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Clinico-pathological study of mucormycosis in post-COVID patients at government general hospital, Nizamabad

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ABSTRACT

Background: Mucormycosis is a rare, opportunistic fungal infection with high morbidity and mortality. Coronavirus disease 2019 (COVID-19) associated mucormycosis has been increasing in frequency since early 2021, particularly among patients with uncontrolled diabetes and systemic corticosteroid treatment. **Aims and Objectives:** The aim of the study was to study mucormycosis occurrence in COVID-19 patients at Government General Hospital, Nizamabad – A tertiary care center and to correlate mucormycosis with comorbidities and previous treatment modalities among admitted post COVID-19 patients. **Materials and Methods:** Cross-sectional retrospective study was done for 3 months, from June 2021 to August 2021. Fifty suspected cases of mucormycosis specimens sent to the Department of Pathology, Government General Hospital, Nizamabad for histopathological diagnosis were considered. Clinical history and treatment details were noted. Tissues obtained were fixed in 10% formalin, routine hematoxylin and eosin (H&E) stains were done. Gomori's methenamine silver stains were done. **Results:** Mucormycosis was most common in males 37 (74%) and common in the age group of 41–50 years (26%). Diabetes mellitus is the most common comorbidity (60%). The mean duration between diagnosis of COVID-19 and development of symptoms of mucor was 6–40 days. Out of 50 cases, 46 patients received steroid therapy and 39 cases were given oxygen support. **Conclusion:** Mucormycosis is a rapidly progressive life-threatening opportunistic infection. Post COVID-19 patients with uncontrolled diabetes are more prone to it and an important predisposing factor. Treatment with steroid therapy and oxygen support must be carefully monitored and used in a vigilant manner. Histopathological examination and clinical correlation are essential for early diagnosis and treatment.

Key words: COVID-19; Diabetes mellitus; Immunosuppression; Mucormycosis

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus.[1] After the first known case was identified in Wuhan, China, in December 2019, the disease spread worldwide and the WHO declared it a pandemic on March 11, 2020. While the recovery period for COVID-19 ranges from 2 weeks to 6 weeks, several post COVID-19 or COVID-19 related complications have been reported in patients who have recovered from it. One such lethal infection is a fungal disease called mucormycosis commonly known as "black fungus." A surge in mucormycosis cases has been observed in many parts of India during the second COVID-19 wave.

Mucormycosis is an emerging angioinvasive infection caused by the ubiquitous filamentous fungi of the Mucorales order of the class of Zygomycetes.[2] It is a rare but serious angio-invasive infection caused by a group of fungi called mucormycetes. Spores of these ubiquitous fungi (commonly

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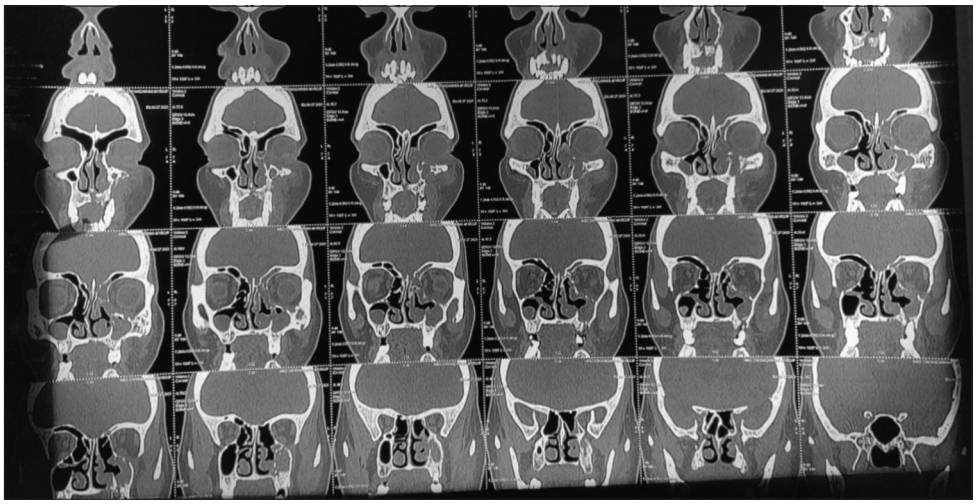

Image 1: Computed tomography scan showing calcific focus measuring approximately 8 mm with no significant perifocal edema seen in right temporo-occipital lobe – likely calcified granuloma

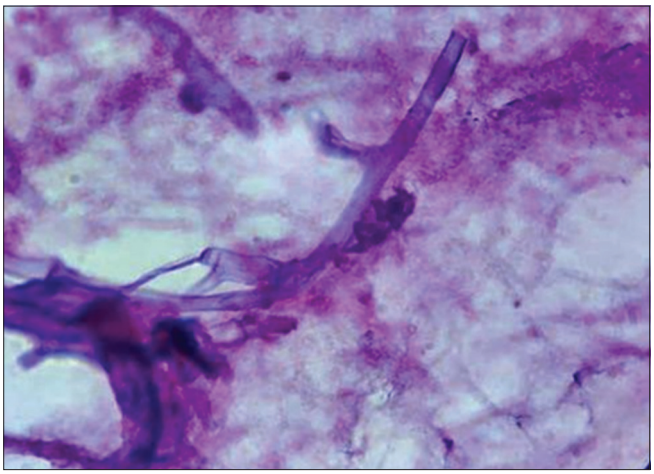

Image 2: Hematoxylin and Eosin Stain of Mucor Showing Broad Nonseptate Hyphae with 90-degree or acute angle Branching (Arrow) (×40)

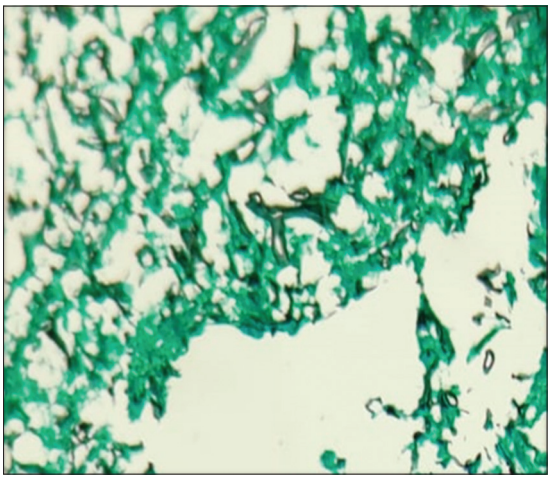

Image 3: GMS Stain highlighting the presence of fungal hyphae of mucormycosis

found in soil, fallen leaves, compost, animal dung, and air) can be inhaled and then infect the lungs, sinuses, and extend into the brain and eyes.[3] It can also occur on the skin after a cut, burn, or other type of skin injury. It affects people who are immunocompromised and high risk groups include those with diabetes, long-term systemic corticosteroid use, solid organ transplantation, neutropenia, and also patients who have been treated with steroids for COVID-19. It is a fulminant and rapidly progressing fungal infection associated with a high morbidity and mortality rate.[4]

When compared to other developed countries the probability of people being affected with mucor spores either in indoor or outdoor air is outrageous in India, the reason being tropical and humid climate.

Due to irregular testing of blood sugar levels by remarkable majority of patients, uncontrolled diabetes is frequently and more common in our country.[5] Blood sugar testing has been drastically declined during the pandemic of COVID-19, this was because of the monumental amount of people that have been affected and rushed to hospitals during this period of time.[6] It has been noticed scores of people have been self-medicating or irrationally utilizing corticosteroids of unreasonably excessive doses, this has cast up to the imminent annihilation. It has been noticed that several patients have developed diabetes in the course of the COVID-19 illness and were formerly unenlightened regarding the blood sugar levels, which might have been a result of damage which has been straight to pancreatic beta cells caused by the SARS-COV-2, due to its similarity for the angiotensin-converting enzyme-2 receptor and incidentally by damaging the smaller blood vessels which supply beta cells.

COVID-19-associated mucormycosis emerged as an epidemic in certain parts of the world amidst the global COVID-19 pandemic.[7] Mucormycosis is therefore an

aggressive, life-threatening infection requiring prompt diagnosis and early treatment. Treatment usually consists of antifungal medications and surgery.

Aims and objectives

The aim of the study was to study mucormycosis occurrence in COVID-19 patients at Government General Hospital, Nizamabad – A tertiary care center.

To correlate mucormycosis with comorbidities and previous treatment modalities among admitted post COVID-19 patients.

MATERIALS AND METHODS

Cross-sectional retrospective study was conducted in post COVID-19 patients who are admitted as suspected mucormycosis. Histopathological examination of biopsy material of mucormycosis from the paranasal sinuses was done. Cases of mucormycosis in post-COVID-19 patients were collected from June 2021 to August 2021. Data were entered into Excel spreadsheet. Post FESS biopsy material sent by the ENT department of suspected post-COVID patients were sent to pathology department. Clinical history and treatment details were noted in predesigned forms. Tissues were fixed in 10% formalin solution, processed and stained with Hematoxylin and Eosin and Gomori's methenamine silver stains.

Inclusion criteria

The following criteria were included in the study:

1. Post-COVID 19 patients presenting to the ENT department
2. Age groups above 20 years.

Exclusion criteria

The following criteria were excluded from the study:

1. Non post-COVID patients
2. <12 years of age.

Ethical committee

This study was reviewed and approved by the institutional ethics committee. Descriptive statistics of the explanatory and outcome variables were calculated by the mean, frequency, and proportions for qualitative variables. Statistical Package for the Social Sciences (SPSS) version 20, IBM SPSS statistics was used to perform the statistical analysis.

RESULTS

In the present study which is a retrospective a total of 50 patients were taken. In which majority were males 37 (74%) and female 13 (26%) with mean age 47.53 most common age group being 41–50 years (26%) with diabetes mellitus is the most common co-morbidity (60%). The mean duration for the development of mucormycosis after the recovery from COVID-19 ranged from a minimum of 6 days to a maximum of 40 days with maximum patients presenting on 15th day of post COVID with the symptoms of mucormycosis. It has been observed that 46 cases of mucormycosis were treated with corticosteroid therapy for COVID-19. Out of 50 cases, 39 cases received oxygen support.

We identified 50 cases of mucormycosis. The age of patients ranged from 21 to 70 years. Majority in the age group of 41–50 years followed by 51–60 years, 61–70 years, 31–40 years, 21–30 years, and >70 years (Table 1) out of 50 cases male were predominant showing 37 (74%) and females being 13 (26%) (Table 1).

Out of the 50 patients, 18 patients were with no diagnosed comorbidities accounting for a total of 36% followed by diabetes mellitus being the most common comorbidity accounting for 60% and diabetes plus hypertension present in an individual being 14 accounting for 28%. Other comorbidities were not observed in our study (Table 2).

Out of 50 cases, 46 cases were treated with steroid therapy and 39 cases were put on oxygen support and both were given for 36 cases (Figure 1).

Steroid therapy and oxygen support showing 46% and 61% respectively in the study by Rao et al.,[8] similar to our study.

Radiological evidence of invasion can be visualized as sinus opacification, bone erosion and obliteration of deep facial planes Image 1. Hematoxylin and Eosin Stain of Mucor Showing Broad Nonseptate Hyphae with 90-degree or acute angle Branching (Arrow) (×40) (Image 2). GMS Stain highlighting the presence of fungal hyphae of mucormycosis (Image 3).

DISCUSSION

In our study, age range from 21 to 70 years, 37 (n=50) cases were males and 14 (n=50) cases were females. A study by Singh et al.,[9] showed that the patient age range was 35–74 years and males being predominant 78.9%. Diabetes mellitus is the most common comorbidity 60%. The mean duration for the development of mucormycosis after the recovery from COVID-19 ranged from a minimum of 4 days to a maximum of 60 days. All 46 (n=50) cases received corticosteroid therapy for COVID-19. Oxygen support was given for 36 (n=50) cases. Steroid therapy and

oxygen support showing 46% and 61%, respectively, in the study by Rao et al.,[8] similar to our study. Eighteen patients were observed to not have any comorbidities this suggests that steroid therapy and oxygen support given plays a role in the development of mucormycosis. According to the WHO guidelines strict recommendation against use of systemic corticosteroid in patients with non-severe COVID-19 was stated.

The majority of cases mucormycosis occurred in India, that is, 71% of world are reported in India and has the highest burden of mucormycosis globally.[11] Globally incidence varies from 0.005 to 1.7 per million population, and case fatality rate is as high as 46%.[12] A study done by Čolović et al., showed that the number of cases of mucormycosis (black fungus) has increased in COVID-19 patients, both in hospitalized and those who have recovered.[13]

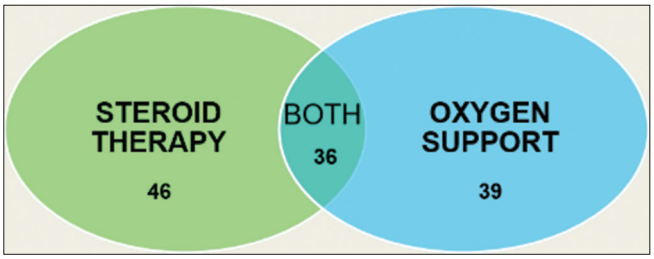

Figure 1: Treatment modalities among mucormycosis positive patients

Table 1: Age- and gender-wise distribution

Age (years)	Gender		Total (%)
	Male	Female	
21–30	7	3	20
31–40	5	2	14
41–50	10	3	26
51–60	4	4	16
61–70	10	1	22
>70	1	0	2
Total	37	14	

There are assorted immunocompromised conditions which influence an individual to mucormycosis, although it is atypical to be found in immunocompetent individuals.[14] There is a need to acknowledge the reciprocity of these influential factors with dysfunctional immune system which might explain how these factor influence to mucormycosis in COVID-19 patients. A dysfunctional intrinsic immune system can be caused by being infected with SARS-COVID-19. Fungal spores germinate into hyphae, which are a result of immune system being incapacitated, the phagocytic potential of macrophages which is present in the airway is also compromised. In a severe case of COVID-19 infection where neutrophilia is present, immature neutrophils are emancipated from the bone marrow which by oneself are impotent of destroying the fungal hyphae, dissimilar to an immunocompetent individual, the hyphae are demolished by mature neutrophils which is a result of action of free radicals. Mucor is a ubiquitous fungi which is present all around us in India as well as in other developing countries. It is most likely that a distinct constellation of factors, few controllable and others uncontrollable ones are there in our settings that may be held responsible for the emerging epidemic of mucormycosis within this COVID 19 pandemic. Uncontrolled diabetes is so common in our

Table 2: Comorbidities in post COVID-19 mucormycosis patients

Age (in years)	21–30	31–40	41–50	51–60	61–70	>70
No comorbidities	9	6	2	1	0	0
Diabetes	1	1	6	3	5	0
Hypertension	0	0	0	1	0	0
Both diabetes and hypertension	0	0	4	3	6	1
Cardiovascular disease	0	0	0	0	0	0
Asthma	0	0	0	0	0	0
Tuberculosis	0	0	1	0	0	0
Hypothyroidism	0	0	0	0	0	0
Chronic kidney disease	0	0	0	0	0	0
Cirrohtic liver	0	0	0	0	0	0
Solid malignant tumor	0	0	0	0	0	0

	Present study	Rao et al.[8]	Singh et al.[9]	Sharma et al.[10]
Age range (years)	21–70	41–60	35–74	41–77
Gender (predominance)	74% Males 26% Females	22 Male	78.9% Males	15 Males 8 Females
Diabetes	60%	96%	80%	91.3%
Hypertension	32%	4%	-	60.86%
Tuberculosis	2%	-	-	-
Steroid therapy	Yes (46/50)	Yes 46%	Yes 76.3%	Yes
Oxygen support	Yes (36/50)	Yes 61%	No	Yes

country and a significant majority of patients do not have regular testing of their blood sugar levels.[5]

Hyperglycemic state advances to growth in adhesion and penetration of Mucorales which is caused by inciting of endothelial receptor glucose regulated protein. The dimensions of invasion and the swiftness of spread is made distinct by the interplay between fungus with the vascular endothelial cells. The thrombosis and tissue necrosis originated by this angioinvasive fungi effectuates to tissue gangrene which consigns black color to the tissue. Unrestricted diabetes with ketoacidosis soars the adherence of the of mucor hyphae to the extracellular matrix which aids in annexation of the endothelial cell lining of blood cells. It is believed by most that the hike of ROCM might have been a result of unrestrained and injudicious usage of corticosteroids in COVID-19 patients.[15] The T cell activity is muffled when glucocorticoids inhibit dendritic cell (DC) matures which gives on to decreased DC activity, this eventually interferes the T cell signaling. Corticosteroids decrease the inflammatory cytokines, chemokines and the inflammatory enzymes (iNOS and COX2). Crippled immune system which is unable to prevent the fungal hyphae is a result of Inducible nitric oxide synthase which plays a consequential role in the formation of free radicals and it's reduced expression.[16] Even in the presence of associated neutrophilia, there is reluctance of neutrophil adhesion to endothelial cells which paves path to decreased trafficking. Glucocorticoids weaken macrophage phagocytic activity and also are a source to declined expression of MHC Class II Molecules. These aforementioned factors steer towards increased vulnerability of the infection.[17] Thus, the usage of corticosteroids during the pandemic has been venial, this suggests that the use likely be rational to intercept the lethal complications of mucormycosis.

Limitations of the study

As we did not do follow up we don't have date regarding recurrence. coinfection with other fungi can be limitation.

CONCLUSION

The present study says that although most patients who do not have comorbidities were susceptible to infections like mucormycosis and this may be due to immune suppression during disease and convalescence. This immune suppression was observed to be due to corticosteroid therapy. This must have also been accentuated by the use of unsterilized oxygen gas and equipment. However, we also noticed that diabetes mellitus can be contributing factor. Clinicopathological correlation with identification of fungus on histopathology aids in effective treatment and management.

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Authors' Contributions:
MT- Concept and design of the study, prepared first draft of manuscript, review of literature, and revision of manuscript; **FB and NCR**- Interpreted the results; reviewed the literature and manuscript preparation; **SVSP and HSD**- Concept, coordination, statistical analysis and interpretation, preparation of manuscript, and revision of the manuscript.

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