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Original Research Article

Covid-19 associated mucormycosis

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ABSTRACT

Background: Corona virus disease-2019 (COVID-19) is associated with various opportunistic, bacterial, and fungal infections. High risk groups include people with diabetes especially diabetic ketoacidosis, solid organ transplantation, long-term systemic corticosteroid use, and iron overload. Many cases of mucormycosis were reported worldwide. Mucormycosis is an invasive fungal infection with high morbidity and mortality. Early diagnosis and treatment with appropriate anti-fungals lead to improved outcomes.

Aims: The aim of this study is to establish the factors associated with mucormycosis in a COVID-19 setting, like comorbidities and treatment protocols for treatment of COVID-19. The histological patterns and tissue reactions to mucormycosis also were studied.

Materials and Methods: In patients with ongoing COVID-19 infection and in a post COVID-19 scenario, we studied the biopsy findings of mucormycosis in various sites like rhino-orbital, lung, gastric and trachea. Material for this study is from a tertiary care hospital in South India. Patient age ranged from 30 years to 74 years. Mean age of the patients was 51 years. Male to female ratio was 1:1.1. Tissue from sino-nasal mucosa, peri orbital tissue, exenterated eyeball, lung tissue and tissue from rare sites like gastric and tracheal mucosal lesions were also included in this study. Tissue was fixed in 10% buffered formalin. Routine Haematoxylin and Eosin(H&E) stains were done. Gomori's Methenamine silver (GMS) stains were done on all cases. Tissue was submitted for fungal cultures in all the cases.

Results: Total of twenty-three cases were diagnosed as mucormycosis based on the morphology and special stains in this analysis. Histology revealed areas of infarction in all cases with neutrophilic infiltration. Granulomatous reaction was seen in seven cases and melanin pigment was seen in two cases.

100% of patients were diabetics. There was neutrophilia in 100% of cases. Lymphopenia was seen in 85.7%, C-Reactive Protein (CRP) was elevated in 100% of cases.

Ferritin was done in 14 patients and D-dimer was done in 17 patients and in all patients, these were elevated. All patients were treated with steroids according to the treatment protocol for COVID-19, Remdesivir was given in 72.7% of cases and second immunomodulator drugs like Tocilizumab in four cases and Baricitinib in one case.

Conclusion: Mucormycosis is an emerging problem with COVID-19. It is important to carefully monitor blood glucose levels and take into account underlying medical conditions of patients before initiation of steroid therapy. Early recognition of symptoms and early diagnosis has a better outcome in patients with mucormycosis associated with COVID-19 infection.

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1. Introduction

COVID- 19 infection does not stop at acute phase. There are many post covid complications like opportunistic infections especially mucormycosis. This is possibly the most feared infection of all the opportunistic infections. Several cases are reported, especially from India.

Prevalence of mucormycosis worldwide is 0.005%. In India the incidence is 80 times more than the rest of the world.[1] Opportunistic infections in COVID-19, especially mucormycosis is associated with underlying diabetes, especially in patients with ketoacidosis, high ferritin levels and decreased white blood cell (WBC) and phagocytic activity. There is a need for relook at these patients because of high morbidity and mortality associated mucormycosis. We reviewed our cases of COVID-19 associated mucormycosis, its association with co morbidities and drugs used during COVID-19 infection and the histological patterns in tissues.

Mucormycosis was first described by Platauf in 1885[2] and the term mucormycosis was coined by Baker in 1957. Mucormycosis is a serious fungal infection caused by a group of molds called Mucoromycetes. Mucormycosis is commonly seen in immunosuppressed people. Diabetes is the most common cause of mucormycosis. India has the second largest population of diabetics worldwide. Poorly controlled diabetes and steroid induced hyperglycaemia secondary to treatment of COVID-19 with steroids are a potent combination for creating a favourable environment for the growth of mucormycosis. Diabetes is an independent risk factor, and in association with COVID-19 infection, especially poorly controlled diabetes, is a fertile ground for occurrence of mucormycosis.

2. Materials and Methods

Material for this study is from a tertiary care hospital in South India. This study included patients who were diagnosed as mucormycosis on biopsy over a period of four months associated with resurgence of COVID-19 in India. Post COVID-19 status was defined as greater than two weeks after being first detected with COVID-19 by polymerase chain reaction (PCR) and a subsequent PCR negative result.

Biopsies from patients in post COVID-19 or ongoing COVID-19 infection were included in the study.

Depending on the site, patients presented with varied signs and symptoms. Patients presented with facial pain, orbital pain and /or decreased vision and sinusitis. Patient with gastric lesion was an ongoing covid patient with dyspeptic symptoms. Lung lesion presented with cough and imageology revealed thick-walled cavity in the upper lobe of lung. Tracheal lesion was seen in a post-transplant, post COVID-19 patient with difficulty in breathing and cough. Biopsies were sent in 10% buffered formalin. 4-micron thin sections were studied. Routine Haematoxylin and Eosin (H&E) stains were done and Gomori's methenamine silver (GMS) was done in all cases. Diagnosis was based on histomorphological features.

3. Results

Twenty-three cases of mucormycosis were diagnosed. Eighteen cases were diagnosed post covid period. Age of the patients ranged from 30 years to 74 years. Male to Female ratio was 1:1.1. Mean age of the patients was 51 years.

In post COVID-19 patients, symptoms developed after two to four weeks after covid illness. Four cases were diagnosed in ongoing covid cases. Mean age of onset of symptoms of mucormycosis after a PCR diagnosis of COVID-19 was 21 days. All patients were diabetic (100%). Ketoacidosis was seen in three patients (13.0%). All the patients were treated with steroids (100%), Remdisivir was used in 16 patients (72.7%). One patient received Baricitinib and four patients received Tocilizumab (27.2%) as second immunomodulator drugs in addition to the steroids due to high inflammatory markers. Ten cases were from rhino-orbital area (43.47%), nine cases from sinuses (39.13%), one each from gastric wall (4.34%), trachea (4.34%) and 2 cases from lung (8.68%) were diagnosed. (Table 1)

Panel of investigations done in these patients included CRP, IL-6, D-Dimer, ferritin, and complete blood counts. (Table 2).

MRI of the brain, orbits and paranasal sinuses revealed soft tissue swelling in the pre-septal, premaxillary and retrobulbar regions with pan sinusitis. (Figure 1)

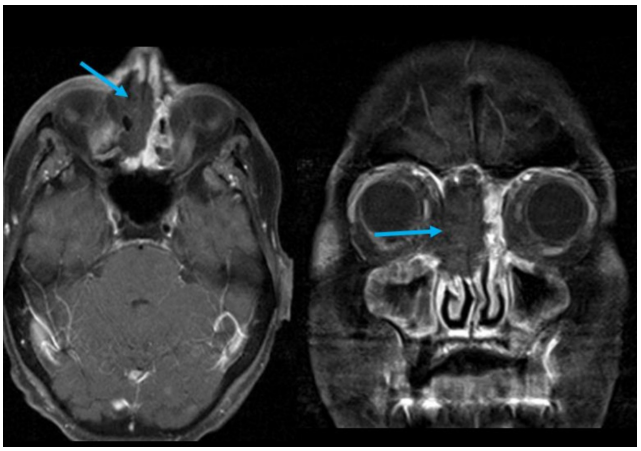

Fig. 1: Axial and Coronal T1 post contrast images show poorly marginated non enhancing area (necrotic tissue) noted in the right ethmoidal sinus involving nasal septum, middle turbinate, meatus, and cribriform plate. Maxillary sinus shows mucosal thickening

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Table 1: Showing clinical details, site of involvement, treatment and prognosis in patients in the study

Age/ Gender	Comorbidities	Time duration since diagnosis of covid	Site of involvement	Clinical presentation	Steroid therapy/ tociluzumab/ Barticinib	Prognosis
70/M	Diabetes & Bronchial Asthma	Post COVID-17 Days	Rhino-orbital	Sinusitis, Diplopia, Orbital Pain	Steroids: +	Recovered Post Exenteration and Debridement
62/F	Diabetes	Post COVID-22 Days	Maxillary Sinus	Sinusitis	Steroid: +	Recovered-Post Debridement
45/F	Diabetes	Post COVID-26 Days	Rhino-orbital	Sinusitis, Diplopia, Headache, Orbital Pain	Steroid: +	Recovered-Post Exenteration and Debridement
47/F	Diabetes, Bronchial Asthma	Post COVID-14 Days	Rhino-Orbital	Sinusitis, Orbital Pain, Decreased Vision, Proptosis.	Steroid: +	Recovered-Post Exenteration and Aggressive Debridement
37/M	Diabetes	Post COVID-23 Days	Rhino-Orbital	Sinusitis, Orbital Pain, Proptosis, Decreased Vision	Steroid: +	Recovered-Post Exenteration and Aggressive Debridement
70/M	Diabetes, Hypertension	Post COVID-18 Days	Rhino-Orbital	Sinusitis, Decreased Vision.	Steroid: +	Recovered-Post Exenteration and Aggressive Debridement.
50/M	Diabetes, Post Renal Transplant Status	Post COVID- 28 Days	Tracheal Nodule	Cough	Steroid: +	Recovered
67/M	Diabetes, Hypertension	On Going COVID-4 Days	Gastric	Dyspepsia	Steroid: + Tocilizumab+	Expired
54/F	Diabetes, Hyperparathyroidism	Post COVID- > 2 Weeks	Lung	Cough	Steroids: +	Recovered
47/M	Diabetes	Post COVID- 20 Days	Maxillary Sinus	Bilateral Sinusitis	Steroids: +	Recovered
50/M	Diabetes	Post COVID-18 Days	Rhino-Orbital	Pan sinusitis, Base of Skull Involvement	Steroids: + Tocilizumab+	Recovered-Post-Aggressive Debridement
45/F	Diabetes, Hypothyroid	On Going COVID- 8 Days	Maxillary Sinus	Sinusitis	Steroids: +	Recovered Post Aggressive Debridement
47/F	Diabetes	Post COVID- 32 Days	Maxillary Sinus	Fever, Sinusitis	Steroids: + Tocilizumab+	Recovered-Post Debridement

Continued on next page

Table 1 continued

40/M	Diabetes	Post COVID- 29 Days	Rhino-Orbital	Headache, Loss of Vision, Cheek Swelling, Diplopia	Steroids: + Baricitinib+	Refused Bilateral Exenteration-Expired
37/M	Diabetes	On Going COVID- 7 Days	Rhino-Orbital	Sinusitis, Proptosis, Loss of Vision.	Steroids: +	Recovered-Post Exenteration and Debridement
45/F	Diabetes	On Going COVID- 11 Days	Maxillary Sinus	Fever, Sinusitis	Steroids: + Tocilizumab +	Recovered-Post Debridement
50/F	Diabetes	Post COVID- 14 Days	Rhino-Orbital	Sinusitis, Orbital Pain, Decreased Vision	Steroids: +	Recovered-Post Exenteration and Aggressive Debridement
30/M	Diabetes	Post COVID-37 Days	Maxillary Sinus	Sinusitis, Cheek Swelling	Steroids: +	Recovered-Post Aggressive Debridement
70/F	Diabetes, Hypertension	Post COVID- 16 Days	Maxillary Sinus	Sinusitis	Steroids: +	Recovered-Post Aggressive Debridement
66/F	Diabetes, Hypertension	Post COVID- 33 Days	Maxillary Sinus	Sinusitis	Steroids: +	Recovered-Post Aggressive Debridement.
74/M	Diabetes, Hypertension	Post COVID- 28 Days	Rhino-Orbital	Pain, Sinusitis, Loss of Vision	Steroids: +	Recovered-Post Exenteration and Aggressive Debridement.
69/M	Diabetes	Post COVID-20 Days	Rhino-Orbital	Eye pain, Sinusitis, Decreased Vision	Steroids: +	Recovered-Post Exenteration and Aggressive Debridement.
55/M	Diabetes, Hypertension	Post COVID-35 Days	Lung	Cough, Bilateral Pnemonia, Fever	Steroids: +	Recovered

Histologically, In all these cases broad aseptate filamentous hyphae with irregular branching was seen. (Figure 2 A&B) There was infarction and necrosis due to angioinvasion with neutrophilic infiltration in all cases (Figure 2 B&C). Granulomatous inflammation was seen in seven cases (Figure 2 D). Melanin pigment was seen in two cases. Sections through the exenterated orbits (Figure 3 A) revealed periorbital tissue necrosis, inflammation and in one case fungal hyphae were seen extending into the pigment layer. (Figure 3 B) These hyphal structures were GMS positive. (Figure 3 C) Pigment was identified as melanin by Masson Fontana stain.(Figure 3 D) Sporangia and sporangiophores were seen in two sino-nasal mucosal lesions.

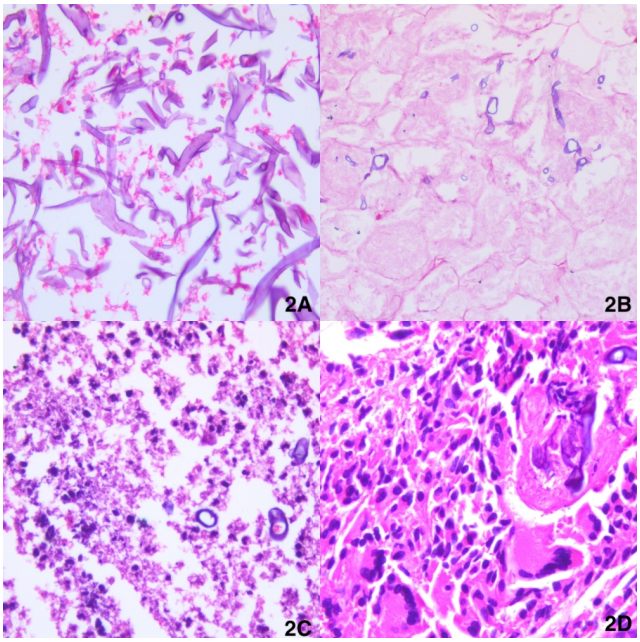

Fig. 2: A): Section showing characteristic ribbon like aseptate branching hyphae. (H&E X 200); **B:** Section showing areas of infarction with fungal elements. (H&E X100); **C:** Sections showing dense neutrophilic infiltration forming abscesses with fungal filaments. (H&EX100); **D:** Sections showing granulomatous reaction with giant cells showing fungal elements. (H&EX200)

After the course of Amphotericin B, these patients were started with Posaconazole delayed release tablet form 300 mg twice daily for two doses followed 300 mg daily for 3-6 months in immunocompetent patients.

4. Discussion

COVID- 19 viral infection is not limited to acute respiratory syndrome. It is associated with many opportunistic infections including bacterial and fungal infections. Mucormycosis is described in these patients and more so from India. Diabetes is the most common

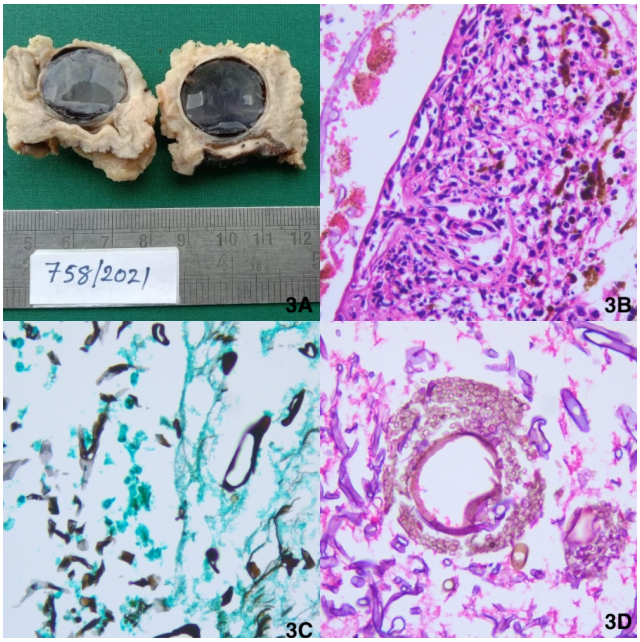

Fig. 3: A: Gross specimen of exenterated eyeball with periorbital tissue; **B:** Section showing pigment layer of orbit with dense neutrophilic infiltration with fungal elements. (H&EX200); **C:** Section showing broad hyphae with right angle branching. (GMSX 200); **D:** Section showing sporangia with sporangiophore with brownish black pigment. (H&EX200)

risk factor for mucormycosis in India. India has the second largest population of diabetics. Large number of diabetic population and steroid induced hyperglycaemia in patients treated with steroids were two important factors for high incidence of mucormycosis in India. Mucormycosis is an airborne infection, and it starts in the upper airways. Mucormycosis is described in diabetics, hematological and other malignancies, organ transplantation, immunosuppression and steroid therapy, iron overload and desferrioxamine therapy, AIDS patients, intravenous drug abusers and malnutrition.[3] In COVID-19 patients, factors which facilitate the spores of mucorales to cause infection are many. Spores germinate to produce hyphae that invade the blood vessels and adjacent tissue, this blocks the blood flow to the tissues causing extensive infarction and necrosis. High glucose levels, low pH is a fertile media for mucormycosis. Decreased phagocytic activity of white blood cells enhances the expression of glucose regulator protein (GRP-78) of endothelial cells. This enables angio-invasion and hematogenous dissemination.[4] The level of unbound serum iron in an acidic environment is an important predisposing factor in growth of mucormycosis. Increase in serum concentration of ferritin causes insulin resistance. Reduced iron binding and excess free iron is taken up by the fungus and further enhances the growth and virulence.[5] Increase in cytokines

Table 2: Showing values of various inflammatory markers

S.No	Investigations	No of patients	% of high values	Value range in pts	Normal ranges
1	D-Dimer	N=17	100%	608-1590	<550 ngs/ml
2	IL-6	N=6	100%	20.9-34.2	0.0-7.0pg/ml
3	CRP	N=22	100%	225-625	<10mgs/l
4	Ferritin	N=14	100%	466-1109	17.9-464ngs/ml
5.	Total WBC Count	N=22	90%	11000-35000	4400-11000 Cells/ul
6.	Neutrophilia	N=22	100%	77-92	40-75%
7	Lymphopenia	N=22	85.7%	7-13	20-40%

in patients with COVID-19 mainly IL-6, increases free iron levels.

Hence, all the parameters were evaluated, and we found good correlation in COVID-19 patients who developed mucormycosis.

It is an angioinvasive fungus, histologically, there will be infarction, necrosis, and neutrophilic infiltration of these areas. Few patients develop a granulomatous reaction.

The management of covid patients is predominantly supportive. There is an exaggerated immune response causing multiorgan failure.[6] There is a cytokine storm described and this led to the use of immunomodulator drugs like Tocilizumab and Baricitinib. Inhibition of IL-6 has adverse consequences as shown in experiments in mice. There is decreased immunity to opportunistic infections[7] including mucormycosis.

The overall mortality rate of mucormycosis as described in literature for India is 36.5%.[8] In our series, one patient who had bilateral orbital involvement expired, as he refused treatment, patient with gastric involvement of mucormycosis also expired constituting 8.6% mortality rate. All the other patients recovered and are on follow up.

5. Conclusion

In early diagnosis of COVID-19, judicious use of steroids, good control of diabetes during on-going COVID-19 treatment and post COVID-19 period, high index of suspicion for mucormycosis and prompt initiation of anti-fungal therapy with aggressive debridement are essential for successful management of COVID-19 associated mucormycosis. Though morbidity was there in the form of loss of vision in our series, overall survival was 91.4%.

6. Source of Funding

None.

7. Conflict of Interest

None.

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