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Clinical Profile and Outcomes of Mucor Mycosis in COVID-19 Patients in a Tertiary Hospital in South India.

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ABSTRACT

India is amongst the worst hit countries by the SARS Corona Virus-2 (COVID-19) pandemic. There has been an alarming rise in incidence of COVID-19 associated Mucormycosis (CAM) in patients suffering from co-morbidities. The present study aimed at studying the epidemiology, clinical features, and outcomes of CAM patients. This is a retrospective study conducted in tertiary care hospital in South India from May 2021 to July 2021. Patients who were admitted with COVID-19 infection and had Mucomycosis were included in the study. The epidemiology, clinical features and outcomes of these patients were studied. Twenty-two patients of CAM were included in the study. There was a male preponderance with the male to female ratio being 1:4.5. Diabetes was the most common comorbidity (n=15; 68.18%). Majority of the patients (n=21; 95.46 %) had suffered from moderate to severe covid illness. Invasive fungal sinusitis was the most common (n=14; 63.64%) radiological finding. Most patients (n=20; 90.9%) had received steroids as part of treatment for covid 19 illness. Patients were managed with the combination of both medical and surgical treatment and 18 patients underwent (81.81%) surgical procedure. Nineteen patients (n=19; 82%) recovered after treatment. COVID-19 associated Mucor mycosis has a worse prognosis when accompanied with poor glycemic control, severe COVID-19 illness, and anatomical extent of fungal infection.

1. Introduction

The occurrence of Mucor mycosis during the second wave of COVID-19 grew exponentially in India. There is an association of severe COVID-19 with diabetes in some studies [1], [2]. CAM (COVID-19

associated mucormycosis) is defined as the occurrence of confirmed mucormycosis infection in COVID-19 patients [3]. This is due to uncontrolled glycemia, prolonged use of corticosteroids [4]. Mucor mycosis is an Angio-invasive fungal disease that may involve the nose, orbit, sinuses, and multiple organs [5]. Injury to the vascular endothelial cells by the fungi is caused by enhanced GRP 78 (Glucose Regulatory Protein 78) expression on endothelial cells [6]. The infection causes Angioinvasion and thrombosis of vessels [7]. COVID-19 causes hyperglycemia by its action on pancreas [8] and causes immune dysregulation [9]. Steroids abuse can cause immunosuppression, hyperglycemia [10]. This study aimed at analyzing the epidemiology, clinical features, and outcome of CAM patients.

2. Materials & Methods

Study design - This is a retrospective observational study in M.S. Ramaiah hospitals.

Ethics statement - The study was conducted after the approval from the Institutional Ethical Committee (MSRMC/EC/AP-05/08-2021).

The data for all confirmed mucormycosis cases following COVID-19 infection reported between May1, 2021 to July 31, 2021, were collected.

A diagnosis of mucormycosis was confirmed when there are compatible clinical and radiologic findings, and in addition there is demonstration of fungi in the tissue by either direct microscopic visualization of broad ribbon-like aseptate hyphae or isolation of Mucorales. Diagnosis of COVID-19 was made by testing respiratory specimens of the patients by reverse transcription polymerase chain reaction (RT-PCR).

A proforma for a detailed data collection was developed. Demographic details such as age, gender, comorbidities, clinical manifestations, site of Mucor mycosis, diagnostic tools used for Mucor mycosis like microscopy, culture, histopathology, radiological findings, and treatment modalities were studied. Diagnostic samples were collected from sinuses, paranasal spaces, nasal cavity, and crust. Samples collected were studied for evidence of mucormycosis by direct microscopy, tissue histopathology and culture. Tissue biopsies from Mucor mycosis-affected anatomical sites were studied for microscopy, culture, and histopathology. Microscopy was done by using potassium hydroxide mount with or without calcofluor stain. The samples were inoculated on 2 sets of Sabouraud dextrose agar and incubated at 25°C and 37°C. Tissue samples for histopathology were examined by using hematoxylin and eosin, periodic acid Schiff, or Gomori methenamine silver stain. Radiological features such as acute invasive fungal sinusitis with vascular extension and extension to neuroparenchyma were studied using Computed Tomography and Magnetic resonance imaging methods. The treatment details such as the dose and duration of steroids, antibiotics received, supportive measures like oxygen, non-invasive ventilation and use of zinc supplements were studied. Details of the surgical procedures like Functional Endoscopic Sinus Surgery (FESS) with debridement, orbital decompression and maxillectomy along with intra and post-operative complications were recorded in the study.

3. Statistical methods

Data was analyzed using statistical software R version 4.1.1 and Microsoft Excel. Categorical variables are represented in the form of frequency tables. Continuous variables are given in Mean ± S.D./ Median (Min, Max) form. Chi square test was used to assess the association variables with outcome and diagnosis. Kruskal -Wallis test was used to compare distribution of age and duration of hospital stay with diagnosis. Mann Whitney U test was utilized to compare age and duration of hospital stay with outcome. “p-value”

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less than or equal to 0.05 was considered statistically significant.

4. Results

Twenty-two cases of COVID -19 associated Mucor mycosis were included in the study. The mean age of patients was 54.7 ± 15.8 years. Out of 22 subjects, 4 (18.1%) were females and 18 (81.8%) were males with gender ratio of 1: 4.5. The clinical presentation of the patients has been described in the Table-1. Out of 22 subjects under study, 1 (4.5%) subject had mild COVID-19 infection, 7 (31.8%) had moderate illness and 14 (63.6%) had severe illness. All patients who died (n=4; 18.8%) had severe illness. Most common underlying comorbidity was Diabetes mellitus (n=15; 68.1%). Two patients were newly diagnosed with Diabetes mellitus and Diabetic ketoacidosis was seen in 1 patient. The comorbidities of the patients are described in Table-2

A greater majority (n=16; 72.7%) of patients with CAM were managed in the ward while 6 (27.2%) patients required ICU care. The rhino-orbital region was the most common infection site, followed by rhino-orbital-cerebral region. The most common clinical features were facial pain and tenderness, facial swelling, crusting, eye pain and swelling.

Diagnosis was made by direct microscopy in 18 (82.6%) patients. The histopathological features are presented in Table 3. Culture identified the etiologic agent in 11 (48.1%) cases. The isolated Mucorales included Rhizopus arrhizus, Rhizomucor pusillus and Apophysomyces variabilis. Table 4 gives the comparison of different clinical variables with the severity of COVID-19 illness. Figure 1 shows Mean plot of Glucometer Random Blood sugar over outcome. Patients who suffered from moderate to severe COVID-19 infection and the non-survivors had poorer glycemic control as compared to patients with mild disease and the survivors in our study.

All patients received conventional Amphotericin B, the antifungal agent in addition to supportive medications. History of corticosteroid use for the treatment of COVID-19 was present in 20 cases and tocilizumab was used in 3 cases. Surgical procedures such as Functional Endoscopic Sinus Surgery (FESS) with debridement, maxillectomy, turbinectomy and orbital decompression were performed on 18 (81.8%) patients. Methylprednisolone was the most used steroid. Corticosteroid therapy was started at a median 1-3 days after admission for moderate and severe cases. We found that 11 (50%) patients received steroids for the duration of 5 -10 days. (Table-5).

5. Discussion

During the second COVID-19 wave in India, many patients presented after recovery with another life-threatening complication of Mucor mycosis which is usually known to occur in immunocompromised individuals. The present study found a male preponderance with a ratio of 4.5:1 (n=18; 81.8% versus n=4; 18.1%). In a study by [11] of 101 cases of CAM, 78.9% were males.

The mean age group was 54.7 ± 15.8 years in this study, whereas in a study from France the average age was 64 years [12]. The average age of non-survivors was 35 years which was two decades lower than the average age. These variations might be based on physiological, immunological, or genetic differences which require further verification.

In a metacentric study across India by [13] it was seen that in patients with CAM rhino-orbital-cerebral was most common presentation (58.1%) as compared to other types. Pain in the tooth, loosening of teeth and involvement of the mandible were noted in many CAM patients. A greater percentage of their patients with

CAM had hypoxemia requiring Intensive Care unit (ICU) care. In our study, 72.7% i.e., 16 patients were managed in wards and 27.2% i.e., 6 patients required ICU care. Consistent with their study we too found that rhino-orbital-cerebral type was the most common presentation in our CAM patients. However, our patients had clinical features widely varying from the most common features like facial pain, facial swelling, crusting, and sinusitis to least common features like ptosis, dysphagia, and blackish discoloration of palate.

It was seen in the present study that Diabetes mellitus was the most common comorbidity. Diabetes as an important risk factor and SARS-CoV-2 has been shown to infect the beta cells of the pancreas by impairing glucose homoeostasis, possibly causing the disease. It is suggested that the pancreas is susceptible to SARS-CoV-2 infection, which can harm β-cell integrity. A study by [14], found diabetes mellitus to be the most common risk factor in developing Mucor mycosis. Patients who suffered from moderate to severe COVID-19 infection and the non-survivors had poorer glycemic control as compared to patients with mild disease and the survivors in our study. In the Indian population risk factors include Diabetes mellitus (73.5%), Cancers (9.0%) and organ transplants recipients (7.7%) [13]. Diabetes leads to immunosuppression by impairing neutrophilic function [15]. In patients with diabetic ketoacidosis, acidic pH prevents binding of free iron to transferrin. Elevated free iron levels in serum can make the host susceptible to Mucor mycosis [16]. Additionally, hyperglycemia leads to glycosylation of transferrin and ferritin which further reduces the iron binding and increases free iron levels [17].

The mortality rate for CAM patients in our study was 18%. In a systematic review conducted by [11] involving 101 patients worldwide, the mortality rate was 30.7%. The mortality rate in our cohort was probably lower due to a high degree of suspicion even among public during the second wave of the pandemic, owing to wide media coverage which increased the public awareness leading to medical attention being sought earlier in the course of the illness. A combination of early surgical intervention and medical therapy contributed to recovery.

Excessive use of steroids for prolonged duration is a risk factor for Mucor mycosis in COVID-19 patients. Since the RECOVERY trial demonstrated that corticosteroids reduced mortality in those COVID- 19 patients who were on oxygen and required ventilatory, steroids had been used extensively [18]. In our study when we analyzed the mortality data, we found that patients who received steroids for 5 to 10 days had a mortality of 13.0%. None of the patients who died received steroids beyond 10 days.

The deadly combination of steroids and a weak immune system due to COVID-19 increased the breach in the immune system. Endothelitis, which is a pathological event seen in severe COVID-19, is another plausible link between COVID-19 and Mucor mycosis [19]. In our study we used methyl prednisolone (1mg/kg) IV in patients with hypoxia in moderate to severe COVID - 19 cases. Methylprednisolone penetrates the lung better, thus it can act as a superior immunosuppressive agent in the treatment of COVID-19 and helps in improvement of respiratory complications [20].

The second wave of COVID-19 which was due to the delta variant (B.1.617.2) led to a rapid rise in both coronavirus and COVID-associated Mucor mycosis cases in India.

Physicians should be cautious regarding development of Mucor mycosis in the patients with COVID-19 illness, especially among the Diabetic patients. Prolonged use of corticosteroids in the treatment of COVID-19, seems to be an additional risk factor and should be avoided. There has been speculation that variants such as Delta and Delta plus have led to increased incidence of Mucor mycosis in these patients as it was

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not seen with any other variant so far. The limitations of were limited sample size; secondly there was lack of a control group for the study. Hence, the findings cannot be generalized.

6. Conclusion

The combination of diabetes mellitus, excessive use of corticosteroids and COVID-19 infection poses a serious risk of developing Mucor mycosis. Proper glycemic control and use of corticosteroids judiciously in patients with COVID-19 as per the latest evidence is recommended to reduce fatal Mucor mycosis. Appropriate preventive measures such as vaccination along with timely diagnosis and early treatment may lead to better outcomes in the setting of COVID19 associated Mucor mycosis.

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Conflict of Interest – All the authors declare that there are no conflicts of Interests.

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Table 1: Clinical features of the patients with COVID-19 associated Mucor mycosis

Facial pain and tenderness	10 (45.4%)
Crusting over face	7(31.8%)
Facial Swelling	7(31.8%)
Eye pain and swelling	5(22.7%)
Nasal Block	4 (18.1%)

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Headache	3 (13.6%)
Epistaxis	2(9.1%)
Sinusitis	2 (9.0%)
Tender nasal cavity	2 (9.0%)
Pain during mastication	2 (9.0%)
Altered sensorium	1 (4.5%)
Para-septal nasal cellulitis	1 (4.5%)
Ptosis	1 (4.5%)
Decreased sensation of right side of face	1 (4.5%)
Eye movement restriction	1 (4.5%)
Dysphagia	1 (4.5%)
Black discoloration of hard palate	1 (4.5%)
Decreased sensation over eye	1 (4.5%)
Vision disturbanc	1 (4.5%)

Table 2 – Comorbidities in the study population

Diabetes Mellitus	15 (68.1%)
Hypertension	9 (40.9%)
Asthma	2 (9.0%)
Acute Kidney Injury	1 (4.5%)
Chronic Kidney Disease	1 (4.5%)
Cerebrovascular Accident	1 (4.5%)
Hypothyroidism	1 (4.5%)
No comorbidities	4 (18.1%)

Table 3- Histopathology and Radiological findings

Histopathology	Mucor mycosis with Aspergillosis	11 (50.0%)
	Mucor mycosis with micro-abscesses	10 (45.4%)
	Mucor mycosis with fungal granuloma	10 (45.4%)
	Mucor mycosis with vascular invasion	9 (40.9%)
Radiology	Invasive fungal sinusitis	14 (63.6%)
	Invasive fungal sinusitis with orbit involvement	2 (9.0%)
	Invasive fungal sinusitis with neuroparenchyma involvement	7 (31.8%)

Table - 4 Comparison of clinical variables with severity of COVID-illness.

Variables	**Subcategory**	**Mild COVID**	**Moderate COVID**	**Severe COVID**	**p-value**
Age (years)	Mean ± S.D.	54	53.7 ± 12.2	55.3 ± 18.3	0.9[K]
Gender	F	1 (100.0%)	2 (28.5%)	1 (7.1%)	0.1[MC]
	M	0	5 (71.4%)	13 (92.8%)	
Diabetes Mellitus	No	1 (100.0%)	4 (57.1%)	2 (14.2%)	0.05[MC]
	Yes	0	3 (42.8%)	12 (85.7%)	

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Duration of hospital stay (days)	Mean ± S.D	12	21 ± 14.3	16.6 ± 8.0	0.7[K]
Random Blood sugar (mg/dl)	<200	1 (100.0%)	6 (85.7%)	7 (50.0%)	0.5[M] [C]
	>300	0	1 (14.2%)	3 (21.4%)	
	200-300	0	0	4 (28.5%)	
	Mean ± S.D.	150	191.5 ± 142.7	214.7 ± 103.5	0.7[K]

Table 5: Comparison of duration of steroid administration with outcome

Steroid administration (days)	**Outcome**	
	Non-Survivors	**Survivors**
< 5	1(4.3%)	5 (22.7%)
>10	0	2 (9.0%)
5-10	3 (13.0%)	11 (50.0%)

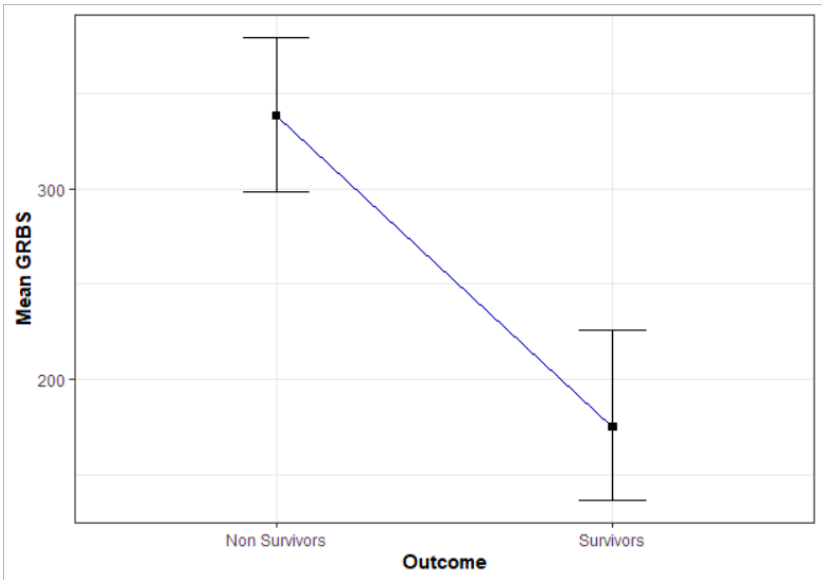

Figure 1– Mean plot of Glucometer Random Blood sugar over OUTCOME.