

Original Article

Study of Admitted Patients of Mucormycosis : Experience of A Tertiary Care Centre

Namita Goyal¹, Lakhan Poswal², Deepika Gupta³, Neera Samar⁴, Priyanka Parakh⁵, Manisha Raut⁶

A prospective study was done during mid-April to mid-June 2021 to analyze the sudden rise in cases of Mucormycosis. We assessed characteristics, related comorbidities, disease locale, steroid administration and disease outcome in patients. Cases of Mucormycosis associated with RTPCR positive for Coronavirus disease (COVID-19) and Mucormycosis occurring in patients with negative RTPCR for COVID-19 were compared. A total of 103 Mucormycosis patients were analyzed, 84 (81.55%) were RTPCR positive. Uncontrolled Diabetes Mellitus (70.87%) was the most common comorbidity present among RTPCR positive and negative Mucormycosis patients. Rhino-orbital and Rhino-sinusoidal sites were equally involved (44.0%), followed by Rhino-orbital-cerebral (11.9%) region. We ascribe the spike in Mucormycosis episodes to the trio of diabetes, excessive corticosteroid usage in the face of the COVID-19 Pandemic.

[J Indian Med Assoc 2022; 120(7): 25-9]

Key words : Mucorales, Diabetes Mellitus, COVID-19, Coronavirus, Corticosteroids, Mucormycosis.

Recently with the increasing trend associated with the second wave, we witnessed an increase in invasive Rhinosinusitis caused by Mucormycosis. On extensive search of previous five-year records of our institution RNT Medical College and associated group of Hospitals, we did not come across any case of Mucormycosis in first wave and in the near Pre-COVID era. This observation was a matter of concern which prompted us to analyze temporal dimensions of Mucormycosis, correlation with co-morbidities, therapies used for Coronavirus along with overall features and prognosis.

MATERIALS AND METHODS

During the prospective study in a period of two months, from mid- April to mid-June 2021, all patients presenting with symptoms of Mucormycosis with or without Coronavirus history or an ongoing COVID-19 illness were included. The observational study was conducted at Rabindra Nath Tagore Medical College and associated hospitals, Udaipur, Rajasthan, India.

Editor's Comment :

- Second wave of COVID-19 witnessed alarming increase in cases of Mucormycosis.
- Triad of SARS-CoV-2, Diabetes and Steroid are Ominous risk factors for Mucormycosis.
- Judicial use of steroids, good glycemic control and monitoring for immunosuppression following steroid use is imperative.

A Mucormycosis case was diagnosed based on their Clinico-radiological features and evidence of fungi either by conventional microscopy or culture of Mucorales from patient's tissue/ body fluids. Microscopy showed presence of broad, aseptate, ribbon like, irregular hyphae. Patients testing positive for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in respiratory specimens using Reverse Transcription (RT)-PCR were diagnosed to be COVID-19 positive. We divided patients with Mucormycosis in two groups based on RTPCR for COVID-19 as Mucormycosis in COVID-19 patients (Group A); and Mucormycosis in patients negative for RT-PCR (Group B). Antibody titer of patients was not available, so the presence of past asymptomatic COVID-19 could not be ruled out in patients with negative RTPCR.

Over the two-month period, the presenting complaints of patient, other co-morbidities including history/current COVID-19 infection, details of biochemical; microbiological; pathological; radiological investigations were obtained. Also, information regarding Diabetes and its control, use of steroids and patient's follow-up details were studied. Hierarchical model was used to classify various underlying illness.

Department of Pathology, Ravindra Nath Tagore Medical College, Udaipur, Rajasthan 313001

¹MBBS, MD (Pathology), Professor and Head

²MBBS, MD (Paediatrics), PhD (Child Psychology), Principal and Controller, Senior Professor, Department of Paediatrics

³MBBS, MD (Pathology), Assistant Professor

⁴MBBS, MD (Medicine), FIACM, Professor, Department of Medicine

⁵MBBS, MD (Pathology), Assistant Professor and Corresponding Author

⁶MBBS, MD, Resident

Received on : 13/07/2021

Accepted on : 14/02/2022

For example, an individual with COVID-19 who is also a known case of uncontrolled Diabetes then, Diabetes was recognized as underlying primary disease.

Comparison between Mucormycosis cases as two Groups – RT-PCR positive and negative, along with underlying diseases, infection site, investigations and outcomes was the prime objective of study.

Tissues from anatomical regions affected by Mucormycosis were biopsied followed by processing using different methods and positive samples were identified based on their macroscopy and microscopy findings. Evaluation of potassium hydroxide tissue mounts was done by routine microscopy. Culture of samples was performed in two sets using SDA (Sabouraud's Dextrose Agar) one at 25°C and other at 37°C. Histopathological examination was done using stains like H&E (Hematoxylin-eosin), Periodic Acid-Schiff (PAS) / Grocott-Gomori's Methenamine Silver (GMS).

Data analysis was performed using Epi info. Descriptive statistics such as frequencies, arithmetic mean (SD), Median, Interquartile Range [IQR] were used. Categorical variables were compared by Chi-square test / Fischer's exact test. p-value of <0.05 was considered statistically significant.

RESULTS

In the second wave of COVID-19 in 2021 a significant rise was noted; as during 2 months of study period, diagnosis of total 103 cases of Mucormycosis was made and admitted at our Institution. Of the 103 cases, 84 (81.55%) had Mucormycosis with RTPCR (+) for COVID-19 (Group A) and 19 (18.45%) had Mucormycosis with RTPCR – for COVID-19 (Group B). The average of study population's age was 50.22 years (SD 11.906 years); age bracket in Group A was 30-81 years and in Group B was 35-70 years. 75.73% were men and 24.27% were women (Table 1). Patients in Group A had a higher proportion (79.76%) of males than in Group B patients. The presenting complaints of patients comprised of face and periorbital discomfort/pain, headache, sudden compromise in vision and nasal discharge.

On presentation 25 (24.27%) cases tested positive for Coronavirus; the remaining 59 (57.28%) were infected before and had thereafter recovered, while 19 patients had no present or previous episode of Coronavirus illness.

Patients in whom minimum 2 weeks had passed, Post COVID-19 detection were characterized as Recovered/ Post-COVID.

Hyperglycemia (Pre-existing or new-onset DM or concomitant Diabetes and Hypertension) was the prime risk factor seen in 86 (83.5%) cases on presentation in patients suffering from Mucormycosis along with COVID-19. Uncontrolled DM (70.87%) with HbA1c values more than 7 percent was the most frequent underlying co-morbidity in both groups. Cases of newly diagnosed Diabetes Mellitus (DM) were more common in Group A (28/84 [33.33%]) than in Group B (2/19 [10.52%]; p = 0.0482). Singly COVID-19 was present as underlying illness in 10/84 (11.9%) of group A patients, among whom Glucocorticoid therapy was received by 07 (70%) patients for COVID management. Risk factors like Hypertension, CAD and HIV were also observed in some patients (Table 1).

A greater percentage of Mucormycosis patients with RTPCR + COVID-19 had hypoxemia during hospitalization than the patients with RTPCR (-). (Table 1). The Rhino-orbital and Rhino-sinusoidal region were equally involved Mucormycosis site (44.0%), followed by Rhino-orbital-cerebral (11.9%) (Table 1). However, both groups were identical in terms of the affected site.

Direct microscopy diagnosed Mucormycosis in 61/103 (59.2%) patients. Aseptate hyphae were visualized by Histopathology in 24/34 (70.59%) cases (Fig 3).

Among RTPCR (+) COVID -19 patients, 32 (38.1%) were hypoxemic. Glucocorticoids were given in 52 (61.91%) patients. As majority of the patients were Post-COVID, they had received treatment for COVID

Variables	Group A, n=84	Group B, n=19	p value
Mean age± SD	50.47 ±11.91	49.10±12.12	0.862
Sex : Male	67 (79.76)	11 (57.89)	0.45
Female	17(20.24)	08 (42.11)	
Underlying disease	84 (81.55)	15 (78.95)	0.0001
COVID-19 only	10 (11.9)	0	0.113
Glucocorticoids for COVID-19	52/84 (61.91)	0	<0.0001
Oxygen for COVID-19	32/84 (38.10)	0	0.001
Remdesivir for COVID-19	29/84 (34.52)	0	0.002
Diabetes Mellitus	58 (69.05)	11 (57.89)	0.027
Diabetes Mellitus and Hypertension	15 (17.86)	2 (10.53)	0.436
Other [†]	1 (1.19)	2 (10.53)	0.028
None	0	4 (21.05)	0.0001
Site of involvement			0.636
Rhino-orbital	37 (44.05)	08 (42.1)	0.877
Rhino-orbito-cerebral	10 (11.9)	01 (5.3)	0.399
Rhino-sinusoidal	37 (44.05)	10 (52.6)	0.4975

*Values are no (%) except as indicated.
[†]Includes Hypertension, Coronary Artery Disease, HIV.



Fig 1 — Gross Specimen of enucleated eye including eyelids measuring anteroposterior diameter 3 cm, horizontal diameter 4 cm, vertical diameter 3.8 cm, optic nerve measured 2 cm in length

as per the protocol of the respective treating Institute. All patients receiving steroid in any dose and for any duration for COVID management were included in the study. However, on tracing back the data where available, parenteral dexamethasone was the commonest steroid used; minimum being 6 mg/day for 5-7 days. Remdesivir was administered to 29 (34.5%) patients for COVID-19 management. Other immunosuppressive drugs like Tocilizumab were used in very few patients so were not included in the study.



Fig 2 — Photomicrograph of KOH mount showing aseptate hyphae (KOH, 10X)

Majority of the patients (79.6%) had not received even a single shot of vaccination.

DISCUSSION

Mucormycosis is now a notifiable disease. There was not a single case of Mucormycosis in the past five-year records of RNT Medical College and associated group of Hospitals comprising of near pre-COVID era and the following first wave. We observed a surge in Mucormycosis with 103 cases between mid-April and mid-June, 2021, indicating an epidemic along with COVID-19 pandemic. A series of 18 cases in a South Indian City also reported a significant risk in COVID-19 associated Mucormycosis⁹. Hypoxemia owing to Coronavirus and inadvertent Glucocorticoids usage were both separately related with Mucormycosis development in RT-PCR (+) COVID-19 patients.

In our study, 75.73% patients of Mucormycosis were males, which is similar to previous studies¹⁰. There is no gender or age dependency of Mucormycosis incidence. However, the notably increased incidence of Mucormycosis amongst males in our study may reflect the higher prevalence of Coronavirus illness in men in India. In India nearly 66.8% cases of COVID-19 were males until May 25, 2020⁶.

Mucormycosis within a time period of four weeks or less causes Hyphal Sinus invasion¹¹. Atypical clinical manifestations like complicated Rhinosinusitis

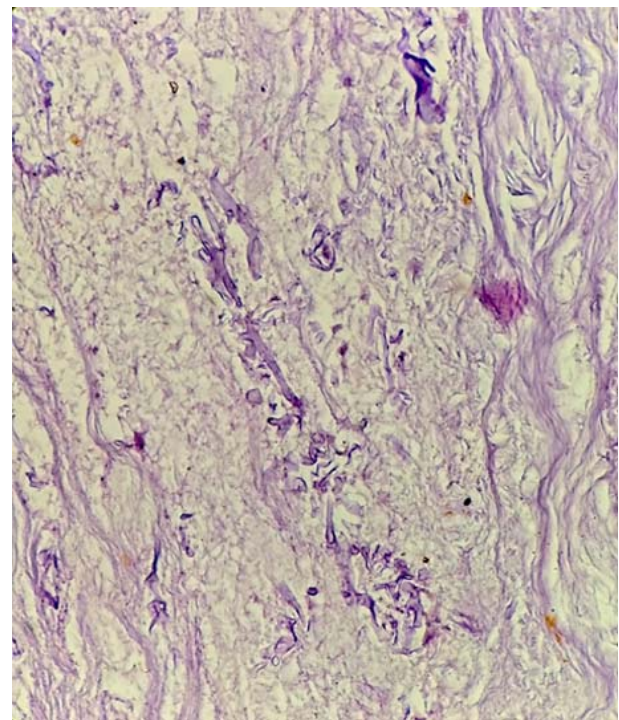


Fig 3 — Photomicrograph of Section examined show broad aseptate irregularly branching fungal hyphae (H and E, 40X)

comprising of Nasal Blockage, Ophthalmoplegia, proptosis, headache, fever, pain in facial region, edema and neurological symptoms may be present in Rhinocerebral Mucormycosis¹². Black eschar is commonly present over hard palate and in nasal cavity; however, it is not typical¹³. Histologically blood vessels show Mycotic infiltration, Thrombosis, Vasculitis; also there is hemorrhage, neutrophil infiltration and infarction of tissue¹⁴. Although CT (Computed Tomography) is used as the initial diagnostic modality to analyze the sinus status; however, MRI (Magnetic Resonance Imaging) is the best technique to determine extra-sinus dissemination¹³. Site involved by Mucormycosis was comparable in both groups, which is in corroboration with other studies¹⁰.

Spores of Mucorales germinate in an environment with increased glucose levels (DM, hyperglycemia either steroid induced or new onset), decreased oxygen (hypoxia), acidic medium (DKA – diabetic ketoacidosis, metabolic acidosis), increased ferritin levels, and reduced phagocytosis by leucocytes because of immunosuppression (corticosteroid mediated / iatrogenic / SARS-CoV-2 mediated / co-morbidities / AIDS / hematological cancers / organ transplantation) combined with other factors like usage of mechanical ventilation and extended hospitalization¹⁵.

Commonest underlying disease in both Groups A and B mucormycosis patients was Diabetes Mellitus. SARS-CoV-2 affects pancreatic beta cells, leading to metabolic derangements, and perhaps resulting in Diabetes mellitus¹⁶. More frequent diagnosis of diabetes mellitus alone or with hypertension in Group A (86.9%) compared with Group B patients (68.4%); it is unclear if this is due to COVID-19 infection, glucocorticoid treatment or a coincident. A multicenter retrospective study across India in September to December, 2020 compared epidemiology among patients of COVID associated Mucormycosis also reported uncontrolled diabetes to be the commonest underlying illness among both CAM and NCAM (non-CAM) cases¹⁰.

In the study of 103 patients, 84 patients of Mucormycosis with RTPCR

(+), among them 10 (11.91%) had COVID as sole underlying illness; of them 3 cases had not received any Glucocorticoids / Immunomodulatory therapy; rest had received steroids for COVID management. Inappropriate usage of Glucocorticoids was observed to be related with Group A patients. These findings are in corroboration with other studies^{8-10,15}. It is unknown if COVID-19 induces immunological dysregulation by itself and thereby predisposing individuals to Mucormycosis¹⁷. CBC analysis showed presence of Neutrophilia in 36 (42.86%) Group A patients (Table 2). Wang *et al* also showed a rising Neutrophil count and a falling Lymphocyte count during the severe phase in several COVID-19 patients¹⁸. Altered innate immune response owing to decrease in T lymphocytes (both CD4+ and CD8+ T lymphocytes) due to immune dysregulation, leads to increase in likelihood of subsequent fungal infections⁸. Both group A and B had comparable sites involved by Mucormycosis.

Table 2 — Baseline investigations among patients with Mucormycosis with RTPCR for COVID-19 positive (history of positive or active infection): Group A and negative: Group B				
Variables		Group A, n=84	Group B, n=19	p value
Diabetes ^{††} :	Uncontrolled	62/73 (84.93)	10/13 (76.92)	0.4712
	Controlled	11/73 (15.07)	3/13 (23.08)	
Microscopy (KOH) : Positive smear		49 (58.3)	12 (63.2)	
	Negative smear	29 (34.5)	05 (26.3)	
	Not available	6 (7.1)	2 (10.5)	
Histopathology diagnostic of Mucormycosis [†]		20/27 (74.07)	04/07 (57.14)	0.381
	WBC Analysis :			0.7758
NLR Ratio:	Neutrophilia	36 (42.86)	09 (47.37)	0.4776
	Lymphocytosis	10 (11.90)	03 (15.79)	
	Normal	38 (45.24)	07(36.84)	
Platelet Analysis:	Raised	68 (80.95)	14 (73.68)	0.7366
	Normal	16 (19.05)	05 (26.32)	
	Thrombocytopenia	12 (14.29)	02 (10.53)	
IL-6:	Thrombocytosis	08 (9.51)	01 (5.26)	0.0482
	Normal	64 (76.2)	16 (84.21)	
	Raised	45 (53.6)	09 (47.4)	
Serum ferritin :	Normal	39 (46.4)	10 (52.6)	0.662
	Raised	31 (36.9)	06 (31.6)	
Vaccination :	Normal	53 (63.1)	13 (68.4)	0.8456
	First dose	13 (15.5)	2 (10.5)	
	Second dose	05 (6.0)	01 (5.3)	
	None	66 (78.6)	16 (84.2)	
Outcome:	Death	03 (3.57)	0 (0)	0.403
	Discharged	03 (3.57)	01 (5.26)	0.730
	Admitted	70 (83.33)	15 (78.95)	0.649
	Shift to ICU	02 (2.38)	01 (5.26)	0.499
	LAMA	06 (7.14)	02 (10.53)	0.618

([†]Histopathological examination was performed in 34 cases, 27 in the mucormycosis with RTPCR (+) for COVID -19 and 07in mucormycosis with RTPCR (-) for COVID-19.IL -6 (>400ng/ml) and Serum Ferritin (>4.4pg/ml). NLR Ratio (>3). ^{††}Diabetes includes patients with only diabetes as well as patients with diabetes and hypertension.

Timely management of Mucormycosis by antifungals like Amphotericin B Liposomal and Isavuconazole along with surgical excision, when possible are imperative¹⁹. Mortality rates did not differ between both groups. However, COVID associated increased mortality risk could not be excluded for RT-PCR(+) COVID-19 patients. The exact effect of vaccination and its bearing on COVID-19 and Mucormycosis needs further analysis and research as limited data is available, and vaccination is still under process in our country.

Our study showed Diabetes to be an important risk factor for developing Mucormycosis, like in other studies from China, Bangladesh, Mexico etc²⁰. Individual exact dosage of steroid and effect of Mucormycosis treatment medical/surgical having an impact on outcome could not be analyzed. Increase in Mucormycosis prevalence in India may be due to undetermined variables, such as genetic susceptibility.

CONCLUSION

The triad of SARS CoV-2, Diabetes and steroid are ominous risk factors for occurrence of Mucormycosis. Usage of Glucocorticoids in the wake of COVID-19 outbreak in India along with existence of co-morbidities, such as Diabetes etc. supports the hypothesis of necrosis of tissue and fungal invasion caused by heightened immune dysregulation. Presence of Diabetes and its poor control are risk factors for Mucormycosis, therefore early diagnosis and good glycemic control are imperative.

Limitations :

- Small sample size
- Short period of study

REFERENCES

- 1 Wang Y, Wang Y, Chen Y, Qin Q — Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. *J Med Virol* 2020; **92**: 568-76.
- 2 Yang W, Cao Q, Qin L, Wang X, Cheng Z, Pan A, *et al* — Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): a multi-center study in Wenzhou city, Zhejiang, China. *J Infect* 2020; **80**: 388-93
- 3 Song G, Liang G, Liu W — Fungal Co-infections Associated with Global COVID-19 Pandemic: A Clinical and Diagnostic Perspective from China. *Mycopathologia* 2020; **185(4)**: 599-606.
- 4 Skiada A, Pavleas I, Drogari-Apiranthitou M — Epidemiology and diagnosis of mucormycosis: An Update. *J Fungi* 2020; **6(4)**: 265.
- 5 Gillespie MB, O'Malley BW — An algorithmic approach to the diagnosis and management of invasive fungal rhinosinusitis in the immunocompromised patient. *Otolaryngol Clin North Am* 2000; **33**: 323-34.
- 6 Ballester DG, González-García R, García CM, Ruiz-Laza L, Gil FM — Mucormycosis of the head and neck: report of five cases with different presentations. *J Craniomaxillofac Surg* 2012; **40**: 584-91.
- 7 Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al* — Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507.
- 8 Hoang K, Abdo T, Reinersman JM, Lu R, Higuera NIA — A case of invasive pulmonary mucormycosis resulting from short courses of corticosteroids—an unholy trinity in invasive fungal infections of the maxillofacial region? A retrospective, multi-centric analysis. *J Maxillofac Oral Surg* 2021; 1-8; [Epub ahead of print].
- 9 Moorthy A, Gaikwad R, Krishna S, Hegde R, Tripathi KK, Kale PG, *et al* — SARS-CoV-2, uncontrolled diabetes and corticosteroids—an unholy trinity in invasive fungal infections of the maxillofacial region? A retrospective, multi-centric analysis. *J Maxillofac Oral Surg* 2021; 1-8; [Epub ahead of print].
- 10 Patel A, Agarwal R, Rudramurthy SM, Shevkani M, Xess I, Sharma R, *et al* — MucoCovi Network. Multicenter epidemiologic study of coronavirus disease-associated mucormycosis, India. *Emerg Infect Dis* 2021 Sep (assessed June 04, 2021). <https://doi.org/10.3201/eid2709.210934>
- 11 Ferguson BJ — Definitions of fungal rhinosinusitis. *Otolaryngol Clin North Am* 2000; **33**: 227-35.
- 12 Scheckenbach K, Cornely O, Hoffmann TK, Engers R, Bier H, Chaker A, *et al* — Emerging therapeutic options in fulminant invasive rhinocerebralmucormycosis. *Auris Nasus Larynx* 2010; **37**: 322-8.
- 13 Mohindra S, Mohindra S, Gupta R, Bakshi J, Gupta SK — Rhinocerebralmucormycosis: the disease spectrum in 27 patients. *Mycoses* 2007; **50**: 290-6.
- 14 DeShazo RD, Chapin K, Swain RE — Fungal sinusitis. *N Engl J Med* 1997; **337**: 254-9.
- 15 Singh AK, Singh R, Joshi SR, Misra A — Mucormycosis in COVID-19: A systematic review of cases reported worldwide and in India, Diabetes & Metabolic Syndrome: Clinical Research & Reviews (2021), doi: <https://doi.org/10.1016/j.dsx.2021.05.019>.
- 16 Müller JA, Groß R, Conzelmann C, Krüger J, Merle U, Steinhart J, *et al* — SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. *Nat Metab* 2021; **3**: 149-65. DOIExternalLinkPubMedExternal Link
- 17 Files JK, Boppana S, Perez MD, Sarkar S, Lowman KE, Qin K, *et al* — Sustained cellular immune dysregulation in individuals recovering from SARS-CoV-2 infection. *J Clin Invest* 2021; **131**: e140491. DOIExternalLinkPubMedExternal Link
- 18 Wang D, Hu B, Hu C — Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020; **323(11)**: 1061-9.
- 19 Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, *et al* — Mucormycosis ECMM MSG Global Guideline Writing Group. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *Lancet Infect Dis* 2019; **19**: e405-21.
- 20 Prakash H, Chakrabarti A — Global Epidemiology of mucormycosis. *J Fungi (Basel)* 2019; **5**: 26. DOI : ExternalLinkPubMedExternal Link