



## ASSESSMENT OF RISK FACTORS IN COVID-19 ASSOCIATED MUCORMYCOSIS PATIENTS AT A TERTIARY LEVEL DENTAL HOSPITAL - AN OBSERVATIONAL STUDY.

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### ABSTRACT

**Introduction:** Corona Virus Disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Several countries have faced multiple waves of COVID-19 pandemic and its serious threats. During or after COVID-19 illness, patients acquired several opportunistic infections. Among which, the most commonly reported infection in Indian population was COVID-19 associated Mucormycosis (CAM). Diabetes mellitus (DM), high use of steroids and contaminated hospital tools are some of the risk factors involved in causing CAM. **Materials and Methods:** A total of 138 patients visited our tertiary level dental hospital for mucormycosis during the time period of June to August 2021. These patients were subjected to a detailed clinical assessment including history of past COVID-19 infection. Several risk factors such as vaccination status, DM, steroid intake and hospitalization during COVID-19 infection were evaluated for each patient. **Results:** In the present study, CAM patients in the age group of 30 to 50 years were commonly affected, 78.3% of patients were male, 91.3% were non-vaccinated and none of them were completely vaccinated with 2 doses. Among risk factors, 45.7% had previous history of DM, 47.1% had acquired DM during or after COVID-19 illness, 78.3% had received steroids, and 58% had received oxygen or ventilator support during COVID-19 treatment. **Conclusion:** Mucormycosis is a major hazard to the individuals affected with severe COVID-19 illness. The major risk factors include lack of vaccination, presence of DM, injudicious use of steroids and contaminated oxygen cylinders used during COVID-19 treatment. Proper measures to control these risk factors are necessary for prevention of mucormycosis infection in the subsequent COVID-19 waves.

**KEYWORDS :** COVID-19, Mucormycosis, Risk factors, SARS-CoV-2.

### INTRODUCTION:

The global pandemic of Corona Virus Disease 2019 (COVID-19) was caused due to infection by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection. The World Health Organization (WHO) announced a public health emergency of international concern on January 30<sup>th</sup> 2020 and later on declared COVID-19 as a pandemic on March 11<sup>th</sup> 2020.<sup>[1]</sup>

India has faced three waves of COVID-19 infection. The symptoms during the first wave were fever, cough, sore throat, confusion, dyspnoea, headache, anosmia and ageusia. The second wave of this infection caused much more serious health complications, with various opportunistic infections being reported.<sup>[2][3]</sup> This higher incidence of secondary infections can be attributed to pre-existing comorbidities like diabetes mellitus, high usage of steroids and immunocompromised conditions.<sup>[4]</sup> The opportunistic fungal infections occurring in COVID-19 patients included Mucormycosis (black fungus), Candidiasis and Aspergillosis.<sup>[5]</sup>

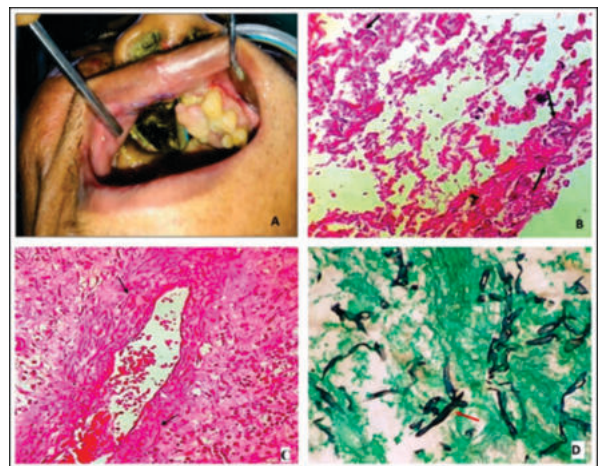
As there was a substantial incidence of CAM cases in the second wave of COVID-19 in India, this study was designed to evaluate the association of CAM infection with various risk factors involved.

### MATERIALS AND METHODS:

An observational study was done on Mucormycosis patients who reported to our Tertiary Level Dental Hospital during the time period of June to August 2021. A total of 138 patients visited our hospital for mucormycosis of the oral cavity and all of them were included in the study. They were informed about the purpose of the study and written informed consent was obtained from them prior to their inclusion. These patients were subjected to a detailed clinical history which included history of past COVID-19 illness. [Figure 1A].

Biopsy tissues were obtained from the patients and were processed and stained with standard Hematoxylin and Eosin stain [Figures 1B]. In

addition, special stain Grocott Gomori methenamine silver stain (GMS) was done to identify the fungal hyphae distinctly in the tissue sections [Figure 1D]. After the histopathological diagnosis of mucormycosis, the various risk factors which included vaccination status, steroid intake, DM and hospitalization with oxygen support during COVID-19 infection were evaluated for each case.



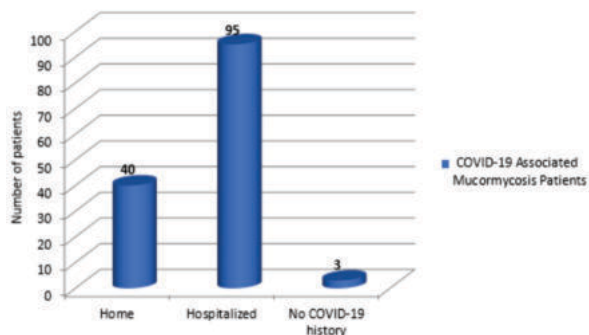
**Figure 1:** A) Mucormycosis involving the oral cavity showing exposed necrotic palatal bone with blackish nasal turbinate. B) Photomicrograph showing filamentous structures resembling fungal hyphae spread throughout the stroma (H&E 40x). C) Mucor hyphae invading the blood vessel (H&E 20x). D) Photomicrograph showing branching aseptate fungal hyphae stained black (GMS 40x).

### RESULTS AND OBSERVATIONS:

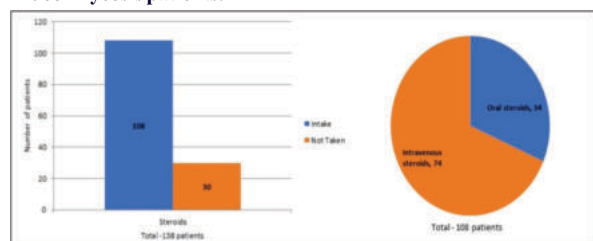
Among the 138 (N) patients evaluated, the highest incidence of

mucormycosis cases was seen in the age group of 30 to 40 years (n=51) followed by 40 to 50 years (n=50). Six cases were in the 20-30 year age group, 26 in the 50-60 year age group and 5 in the age group greater than 60 years. Among the total cases, 78.3% (n=108) were males and 21.7% (n=30) were females. Amongst all the patients, 91.3% (n=126) were non-vaccinated, 8.7% (n=12) were vaccinated with only first dose and none were completely vaccinated (2 doses). During the phase of COVID-19 infection, out of 138 patients, 95 (68.9%) were hospitalized, 40 (29%) were in home isolation and three patients (2.1%) had no symptoms (subclinical infection) [Graph 1]. Among the 95 patients who were hospitalized, 74 (53.6%) were given mild oxygen support therapy, 6 (4.3%) were given ventilator supported therapy and remaining 15 patients had no oxygen therapy. Out of 138 patients, 108 patients received steroids for COVID-19 management, out of which 53.6% (n=74) received intravenous (IV) steroids while 24.6% (n=34) of patients received oral steroids [Graph 2].

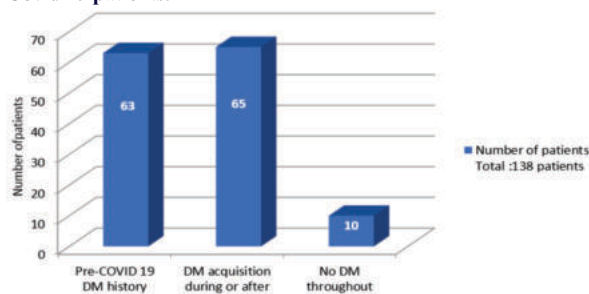
Among the total patients, 63 patients (45.7%) had a history of DM and 65 patients (47.1%) had acquired DM during or after COVID -19 illness whereas 10 patients (7.2%) had no DM history throughout [Graph 3]. Among these 63 known DM patients, 87.3% (n=55) were using oral hyperglycemic drugs and 11.1% (7) were using injectable form of insulin and one patient (1.6%) was not taking any diabetic medication before COVID-19 illness. Whereas after COVID-19 infection, 17 were using oral hyperglycemic tablets and 46 were using injectable form of insulin to control DM. Among 65 patients who acquired diabetes following COVID-19 infection, 32 were using oral hyperglycemic tablets and 30 were using injectable form of insulin and 3 were not taking any medication for DM.



**Graph 1: Mode of treatment among COVID-19 Associated Mucormycosis patients.**



**Graph 2: A) Frequency of steroid intake for Covid-19 illness in Mucormycosis patients. B) Mode of steroid intake among the Covid-19 patients.**



**Graph 3: Correlation between Diabetes Mellitus and COVID-19 Associated Mucormycosis**

## DISCUSSION:

Mucormycosis is an uncommon, highly invasive opportunistic fungal

disease caused by fungi of the Mucoraceae family of the class Phicomycetes or Zygomycetes. On the basis of clinical presentation, it is classified into rhino-cerebral, cutaneous, pulmonary, gastrointestinal and disseminated forms.<sup>[6]</sup> This fungus is a commensal organism of the nasal mucosa. In certain conditions like immunosuppression caused by DM, and long-term corticosteroid therapy, it can rapidly germinate within the nasal cavity and paranasal sinuses and can invade the palate, eye and brain even leading to death.<sup>[7]</sup> Majority of mucormycosis cases reported recently in India, showed a past history of COVID-19 infection indicating that this disease and the medical care provided for it are causative factors for mucormycosis infection.

Diabetes mellitus is a major predisposing condition for the incidence of mucormycosis. Previous studies suggested that SARS-CoV-2 damages the islets cells of pancreas by binding to Angiotensin-Converting Enzyme 2 (ACE 2) receptors and inducing beta cell apoptosis resulting in acute insulin dependent DM.<sup>[8]</sup> Similarly, SARS-CoV-2 infection causes cytokine storm which also induces hyperglycemic condition. Hyperglycemia triggers phagocytic malfunction, poor neutrophil chemotaxis, inefficient intracellular killing by oxidative and non-oxidative pathways and increasing serum iron level.<sup>[7]</sup> In addition, SARS-CoV-2 infection may raise stress levels and induce secretion of several hyperglycemic hormones<sup>[9]</sup>

This virus also attacks endothelial cells and causes diffuse endothelitis which favours adhesion of mucor fungi to the endothelial cells of blood vessels thereby facilitating angiogenesis. Mucor species possess a fungal ligand (CoH3) which binds to the specific endothelial cell receptor GRP78 thereby facilitating fungal endocytosis. This unique interaction potentiates mucor species to invade the blood vessels, resulting in thrombosis and consequent necrosis of tissues.<sup>[10]</sup> A previous study conducted by **Ramazan et al (2021)** showed that the serum GRP78 levels were elevated in COVID-19 patients when compared to normal individuals.<sup>[11]</sup>

Another substantial risk factor for mucormycosis is the high intake of corticosteroids during COVID-19 treatment. This predisposes an individual to immune dysregulation by impairment of innate immunity, uncontrolled steroid induced hyperglycemia and precipitation of diabetic ketoacidosis that causes high acidic pH favouring germination of mucor species.<sup>[12][13]</sup>

Iron also promotes the growth of fungal hyphae. Induced hyperglycemia after COVID-19 infection triggers glycosylation of iron binding proteins like ferritin and transferrin which reduces bound iron and increases free iron content in serum. Furthermore, increased interleukin-6 levels and acidosis in COVID-19 patients also augment free iron level by increasing its synthesis and decreasing iron transport.<sup>[12]</sup> Thus, all these mechanisms increase the serum iron level and favour the growth of mucor species.<sup>[13]</sup>

In our study, patients of 30 to 50 years of age were most commonly affected with CAM. In accordance with our results, in the study conducted by **Shweta et al (2021)** the most common age group affected by CAM was 41–50 years.<sup>[14]</sup> The probable reason might be that during the second wave of the pandemic, majority of the aged patients (51 to 60 years) were vaccinated hence the COVID-19 illness was less severe among them, whereas the middle-aged patients experienced severe infection due to lack of vaccination.

In our study, males were more commonly affected than females. Similar results were obtained by **Sahar et al (2022)** and **Reshma et al (2021)**<sup>[15][16]</sup> Males presented with more severe COVID-19 infection at a higher rate than females. This can be attributed to their higher exposure to the external environment and engagement in more risky behaviors like smoking which further compromises the lung condition.<sup>[17]</sup> Higher expression of ACE 2, a SARS-CoV-2 receptor in males is also a contributing factor.<sup>[18]</sup>

Our results showed that most patients were non-vaccinated (91.3%) and few were vaccinated with only first dose (8.7%) and none of them were completely vaccinated with 2 doses. In compliance with our findings, results obtained by **Kasturi et al (2022)** and **Rohit et al (2022)** showed that CAM incidence was greater among the non-vaccinated patients compared to vaccinated patients.<sup>[19]</sup> This might be because, the severe form of COVID-19 illness was reported in non-vaccinated or partially vaccinated (only first dose) compared to

completely vaccinated patients (two doses).<sup>[19]</sup> Thus, during the recovery period of severe COVID-19 infection, there is increased vulnerability to acquire medication (steroids) related mucormycosis and complete vaccination against SARS-CoV-2 will decrease the incidence of CAM.<sup>[20]</sup>

In our study, home isolated patients (29%) also acquired CAM infection. This could be due to self-medication with heavy doses of steroids and iron supplements even for mild COVID-19 illness.<sup>[21]</sup> In our study, 53.6% had received oxygen therapy and 4.3% had received ventilator therapy during COVID-19 hospitalization. Our results coincided with the findings of **Subashree et al (2022)** who noted that among the total, 54.68% of mucormycosis patients received oxygen therapy during COVID-19 management.<sup>[21]</sup>

Increased occurrence of CAM following COVID-19 infection was seen in hospitalized patients receiving oxygen therapy. This may be because of contamination of the water used to hydrate the oxygen used in hospitals with mucor spores, which can then pave their way through the nasal passages.<sup>[15]</sup> Prolonged ventilators and humidifier use, use of unhygienic masks may have increased the spread of fungal spores in the hospital environment.<sup>[22][23]</sup>

In our study, greater number of patients (78.3%) were administered oral or IV steroids during their COVID -19 illness. Our findings coincided with those of **Subashree et al (2022)**, who found that 70.14% of mucormycosis patients received steroidal therapy during COVID-19 illness.<sup>[21]</sup> Likewise, **Nurani et al (2022)** in their study observed that 87% of mucormycosis patients had history of steroid usage during COVID-19 infection.<sup>[24]</sup> Thus, glucocorticoid induced immunosuppression may predispose to mucormycosis.<sup>[13]</sup> Hence, injudicious administration of corticosteroids to patients with mild COVID-19 illness must be avoided.<sup>[25]</sup>

In our study, 63 patients (45.7%) had a history of DM and 65 (47.1%) acquired DM during or after COVID-19 illness. Our results were similar to those of **Varsha et al (2022)** where 42.9% patients had pre-existing DM at the time of diagnosis of COVID-19, and 36.7% acquired DM after COVID-19 infection.<sup>[7]</sup> Similar results were obtained by **Shweta et al (2021)** where 65% of mucormycosis patients had acquired DM during or after COVID-19 illness.<sup>[14]</sup> This may be ascribed to the undiagnosed pre-diabetic condition of the patients at the time of SARS-CoV-2 infection which was stimulated as a sequel of the effect on pancreas.<sup>[7]</sup>

Another remarkable finding of our study was that among the known diabetic patients (n=63), 39 had to be shifted from oral hypoglycemic medication to injectable form of insulin after SARS-CoV-2 infection. It signifies the aggressiveness of the hyperglycemic condition induced by SARS-CoV-2.

## CONCLUSION:

COVID-19 infection in the background of DM and steroid intake triggers triple immune suppression leading to acquisition of mucormycosis infection.<sup>[7]</sup>

Hence, there is a need to alter the treatment for COVID-19, by ensuring judicious use of corticosteroids and multivitamins like iron supplements and avoiding irrational use of antibiotics.<sup>[7]</sup> Optimum glycemic control in individuals with preexisting or new-onset diabetes mellitus must be ensured.<sup>[25]</sup> Likewise, patients administered with high dose of corticosteroids and under prolonged Intensive Care Unit stay during COVID-19 illness require careful monitoring to prevent occurrence of CAM.<sup>[24]</sup>

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