Clinical-epidemiological Profile of Coronavirus Disease 19 Associated Mucomycosis (CAM) and Relation with Zinc and Iron Levels

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Received: 07.10.2021; Accepted: 29.11.2021

Abstract

Introduction: Coronavirus disease associated mucormycosis (CAM), perturbed a lot by reaching to epidemic proportions particularly during the second wave of the pandemic.

Material and Methods: This was a retrospective, observational study of patients with COVID-19-associated mucomycosis admitted in April-May 2021 at a tertiary care teaching hospital. Demographic profile, clinical and laboratory parameters were recorded. Multidisciplinary treatment including antifungals and surgical interventions were noted.

Results: This study included 98 patients of mucormycosis, diagnosed on the basis of clinical and radiological findings and later were confirmed by microbiological investigations. Out of 98 patients, 72 had rhino orbital, 24 had rhino-orbital-cerebral and 2 had pulmonary mucormycosis. Twelve had coinfection of covid 19 while 86 had developed mucormycosis within 3 weeks.

Conclusion: CAM has posed as a continuum of challenges faced during the pandemic of covid 19. This rare and life threatening complication requires high index of suspicion for early diagnosis. Multidisciplinary involvement and timely interventions including antifungal pharmacotherapy, stringent glycemic control and surgical debridement can reduce the mortality. Mucormycosis is uniformly associated with low iron levels but role of zinc needs to be further studied.

Introduction

The Covid-19 symptom spectrum ranged from common clinical features that include fever, cough, fatigue, myalgia and pneumonia to involvement of many organ systems. It is associated with increased incidence of secondary bacterial and fungal infections. The fungal co-infections associated with COVID-19 have been reported last year too but it increased exponentially during the second wave. Mucormycosis is an uncommon but serious infection that complicates the course of severe COVID-19. In India, the prevalence of mucormycosis is already disproportionate high, approximately 0.14 cases per 1000 population, which is about 80 times the prevalence of mucormycosis in western countries.
Laboratory data consisted of complete blood count, liver and renal function test, examination of haemostasis parameters, inflammatory markers in form of high-sensitivity C-reactive protein (hsCRP), interleukin 6, lactate dehydrogenase and procalcitonin for presence of secondary bacterial infection. The normal reference range for the zinc concentration was considered 80–120 mg/dL. A zinc level $<80$ mg/dL was defined as ‘deficient while iron (60–180 µg/dL), ferritin (10–125 in females and 15–250 ng/mL) were considered normal.

Microbiological confirmation was done by microscopy for presence of fungal hyphae using potassium hydroxide mount. For fungal culture the samples were inoculated on Sabouraud dextrose agar. Tissue samples after debridement were submitted for histopathological analysis by using, periodic acid Schiff and Gomori methenamine silver stain.

**Statistical analysis**

A descriptive statistical analysis was performed for all variables using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA), consisting of mean, standard deviation, percentage. Proportions and associations between characteristics of the study groups were compared by Fisher’s exact test. The Mann–Whitney U-test and t-test were used to compare continuous variables between the study groups. Multivariate logistic regression analysis was conducted to determine the odds ratio (OR) and 95% confidence intervals (95% CI) for the variables affecting mortality. Results were considered statistically significant when the p-value was $<0.05$.

**Results**

A total of 98 patients of mucormycosis clinically and microbiologically confirmed were included in the study. Out of 98 patients, 72 had rhino orbital, 24 had rhino-orbital-cerebral and 2 had pulmonary mucormycosis whom bronchoalveolar lavage fluid confirmed presence of asperate hyphae.

There was male preponderance noted in the study population. Mean age of the patients was 52 ±14 years. Majority were from rural areas. Out of 98, 12 patients were diagnosed to have covid 19 first and subsequently they developed symptoms of mucormycosis. Amongst them, 8 patients had severe covid 19 illness while 4 had mild disease. Rest of the 86 had history of covid 19 within 3 weeks. The mean duration between RTPCR covid negative test and onset of symptoms of mucormycosis was 13± 8 days. All the patients who had fungal coinfection had severe covid 19. Demographic features of patients who were diagnosed to have mucormycosis during covid 19 illness were similar to those in post covid state except prevalence of ICU admissions, presence of DKA and inflammatory mediators as shown in Table 1.

The common presenting symptoms and signs were proptosis (90%), orbital pain (85%), headache (80%), facial pain (75%) conjunctival hyperemia or chemosis (70%), ptosis (70%), diplopia (65%), fixed and dilated pupil (63.6%), vision loss (63.6%), nasal stuffiness (50%) epistaxis (45%) and endophthalmitis (34.5%). Left eye was involved in 55% while right eye involvement was noted in 45% patients.

Paranasal sinuses were involved in all patients and 90% had multiple sinus involvement. Ethmoidal (72%), maxillary (65%), sphenoidal (43%) and frontal sinuses (23%) were involved in decreasing frequency.

Intracranial extension was evident in 24 patients. They presented with altered consciousness, seizures and hemiparesis. MRI brain suggested cavernous sinus thrombosis in 4 patients.

Laboratory parameters revealed significantly increased inflammatory markers including neutrophil lymphocyte ratio, hsCRP, ferritin and...
In our study, we observed that all patients with mucormycosis had uncontrolled diabetes. In the present study, 16/98 (17%) patients were expired. On multivariate logistic regression analysis, age, site of involvement (rhino-orbital-cerebral), presence of DKA and sepsis were associated with increased mortality as shown in Table 4.

### Discussion

Covid 19 has presented us with a major challenge to find effective ways to mitigate this pandemic. The pathogenesis of COVID-19 is not fully understood, but it is probably multifactorial, resulting in a systemic hyperinflammatory response and associated thromboembolic complications in severe cases. It resulted in higher morbidity and mortality in patients with diabetes and hypertension.

Covid 19 is associated with immune dysregulation caused by SARS-CoV-2 virus itself. It leads to over expression of inflammatory cytokines and impaired cell-mediated immunity with decreased cluster of differentiation (CD4+ T and CD8+ T) cell counts that cause more susceptibility to fungal co-infections. White et al and Song et al have reported increased incidence of invasive fungal disease in patients with covid 19.

Mucormycosis is a rare, rapidly progressive disease associated with high morbidity and mortality. Diagnosis is often delayed and management needs multidisciplinary involvement.

In India, particularly we saw a new epidemic of mucormycosisesembbedded in covid pandemic as coinfection in patients of Covid 19 or when they were in convalescent phase.

In our study, we observed that all our patients had uncontrolled diabetes. Patients with diabetes are prone to have opportunistic infections and mucormycosis. This is contributed by use of corticosteroids which were intensively used as lifesaving drugs. According to existing literature India contributed to 81% of the cases of the global burden of this ‘rare mould’ infection. Patel et al conducted an important study involving 16 centers across the country on prevalence of fungal infections in patients with covid 19 noted a 2.1-fold rise in mucormycosis, uncontrolled diabetes was the most common comorbidity. Presence of COVID-19-related hypoxemia and improper glucocorticoid use independently were associated with CAM. Sen et al have also conducted a multicentric study involving 2826 patients of rhino-orbital-cerebral mucormycosis from states of Gujarat and Maharashtra and reported diabetes and steroid use as most common risk factors. They highlighted the importance of surgical interventions to reduce mortality in these patients.

Mucor is a saprophytic fungus; ubiquitous in nature, especially in soil, air, decaying vegetation and organic matter. It may be present in nasal mucosa as commensal. When there is any breach or immunocompromised state, it can invade paranasal sinuses, orbit and brain. Mucormycosis describes infections caused by fungi of the order Mucorales. The most frequently reported pathogens in mucormycosis are Rhizopus spp, Mucor spp, and...
In present study *Rhizopus* spp. were grown in all the culture specimens.

Hyperglycemia leads to increased expression of the endothelial receptor GRP78, resulting in polymorphonuclear dysfunction, impaired chemotaxis and defective phagocytosis. Hyperglycemia and ketoacidosis favor germination and growth of fungal spores which are present in the environment. Corticosteroids used in COVID-19 by causing impaired neutrophil function and hyperglycemia make diabetic patient more vulnerable for development of mucormycosis.

Acidic pH, a hall mark of DKAlkEads to more availability of free iron which is taken up by the mucorales through siderophores or iron permeases. Rhizoferrin produced by fungal hyphae forms iron-rhizoferrin complexes after binding to serum iron.[15] Iron is an essential element for fungal cell growth and development and iron acquisition is adds virulence to fungi. It was noted in all the patients that serum iron levels were uniformly low and most of the patients had severe anemia. Mucorales, have extremely restricted growth in normal serum unless exogenous iron is added.[16] High-affinity iron permease (*FTR1*) has a role in iron uptake and transport in mucorales especially during the lack of iron in the environment.[17]

Experimental data have shown that compounds interfering with zinc homeostasis process would inhibit fungal growth. Zinc deficiency increases stress in fungal cells and inhibits fungal growth by restricting the activity of zinc-binding proteins, which are mainly transcription factors involved in many biological processes.[18] Further, some zinc chelators have shown promising results against *Aspergillus fumigatus* strains.[19] Another objective of present study was to estimate the zinc levels in our patients to determine any association with mucormycosis. It was observed that none of the patients in study cohort had zinc deficiency or zinc excess.

We had few notable findings in the present study, firstly not all patients had suffered from severe covid 19 requiring hospitalization and supplemental oxygen therapy, and secondly, significant proportion of the patients did not receive corticosteroids. However, some of them received in large doses for prolonged duration. Therefore, we conclude that covid 19 infection, steroid use and uncontrolled diabetes, were the important factors which were present in our patients similar to the results reported by other studies.[20,21] Sharma et al have reported increased prevalence of mucormycosis in patients with covid 19.[22]

Large proportion of the patients in the present study were diagnosed to have mucormycosis at the early stage, probably because of the increased awareness of this life threatening condition and that was reason behind relatively low mortality rate recorded in present series. High mortality rates (50-80%) have been reported from intra-orbital and intracranial complications of mucormycosis which is observed in present study also.[18] Is there any mucospecific immunity disturbed during COVID-19 induced immune dysregulation or any other host or environmental risk factor responsible for surge is the question which should be answered. Clinicians and laboratory researchers should pay critical attention to the increased incidence of fungal infections in covid-19 affected or recovered patients.

**Conclusion**

CAM has posed as a continuum of challenges faced during the pandemic of covid 19. This rare and life threatening complication requires high index of suspicion for early diagnosis. Multidisciplinary involvement and timely interventions including antifungal pharmacotherapy, stringent glycemic control and surgical debridement can reduce the mortality. Mucormycosis is uniformly associated with low iron levels but role of zinc needs to be further studied.

**References**

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