate and orbit with prior COVID-19 infection.
- Patients belonging to all age groups and both gender with above mentioned complaints.

### Exclusion criteria:

- Apparently healthy individuals.
- Patients of COVID-19 with no history of lesions (of mucormycosis) of nose, maxillary sinus, palate and orbit.

All the specimens pertaining to the study received in the Department of Pathology were fixed in 10% formalin. Proper orientation in eye exenteration specimens and meticulous examination of the debridement specimens was done along with submission of representative sections in all the cases. Then these sections were stained routinely with Haematoxylin and Eosin (H&E) and special stain i.e., PAS and a descriptive study was conducted.

## STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) software (version 1.0.0.1406) was used. Chi-square test was performed. The p-value <0.05 was considered significant.

## RESULTS

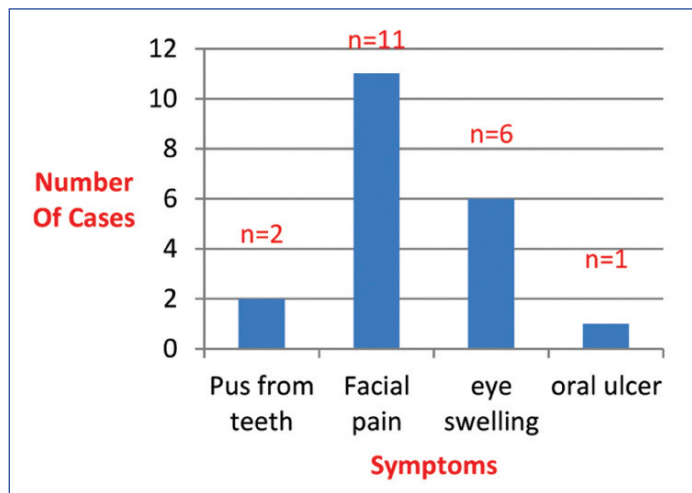
Total 20 cases of mucormycosis were examined during the study period. The study included 14 (70%) males and 06 (30%) females with male to female ratio of 2.3:1. The patients of all age groups were studied, the youngest being a 26-year-old male and oldest being a 75-year-old female. Maximum number of cases was present in the age group of 20-40 years (11 cases, 55%) [Table/Fig-1].

Specimens were resected from different sites. In maximum number of cases (n=11) they were obtained from maxillary sinus, which was seen commonly affecting the age group of 20-40 years and that too in males. Total 04 cases (20%) of eye exaneration were studied, amongst which 3 were male and only one female, most of these were in 20-40 years age group. There were total 03 cases (15%) where palate was the site of involvement, here females were more commonly affected but the cases were equally distributed among all age groups. Least affected sites were nasal septum and inferior temporal fossa [Table/Fig-1].

Site	Gender		Age (years)		
	Male	Female	20-40	41-60	>61
Maxillary sinus	10	1	6	1	4
Palate	1	2	1	1	1
Septum	0	1	1	0	0
Maxillary sinus+orbit	3	1	3	1	0
Maxillary sinus+ITF	0	1	0	1	0
Total	14	06	11	04	05

[Table/Fig-1]: Site according to age and gender.  
ITF: Infratemporal fossa

Amongst all the cases, patients presented with varying clinical features, in decreasing order of frequency these were facial pain (n=11, 55%), eye swelling (n=06, 30%), pus from teeth (n=02, 10%) and oral ulcers (n=1, 5%) [Table/Fig-2].



[Table/Fig-2]: Clinical complaints of cases.

Total, 12 (60%) mucormycosis cases were found to be associated with a positive Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test result, whereas 08 (40%) cases were found to be negative, but in all these (n=8) cases findings like positive CT scan, raised C-Reactive protein, increased D-dimer and interleukin-6 and signs and symptoms favouring COVID-19 infection were present. Most of the study cases had a total leucocyte count in the normal range. A raised count was observed in (n=05, 25%) of cases [Table/Fig-3]. However, lymphopenia was encountered in (n=07, 35%) cases. Corticosteroids were given as a part of COVID-19 management in all the cases (n=20).

A strong association of mucormycosis with DM was found. In maximum number of cases (n=11) 55%, patients were known cases of DM, however in (n=05) 25% cases DM was freshly diagnosed at the time of presentation and in (n=04) 20% cases, no such

Range (/cumm )	Cases (%)
<4000	0
4-11000	15 (75%)
>11000	05 (25%)

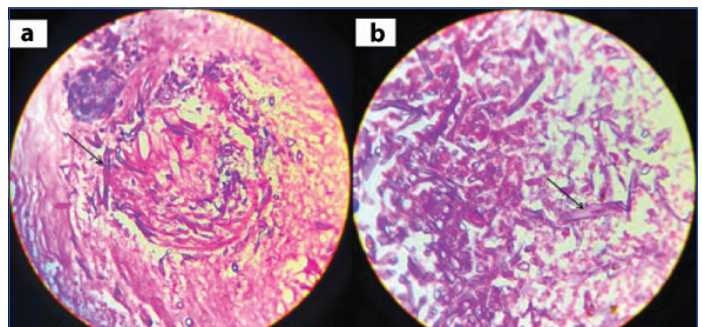
[Table/Fig-3]: Total leucocyte count in mucormycosis.

association was found. Chi-square test was applied and the p-value was calculated, which was found to be significant (p=0.05).

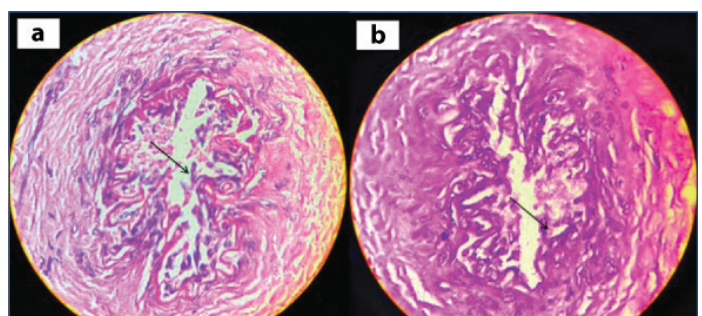
Preoperative KOH was positive in all 20 cases [Table/Fig-4]. Detailed histopathology study along with PAS was performed in all cases for confirmation of fungal mucormycosis [Table/Fig-5]. Additional microscopic findings like angioinvasion [Table/Fig-6], granuloma formation and giant cell reaction [Table/Fig-7] were also recorded.

Site	Preoperative KOH	Histopathology and PAS stain	Additional findings		
			Angio-invasion	Granuloma	Giant cell reaction
Maxillary sinus	Positive	Hyphae seen	01	0	11
Palate	Positive	Hyphae seen	0	01	01
Septum	Positive	Hyphae seen	0	0	0
Maxillary sinus+orbit	Positive	Hyphae seen	02	0	0
Maxillary sinus+ITF	Positive	Hyphae seen	0	0	01

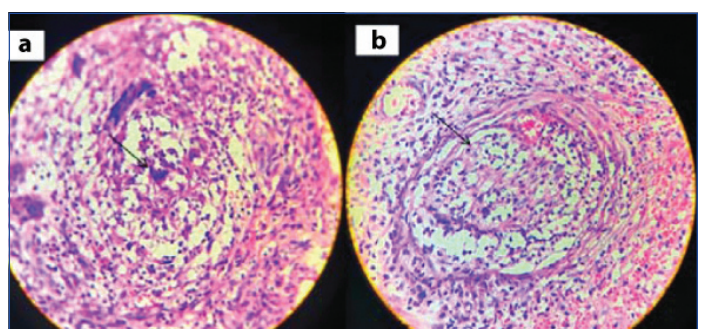
[Table/Fig-4]: KOH, Histopathology and PAS stain with additional findings.



[Table/Fig-5]: a) Short, broad pauciseptate hyphae with 90° angle branching in H&E stain (40X); b) PAS stain (40X).



[Table/Fig-6]: a) Fungal hyphae seen within vessel wall and lumen (angioinvasion) on H&E stain (40X); b) PAS stain (40X).



[Table/Fig-7]: a) Giant cell reaction on H&E stain (40X); b) Ill-formed granuloma on H&E stain (40X).



## DISCUSSION

The COVID-19 infection still continues to be the nightmare amongst the human population worldwide, as we have been witnessing an obdurate rise in the cases during last few months. This infection has been associated with a wide range of patterns varying from mild cough to life threatening pneumonia. An immeasurable number of manifestations and complications have been documented so far and at the same time, new ones are continuously being emerging and being reported, as the world continue to learn more about the novel COVID-19 pandemic [6]. There are many common invasive-fungal infections (aspergillosis, candidiasis, cryptococcosis and mucormycosis) which can be seen arising in a setting of immunosuppressive state or chronic pulmonary disease [2]. But recently, there has been a sudden and significant increase in the incidence of mucormycosis in a background of ongoing COVID-19 pandemic [6]. Mucormycosis or Zygomycosis, is a rare and aggressive fungal infection that usually affects patients with compromised immune system [7]. It is a lethal fungal disease and most commonly affects the rhino-orbital-cerebral region [8]. What has been observed is a complex interplay of factors, responsible for sudden rise in cases of CAM like, use of immunosuppressive drugs, DM, nosocomial infection sources (e.g., use of tap water in humidifier of oxygen cylinder, contamination taking place in oxygen production plants) and systemic immune alteration or dysregulation caused by COVID-19 infection itself [9].

The present study observed a male preponderance in cases of CAM, as observed by Singh AK et al., [10]. The disease incidence was high in patients falling between 20-40 years of age (n=11, 55%). All the 20 cases of CAM in present study had rhino-orbital region involvement. The maxillary sinus being the commonest site, similarly Patel A et al., has also shown rhino-orbital region as the most common site of involvement in the patients of mucormycosis but without a background of COVID-19 infection [11]. The most common presenting complaint in present study patients was facial pain followed by eye swelling and the oral ulcers were rarely encountered. The DM (73%), organ transplantation (7.7%) and malignancy (9.0%) are some of factors associated with mucormycosis in India [11]. DM significantly increases the chance of rhino-orbital-cerebral mucormycosis by 7.5 fold [12]. Present study observed a strong association of mucormycosis with DM, in maximum cases (n=11) 55% patients were known cases of DM however in (n=05) 25% cases it was freshly diagnosed. In all the 20 cases in present study, corticosteroids were given as a part of COVID-19 management. These findings were in concordance with John TM et al., who also found a strong association between mucormycosis, DM and the corticosteroid therapy [13]. Collectively, these observations suggest an atrocious triad of mucormycosis, DM and steroid therapy in people with COVID-19.

The RT-PCR test is considered as one of the confirmatory test for diagnosing COVID-19 infection, but at the same time, it has been found to be associated with considerable number of false negative test results [14]. In the present study, 12 (60%) cases were RT-PCR positive and 08 (40%) cases were RT-PCR negative. In all these (08 cases), findings favouring or confirming the presence of COVID-19 infection were found, like positive CT scan, raised C-Reactive protein, increased D-dimer and interleukin-6 and signs and symptoms favouring COVID-19 infection. Therefore, a negative RT-PCR doesn't exclude COVID-19 infection this should be kept in mind, because neglected cases of COVID-19 are a big threat for developing deadly mucormycosis disease.

Present study encountered total 05 (25%) cases of leucocytosis with neutrophilia and lymphopenia. Amongst the remaining 15 (75%) cases having normal leucocytes count, seven cases also had lymphopenia.

In all the 20 cases, a detailed histopathological examination was done along with the use of PAS stain. Association with preoperative KOH findings was also done. Typical broad pauciseptate ribbon like hyphae with 90° angle branching were encountered in the tissue

sections with often a marked inflammatory response of neutrophilic type. Reactive inflammatory tissue response to fungal hyphae in the form of giant cell reaction (n=13, 65% cases), well-formed granulomas (n=01, 50% case) and areas of coagulative necrosis were also observed. Angioinvasion (presence of fungal hyphae within blood vessel wall) was encountered in total (n=03, 15%) cases, which also had associated lymphopenia. It has been shown that in severe COVID-19 cases, there is reduction in the absolute number of lymphocytes and T-cells. There are some specific type of T-cells i.e., Mucorales-specific T-cells (CD4+ and CD8+) which produce cytokines and interferon gamma (INF-γ) that damage the fungal hyphae. It might be speculated that, in presence of lymphopenia, there is a deficiency created, of these specific T cells, which could increase the risk of developing angioinvasive mucormycosis, having worst outcomes. Recovering of lymphocyte count could improve the adaptive immune system and induce the production of mucorales-specific T-cells, which might have a role in contracting the invasive infection [15].

## Limitation(s)

The present study was a hospital based study conducted on 20 cases, therefore more studies are required to understand and develop better hypothesis of association of mucormycosis with COVID-19 infection.

## CONCLUSION(S)

The world continues to fight with COVID-19 pandemic, at the same time the survivors of the same along with the medical fraternity are learning to deal with the annoying long term complications associated with the disease. Only to add to this struggle, is the recently emerged epidemic of mucormycosis (black fungus). Meticulous screening of DM and any other immunocompromised states and judicious use of steroids have to be stressed upon. Hence, awareness about the chance of severely ill that COVID-19 patients developing fungal infection is important. Therefore, all the physicians, ophthalmologists, otolaryngologists and pathologists should have a united team approach for the management of this deadly and debilitating mucormycosis disease in COVID-19 patients.

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