

Prevalence of Fungal Infection Mucormycosis in COVID-19 Induced Viral Pneumonia Disease - A Prospective Study at A tertiary Care Centre

Dr Neeru Dave¹, Dr V. C Popat², Dr Dhaval Mehta³, Dr Krishna Mansuriya⁴, Harsh Anadkat⁵

¹Associate Professor, Department of Pathology, Shri M. P. Shah Medical College, Jamnagar – 361008, India

²Professor & Head, Department of Pathology, Shri M. P. Shah Medical College, Jamnagar – 361008, India

³Assistant Professor, Department of Pathology, Shri M. P. Shah Medical College, Jamnagar – 361008, India

⁴Senior Resident, Department of Pathology, Shri M. P. Shah Medical College, Jamnagar – 361008, India

⁵Third Year Medical Student, Department of Pathology, Shri M. P. Shah Medical College, Jamnagar – 361008, India

Corresponding author: Dr Krishna Mansuriya

Email id: [krishnaghetia07\[at\]gmail.com](mailto:krishnaghetia07[at]gmail.com)

Contact no: 9426911045

Abstract: *Coronavirus Disease 2019 (COVID-19), during the second wave in early 2021, had caused devastating chaos in India. As daily infection rates rose alarmingly, the number of severe cases had increased dramatically. The country had encountered health infrastructure inadequacy and excessive demand for hospital beds, drugs, vaccines, and oxygen. Adding more burden to such a challenging situation, mucormycosis, an invasive fungal infection, had seen a sudden surge in patients with COVID-19. The rhino - orbital - cerebral form was the most common type observed. In particular, approximately three - fourths of them had diabetes as predisposing comorbidity and received corticosteroids to treat COVID-19. Possible mechanisms involved immune and inflammatory processes. Diabetes, when coupled with COVID-19-induced systemic immune change, tend to cause decreased immunity and an increased risk of secondary infections. Since comprehensive data on this fatal opportunistic infection were evolving against the backdrop of a major pandemic, prevention strategies primarily involved managing comorbid conditions in high - risk groups. The recommended treatment strategies primarily included surgical debridement and antifungal therapy using Amphotericin B and selected azoles. Several India - centric clinical guidelines had emerged to rightly diagnose the infection, characterize the clinical presentations, understand the pathogenesis involved, and track the disease course. A staging system has recently been proposed, and a dedicated registry has been started. In this original article, we extensively analysed the recent evidence on COVID-19 associated Mucormycosis diagnosis & hence early treatment to decrease the morbidity & Mortality.*

Keywords: Fungal infections, Mucormycosis, Viral pneumonia

1. Introduction

The common COVID-19 manifestations, as observed in a study of over 136, 000 cases during the initial months of infection spread in India, included high fever, dry cough, fatigue, dyspnoea and muscle pain while few were asymptomatic. [1]

A very recent (July 15, 2021) study reported an international cohort (n = 3, 762) spanning 56 countries with confirmed or suspected “Long COVID,” a condition with a majority recovered only after 35 weeks since onset. The authors assessed the prevalence of over 200 symptoms affecting 10 bodily systems and revealed that the mean number of symptoms per patient was 55.9 (± 25.5). Researchers warned that the impact of COVID-19 has been greatly neglected as patients continue to develop multi - systemic manifestations for more than 7 months. COVID-19 deteriorated immune status of patients, paved the way for opportunistic and secondary infections, and worsened pre - existing clinical conditions. [2]

A fungal infection that could be life threatening was emerging as a new problem associated with COVID-19

patients who had diabetes and other co - morbidities like dental problems, cancer & immune - compromised status. COVID 19 patients got heavy doses of steroids and antibiotics during hospitalisation causing dip in immunity levels. Those with diabetes had sugar levels several times higher than normal, which severely compromised their immunity. So, such patients got fungal infection both during and after recovery. Mucorales infections were emerging as a matter of concern in COVID-19 with poorly controlled diabetes mellitus and steroids as their risk factors. [3]

Mucormycosis is rare and lethal fungal infection caused by the family of mucoracea. Mucormycosis is a formidable angio - invasive opportunistic infection in an immuno - compromised host. This fungus usually resides as a commensal in the nasal mucosa and in immunosuppression like diabetes, cancers; organ transplants etc. can germinate in nasal cavity and paranasal sinuses to invade the palate, orbits and brain. [4]

Mucormycosis mainly affects people who are immune - compromised, or patients already infected with other diseases. High risk groups include people with diabetes (especially diabetic ketoacidosis), solid organ

Volume 11 Issue 7, July 2022

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

transplantation, neutropenia (low neutrophils, a type of white blood cells), long - term systemic corticosteroid use, and iron overload (hemochromatosis). The risk is high for people living with HIV, and those using immune - modulating drugs, including the anti - fungal voriconazole in some high - risk groups. [5]

It is not contagious and does not spread by contact of person to person. This fungus is found in the environment. Transmission occurs through inhalation, inoculation, or ingestion of spores from the environment. [6]

Corticosteroids have proven to be lifesaving in severe to critical COVID-19 patients, however its overuse comes with severe side effects such as rhino - orbito - cerebral mucormycosis epidemic especially in patients with pre - existing diabetes mellitus. Corticosteroid use should be therefore monitored and all COVID-19 patients should be closely evaluated/monitored for sequelae of immunosuppression following treatment. [7]

Therefore we undertook this study to find out the correlation of Mucormycosis with other co - morbid conditions in COVID 19 positive patients as well its relation to the steroid doses administered to combat the high inflammatory conditions in such patients.

Aims and Objectives

- To correlate presence of Diabetes & various comorbidities and fungal infections in COVID-19 induced viral pneumonia.
- To study of the effect of steroid therapy & oxygen therapy on the occurrence of fungal infections in COVID-19 positive cases.
- To confirm the Histo - pathological diagnosed cases with gold standard microbiological culture.

2. Material & Methods

Type of study: Prospective and retrospective

Duration of study: 1 year.

Inclusion criteria:

- COVID-19 induced viral pneumonia cases treated with steroids were included.
- Patients with type II diabetes mellitus with COVID induced viral pneumonia.
- All COVID 19 positive cases with various co - morbidities & immunosuppressive states were included.
- All radiologically suggested fungal infection cases in COVID induced pneumonia with active disease or in recovery phase.

Exclusion criteria:

- Incomplete patient treatment information and incomplete comorbidity history cases were excluded.

Materials required:

Documentation of specimen at the histopathology section were reviewed and paraffin embedded tissue blocks retrieved from the repository of the department of Pathology

for retrospective cases, while for prospective cases, specimens received at histopathology section of pathology department were processed. The surgery, Dental, Otolaryngology and ophthalmology department were informed regarding the study in order to obtain proper reception of specimen.

3. Method

The scrapings/biopsy specimen submitted for histo - pathological examinations was used as samples. Tissue blocks of (2cm x 2cm x 0.5cm) were collected and fixed in 10% neutral buffered formalin solution for 24 hours and subsequently subjected to histological processing and paraffin embedding. Histological paraffin sections, 3 - 5um thick, were taken. H & E staining was performed and slides were examined by light microscopy. The diagnosis was given according to the morphology of the fungi.

Special stain P. A. S was done whenever possible. Sections were De - paraffinized and hydrated. Slides were placed into 1% periodic acid for 10 mins, Rinsed in tap water, Schiff's reagent for 15 - 30 mins, Washed in running tap water for 5 mins, Counterstained in haematoxylin for 5 mins, differentiated in warm distilled water, Dehydrated in alcohol, cleared and cover slipped.

All the cases were compared with the Gold Standard Microbiological fungal culture examination. (In Sabouraud - dextrose Agar cottony floppy colony were seen).

4. Results

The study was conducted in the department of pathology (Histopathology section) in the government medical college for a period of about 2 years. During this time period total 90 cases were enrolled with various parameters. Ninety patients of COVID 19 cases by either RT PCR or RAPID antigen test presenting with Mucormycosis (fungal infection) were studied for Diabetes, co - morbidities & other immunosuppressive states in our medical college attached tertiary hospital. They were correlated with the steroid therapy doses. Here we are showing the results of our study in a tabulated form.

Table 1: Age & Gender distribution of cases (n = 90)

Age Group	Male	Female	Total
<20	0 (0 %)	0 (0 %)	0 (0 %)
21 - 30	2 (4 %)	0 (0 %)	2 (2 %)
31 - 40	8 (14 %)	4 (11 %)	12 (13 %)
41 - 50	5 (9 %)	12 (34 %)	17 (19 %)
51 - 60	19 (35 %)	12 (34 %)	31 (34 %)
61 - 70	14 (25 %)	2 (6 %)	16 (18 %)
71 - 80	5 (9 %)	3 (9 %)	8 (9 %)
81 - 90	2 (4 %)	2 (6 %)	4 (5 %)
TOTAL	55 (61 %)	35 (39 %)	90 (100 %)

In present study, most common age group of presentation for mucormycosis in COVID-19 positive cases was 5th decade followed by 4th decade and then 6th decade. Lowest presentation was seen in 2nd decade.

Incidence of COVID-19 and mucormycosis was more in male than female. In male, most common age of presentation was 5th decade followed by 6th decade where as in female, most common age of presentation was 4th and 5th decades.

Table 2: Organ wise & Operative procedure wise distribution of cases (n=90)

S. no	Organ	Operative details / Cases	Percentage
1	Nasal	Debridement - 52 Scrappings - - 02	60
2	Maxillary	Sinus Scrapings - - 05	30
		Maxillectomy (Total) - 07	
		Macillectomy (Partial) - 15	
3	Orbital	Evisceration - 02	2.22
4	Nasal + orbital	Debridement - 03 Maxillectomy - - 04	7.78
	Total	90	

Table no 2 shows that maximum cases were of nasal mucormycosis followed by maxillary sinus one.

While most common procedure was nasal debridement (52 Cases) followed by partial maxillectomy (15 cases).

Table 3: Diabetes, Co - morbidities & immune - compromised states in cases (n = 90)

Age	Diabetes Present	Immuno - compromised state present	Comorbidities present
<20	0 (0 %)	0 (0 %)	0 (0 %)
21 - 30	0 (0 %)	0 (0 %)	0 (0 %)
31 - 40	3 (6 %)	0 (0 %)	1 (4 %)
41 - 50	9 (19 %)	2 (50 %)	6 (22 %)
51 - 60	17 (36 %)	0 (0 %)	9 (33 %)
61 - 70	11 (23 %)	2 (50 %)	7 (26 %)
71 - 80	4 (8 %)	0 (0 %)	4 (15 %)
81 - 90	4 (8 %)	0 (0 %)	0 (0 %)
Total	48 (53 %)	4 (4 %)	27 (30 %)

In present study, total 48 patient presented with diabetes, 4 cases presented with immune - compromised condition where as 27 patient present with comorbidities.

In present study, most commonly used oxygen therapy was NRBM therapy (40 cases) followed by BiPAP therapy (29 cases) and nasal prong therapy (19 cases).

Table 4: Steroid dose distribution in cases (n = 90)

< 08 mg	38 (42 %)
09 - 16 mg	29 (32 %)
17 - 24 mg	17 (19 %)
25 - 32 mg	6 (7 %)
Total	90 (100%)

Most commonly low steroid dose (<08 mg) was used in 38 cases where as high steroid dose (25 - 32mg) was used in only 6 cases.

GOLD STANDARD fungal culture was positive in all 90 cases (100%) & all those cases were treated by antifungal therapy.

5. Discussion

COVID 19 induced Mucormycosis had created havoc in the near past. We have focussed on their causes & correlated with various factors. Our study was also compared with the other authors & comparable results were observed.

Mucormycosis is rare in healthy individuals but several immune - compromised conditions predispose it. These includes uncontrolled DM with or without DKA, haematological and other malignancies, organ transplantation, prolonged neutropenia, immunosuppressive therapy, severe burns, acquired immunodeficiency syndrome (AIDS), intravenous drug abusers, malnutrition and open wound following trauma. Mucormycosis can involve nose, sinuses, orbit, central nervous system (CNS), lung (pulmonary), gastrointestinal tract (GIT), skin, jaw bones, joints, heart, kidney and mediastinum (invasive type).⁽⁵⁾ WHO have categorised mucormycosis into 5 types according to site viz. Rhino - cerebral (sinus & brain), pulmonary, cutaneous, Gastrointestinal & Disseminated [6].

Hyperglycaemia occurs in people with undiagnosed or uncontrolled diabetes, but it can also be induced by corticosteroids. Patients with diabetes and hyperglycaemia often have an inflammatory state that elicits constant recruitment and local activation of immune cells, including macrophages and neutrophils, which secrete pro - inflammatory cytokines and generate persistent inflammation. In these patients, the activation of antiviral immunity to SARS - CoV2 could potentiate this inflammatory phenotype, which could favour secondary infections. Further uncontrolled use of steroids & antivirals early in disease can cause a dip in the immunity further which could potentiate the opportunistic fungal infections such as mucormycosis.

Awadesh kr Singh et all⁽⁵⁾ studied 101 cases of mucormycosis in people with COVID-19, of which 82 cases were from India and 19 from the rest of the world. Mucormycosis was predominantly seen in males (78.9%), both in people who were active (59.4%) or recovered (40.6%) from COVID-19. Pre - existing diabetes mellitus (DM) was present in 80% of cases, while concomitant diabetic ketoacidosis (DKA) was present in 14.9%. Corticosteroid intake for the treatment of COVID-19 was recorded in 76.3% of cases. Mucormycosis involving nose and sinuses (88.9%) was most common followed by rhino - orbital (56.7%). Mortality was noted in 30.7% of the cases.

Prof Martin Hoenigl et all⁽⁸⁾ studied 80 patients. Following is the Summary of clinical characteristics, treatment, and outcome of 80 cases of patients with COVID-19 - associated mucormycosis. Median age was 55 years & included 59 patients with rhino - orbital cerebral disease, 20 patients with pulmonary disease (three of whom had disseminated pulmonary disease) and one patient with gastrointestinal disease. 28 patients were given dexamethasone 6 mg for 10 days, and 12 were given methylprednisolone (40-120 mg for 5-28 days after hospital admission).

Most patients (76 [95%] of 80) had additional risk factors. Diabetes was the most prevalent condition overall (66 [83%] of 80), with type 2 diabetes being more common than type 1 diabetes (59 [89%] of 66 patients with diabetes had type 2 and 6 [9%] had type 1). Most of the 66 patients with diabetes had uncontrolled or poorly controlled diabetes (reported in 55 [83%] of 66), as characterised by diabetic ketoacidosis (27 [49%] of 55), hyperglycaemia (23 [42%] of 55). Hypertension was the second most common underlying condition, identified in 15 (19%) of 80 patients, followed by chronic kidney diseases (five [6%]), and haematological malignancies (five [6%]). No apparent risk factor, aside from COVID-19, was noted in only four (5%) of 80 patients.

Theresa M. Dulski et al ⁽⁹⁾ studied 10 mucor patients in three locations. Among all 10 patients, the median age was

57 years, all patients, seven were male, one had a history of solid organ transplantation, and one had a history of recent traumatic injury at the body site where mucormycosis later developed. Eight patients had diabetes; among these, the median hemoglobin A1c was 8.6%. During hospitalization, three patients with diabetes experienced diabetic ketoacidosis. Mucormycosis clinical signs and symptoms included those that were rhino - orbital (four patients, including three with cerebral involvement), pulmonary (three), disseminated (two), and gastrointestinal (one). COVID-19 treatment included supplemental oxygen therapy (eight patients), invasive mechanical ventilation (five), corticosteroids (nine), tocilizumab (two), and baricitinib (two). Five patients received surgical treatment to excise mucormycosis - affected tissue. Six of the 10 patients died during hospitalization or within 1 week of discharge.

Table 5: Showing comparison of various authors

Author	No of cases	Median age	Most common site	Association with Diabetes	Other Co - morbidities	Steroid administration & doses
Awadesh Singh et all 2021 ⁽⁵⁾	101	- - -	Nose & sinuses (88.9%)	80%	Cancer (3%)	In 76 patients
Prof Martin Hoenigl et all 2022 ⁽⁸⁾	80	55 yrs	Rhino - orbital cerebral (59cases)	83%	Hypertension (19%) CKD (6%) Hemat malignancy (6%)	28 pts (6 mg) 12 pts (40 - 120 mg)
Theresa M. Dulski et all 2021 ⁽⁹⁾	10	57 yrs	Rhino - orbital (4 cases)	80 %	20% solid organ transplantation, 20% traumatic injury	In 9 patients
Present study 2021 - 22	90	55 yrs	Rhino - Nasal	53%	30% co morbid conditions, 4% Immuno - compromised patients	<8 mg (42%) 25 to 32 mg (7%)

6. Conclusion

There was a significant increase in the incidence of mucormycosis fungal infections in COVID-19 induced viral pneumonia associated with diabetes & other co - morbidities treated with corticosteroids.

Diabetes mellitus (DM) is an independent risk factor for both severe COVID-19 and mucormycosis. Other co - morbidities such as hypertension & CVA as well as immune - compromised states such as tuberculosis & HIV also plays a role in inducing a favourable environment for mucormycosis in COVID 19 patients. High Steroid doses as well as inadvertent use of corticosteroids goes a long way in flaring up the life threatening fungal lesions. Repeated oxygen therapy in patients with incompletely cleaned instruments also added fuel to the already favourable condition for the growth of the fungus/mould specially mucormycosis.

Despite a difficult - to - treat fungal/mould infection and its high mortality in patients with COVID-19, a careful management plan can be successfully implemented for rhino - orbital mucormycosis disease if there is early diagnosis of infection through histo - pathology & culture findings and proper & timely control of COVID-19.

Prevention of COVID 19 associated mucormycosis needs to focus on addressing the underlying risk factors by better glycaemic control in patients of diabetes, proper & timely treatment of other co - morbidities, appropriate use of

systemic corticosteroids and prevention of unnecessary use of antibiotic, antifungal and other immune - modulators. Proper & hygienic use of oxygen therapy is also recommended to prevent the deadly infection in COVID 19 patients.

7. Limitations

The complex relationship between COVID-19 and mucormycosis still cannot be concluded with certainty due to smaller sample size. Still more cohort studies may be required to confirm our findings.

Conflict of interest: None identified so far.

Source of funding: None

Ethical approval: From Institutional ethics committee

Acknowledgement: To all the patients, Dean Madam & superintendent Sir of our college & hospital respectively.

References

- [1] Chanda A. COVID-19 in India: transmission dynamics, epidemiological characteristics, testing, recovery and effect of weather. *Epidemiol Infect.* 2020; 148:e182
- [2] Hannah E. Davis 1Gina S. Assaf 1Lisa McCorkell 1Hannah Wei , Ryan J. Low, 1Yochai Re'em et al. Characterizing long COVID in an international cohort:

- 7 months of symptoms and their impact. LANCET: VOLUME 38, 101019, AUGUST 01, 2021
- [3] Ashley Hagen, COVID-19-Associated Mucormycosis: American Society for Microbiology. July 15, 2021. July 15, 2021
- [4] Rodriguez-Morales AJ, Sah R, Millan-Oñate J, Gonzalez A, Montenegro-Idrogo JJ, Scherger S, et al. COVID-19 associated mucormycosis: the urgent need to reconsider the indiscriminate use of immunosuppressive drugs. *Ther Adv Infect Dis.* 2021; 8:20499361211027065. <https://doi.org/10.1177/20499361211027065> PMID: 3421171
- [5] Awadhesh Kumar Singh , Ritu Singh, Shashank R Joshi , Anoop Misra . Mucormycosis in COVID-19: A systematic review of cases reported worldwide and in India. *Diabetes Metabolic Syndrome.* Jul-Aug 2021;15(4):102146. E pubmed 2021 May 21
- [6] [https://www.WHO.int/india/emergencies/coronavirus-disease-\(covid19\)/mucormycosis](https://www.WHO.int/india/emergencies/coronavirus-disease-(covid19)/mucormycosis).
- [7] Anusuya Bhattacharyya et al. Rhino-orbital-cerebral-mucormycosis in COVID-19: A systematic review. *Indian J Pharmacol*, Jul-Aug 2021
- [8] Prof Martin Hoenigl, MD , Danila Seidel, PhD , Prof Agostinho Carvalho, PhD, Prof Shivaprakash M Rudramurthy, MD, Amir Arastehfar, PhD, Prof Jean-Pierre Gangneux, MD et al. The emergence of COVID-19 associated mucormycosis: a review of cases from 18 countries. *The LANCET Microbe.* Open Access Published: January 25, 2022.
- [9] Theresa M. Dulski, Megan DeLong; Kelley Garner, Naveen Patil, Michael J. Cima, Laura Rothfeldt, Trent Gulley, Austin Porter, Keyur S. Vyas, Hazel K. Liverett, Mitsuru Toda, Jeremy A.W. Gold, Atul Kothari. “*Notes from the Field: COVID-19–Associated Mucormycosis - Arkansas*”, *July–September 2021 C.D.C Weekly / December 17, 2021 / 70(50);1750–1751.*