

Mucormycosis in Post Covid Patients Attending a Tertiary Care Hospital in West Bengal - A Cross-Sectional Study

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Abstract

Background: The COVID-19 pandemic has been accompanied by a surge of secondary fungal infections, particularly mucormycosis, in India. Post-COVID mucormycosis has emerged as a severe opportunistic infection, largely affecting patients with uncontrolled diabetes mellitus and those treated with corticosteroids. We undertook the study to determine the occurrence of COVID associated mucormycosis, establish the association of risk factor(s), identify and characterize the agents of mucormycosis.

Methods: This prospective observational and cross-sectional descriptive study was conducted at a tertiary care hospital in West Bengal, India. A total of 107 COVID-19 patients with clinical suspicion of mucormycosis were included. Microbiological diagnosis relied on KOH wet mount examination, fungal culture, histopathological staining, and molecular methods like RT-PCR for confirmation.

Results: The study revealed a high mortality rate of 21.5% among post-COVID mucormycosis patients. Uncontrolled diabetes with diabetic ketoacidosis, chronic kidney disease, chronic liver disease, and shorter interval between COVID recovery and mucormycosis onset were significant predictors of mortality. *Rhizomucor pusillus* was the predominant fungal isolate. Combined surgical and medical therapy, including aggressive debridement and antifungal therapy, was the mainstay of treatment.

Conclusion: Post-COVID mucormycosis was a major public health concern in India, with high mortality rates despite early intervention. Strict glycemic control, early diagnosis, and comprehensive management of comorbidities are essential to improve survival outcomes. Our study highlights the need for rational corticosteroid use, diabetes control, and multidisciplinary management to prevent future outbreaks. Future research should focus on understanding host-pathogen interactions, resistance patterns, and developing rapid diagnostic assays to enable timely intervention in high-risk patients.

Keywords: Post-COVID mucormycosis, Diabetes mellitus, Corticosteroids, *Rhizomucor pusillus*.

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Introduction

The COVID-19 pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), had multifaceted impacts on human health, extending beyond acute respiratory illness to a range of secondary infections.[1] Among these, COVID-19-associated mucormycosis (CAM) emerged as a significant and devastating opportunistic fungal infection, particularly during the second wave of COVID-19 in India in 2021.[2,3] Mucormycosis, caused by fungi of the

order Mucorales, is characterized by aggressive tissue invasion, vascular thrombosis, and necrosis, leading to high morbidity and mortality despite antifungal and surgical interventions.[4,5] The incidence rate of mucormycosis globally varies from 0.005 to 1.7 per million populations.[6] Whereas, in Indian population its prevalence is 0.14 per 1000, which is about 80 times higher than developed countries.[7] The fatality rate of mucormycosis is 46% globally, however, factors

like intracranial or orbital involvement, irreversible immune suppression increases fatality to as high as 50% to 80%. Classically, uncontrolled diabetes mellitus (DM) and other immunosuppressive conditions such as neutropenia and corticosteroid therapy, patients on iron chelating agent, etc are known risk factors for mucormycosis. Chakrabarti et al. have estimated a prevalence of 0.14 per 1000 cases of mucormycosis in India, which is about 80 times the prevalence of mucormycosis in developed countries. If we consider that currently the number of diabetics in India is nearly 62 million and they are likely to cross the 100 million mark by 2030, the burden of mucormycosis in India in future can be well imagined.[8] The clinical presentations of mucormycosis are classified on the basis of anatomic localization, such as rhino-orbital-cerebral (ROCM), pulmonary, gastrointestinal, cutaneous, renal, and disseminated mucormycosis.[3] The most common manifestation of the disease is rhino-orbital-cerebral (ROCM) mucormycosis, mostly seen in diabetics.[9]

India witnessed an unprecedented surge in CAM cases, leading to its designation as the global epicenter of this infection.[2] The MucoCovi Network multicentric study reported that over two-thirds of mucormycosis cases observed during the pandemic were temporally associated with COVID-19, marking an exponential rise in incidence.[2] The epidemiologic shift was further supported by the COSMIC collaborative registry, which documented more than 2,800 CAM cases across India within a few months.[3] Multiple factors contributed to this outbreak. The widespread and often inappropriate use of corticosteroids during COVID-19 management, combined with uncontrolled diabetes mellitus, created an ideal immunocompromised state for Mucorales infection.[1,10,11] Additional factors such as prolonged oxygen therapy, zinc supplementation, and hospital environmental exposure may have also played contributory roles.[12,13] Clinically, rhino-orbital-cerebral mucormycosis (ROCM) was the most prevalent manifestation, typically developing 2–3 weeks after recovery from COVID-19.[3,14] The infection often progressed rapidly, with symptoms such as facial pain, nasal obstruction, and orbital swelling, frequently leading to vision loss and cerebral involvement if untreated.[5,14]

The experience in India highlights the critical importance of judicious corticosteroid use, strict glycaemic control, and public health surveillance to prevent future fungal epidemics in the context of viral pandemics. We undertook the study to determine the occurrence of CAM, establish the association of risk factor(s), identify and characterize the agents of mucormycosis.

Methodology: Study Design and Sample

Collection: This was a prospective observational & cross-sectional descriptive study conducted at a tertiary care hospital in West Bengal. A total of 107 COVID-19 patients with clinical suspicion of mucormycosis (CAM) were included. The study was conducted over a period of 6 months from April 2021 to September 2021. Patients who had a proven diagnosis of COVID -19 either prior to or at the time of development of Mucormycosis were included in the study. Patients having fungal infections other than mucormycosis were excluded. Patients without a confirmed diagnosis of COVID-19 (or serological evidence of prior COVID-19) were also excluded.

Appropriate clinical specimens were collected based on the site of infection: For clinically diagnosed Rhino-orbital mucormycosis, nasal swabs, nasal crusts, sinus tissue, and orbital tissues were collected.[3] For Pulmonary mucormycosis, broncho-alveolar lavage (BAL),[4] and for Disseminated or cutaneous mucormycosis, Blood or tissue biopsies from affected sites were processed.[5] Specimens were collected under strict aseptic conditions and transported immediately to the microbiology laboratory using sterile containers, maintaining cold chain wherever applicable.[10]

Microbiological Evaluation: Direct Microscopy: Specimens were examined using 10% potassium hydroxide (KOH) wet mount to visualize broad, coenocytic, aseptate, ribbon-like hyphae characteristic of Mucorales. [11] Lactophenol cotton blue staining was performed for better morphological assessment of tissue scrapings.[12]

Fungal Culture: Samples were inoculated on Sabouraud Dextrose Agar (SDA) with Chloramphenicol (SDCA) but without Cyclohexamide and incubated at 25–30°C for 5–7 days.[11,12] Growth was assessed for colony morphology (cottony, fluffy) and microscopic identification using lacto phenol cotton blue mounts.[12] Identification to genus and species level was performed based on morphological features, including sporangiophore and sporangiospore characteristics. [13]

Histopathological Examination: Tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 3–5 µm.[14] Sections were stained with Hematoxylin and Eosin (H&E) for general morphology and inflammation,[14] and Periodic Acid-Schiff (PAS) and Gomori Methenamine Silver (GMS) for specific fungal detection.[15] Fig 1 Histopathology confirmed angioinvasion and tissue infiltration by broad, aseptate hyphae.[14,15]

Molecular Diagnostics (RT-PCR): DNA was extracted from tissues or other clinical specimens using commercial fungal DNA extraction kits.[16] We used Spin Star Extraction Kits (manufactured by ADT BIOTECH) for extracting fungal DNA from various specimens obtained from clinically diagnosed Mucormycosis cases.[3,4,5] Real-Time Polymerase Chain Reaction (RT-PCR) targeted conserved regions of Mucorales rDNA (18S rRNA, ITS regions).[16,17] Positive and negative controls were included.

We used MucorGenius® kit by PathoNostics which detects Mucorales DNA by real-time PCR targeting specific fragments of the 28S ribosomal RNA (rRNA) gene. It also has an internal control targeting an M13 bacteriophage sequences. A sensitivity of >95% and specificity of >98% in BAL fluid, blood also comparable data in tissue samples. RTPCR results were interpreted based on the amplification organism-specific genetic targets and corresponding Cycle threshold (Ct) values. A positive RT-PCR result was defined by the detectable amplification of target nucleic acid within the established Ct cutoff (<40), indicating the presence of fungal genetic material in the sample. Lower Ct values were considered reflective of a higher fungal load, whereas higher Ct values suggested a low burden of organisms. A negative RT-PCR result indicated the absence of detectable nucleic acid; however negative results were interpreted cautiously considering low fungal burden, suboptimal sample quality, or PCR inhibitors which may lead to false-negatives. Samples showing weak or non-specific amplification or failure of internal controls were categorized as Indeterminate and repeat sampling was recommended to confirm the result. RT-PCR findings were correlated with clinical presentation, imaging evidences, KOH mount examination and culture results to ensure diagnostic accuracy.

Cases with RT-PCR positivity despite negative microscopy and culture were interpreted as early or low-burden infections, while culture positive but RT-PCR negative results were considered suggestive of possible technical inhibition or genetic sequence variations affecting amplification. Overall RT-PCR served as a rapid and sensitive diagnostic tool, enabling early detection and supporting clinical decision-making when integrated with conventional and clinical parameters.

So RT-PCR complemented culture and histopathology by providing rapid, species-specific confirmation.[17] All laboratory work was performed under Biosafety Level 2 (BSL-2) conditions using appropriate PPE.[11,12] Assays were validated with control strains to ensure reproducibility and accuracy.[17,18]

Ethical statement: the study was approved by institutional ethics committee prior [vide Memo no. RKC/ 465 Dated 11/06/2021]. All the data were collected after proper patient consent and predesigned standardized case record forms.

Results & Analysis:

A total of 107 patients with clinical suspicion of post-COVID mucormycosis were included in this study. Of these, 84 (78.5%) patients survived, while 23 (21.5%) patients succumbed to the disease. The mean age ranged from 21 to 80 years. Gender distribution showed 76 males and 31 females. Age distribution is shown in Fig. 2. Majority of case were Males (71%) Mean age of Male and Female were 50.84 years and 50.34 years respectively. The Mann-Whitney U test was applied to compare the age distribution between male and female participants and no significant difference were observed. Therefore, the occurrence of mucormycosis in this study population was not influenced by age difference between males and females.

A total of 107 samples were collected from suspected cases of mucormycosis. Out of these, 37 samples were negative by all three diagnostic modalities (KOH, Culture, and RT PCR), while 70 samples showed positivity by at least one diagnostic method. Among the isolates Majority were Rhizomucor (55%) followed by Mucor spp. (36%).

Among 107 suspected samples, 70 were positive by any diagnostic test, giving an overall positivity rate of 65.4%. The 95% confidence interval for this proportion (calculated by binomial approximation) is 56.1% – 74.0%. This suggests that nearly two-thirds of the suspected mucormycosis cases were confirmed by at least one diagnostic modality.

The cases were thus classified according to The 2020 revised EORTC/MSGERC were criteria used to standardize the diagnosis of invasive fungal diseases (IFD) into three categories—Proven, Probable, and Possible. These definitions are based on a triad of host factors, clinical-radiological features, and mycological evidence. [19] Table 1&2.

Comparison of positivity among diagnostic tests was performed using Chisquare (2×2) tests. KOH showed significantly higher positivity than culture ($p = 0.0006$), RTPCR positivity was significantly higher than both KOH ($p = 0.0346$) and culture ($p < 0.0001$). Thus, RTPCR is the most sensitive test among the three.

Among 107 suspected mucormycosis samples, 65.4% were positive by at least one diagnostic method. RTPCR demonstrated the highest positivity (87.1%), followed by KOH (72.9%) and

culture (44.3%). Statistical comparison showed that RTPCR was significantly more sensitive than both KOH and culture. These results indicate that molecular detection by RTPCR substantially enhances diagnostic yield when used along with microscopy and culture. Further evaluation of test agreement (κ statistics) and correlation with clinical parameters can provide a more comprehensive understanding of diagnostic performance.

Agreement between Diagnostic Tests Using Cohen's Kappa Statistics: This analysis evaluates the agreement between different diagnostic methods (RTPCR, KOH, and Culture) used for the detection of fungal infection in 70 clinical samples. The level of agreement between the tests was assessed using Cohen's Kappa (κ) statistic, which measures inter-rater reliability beyond chance.

Based on the Landis and Koch (1977) interpretation scale, values below 0 indicate poor agreement, 0.00–0.20 indicate slight agreement, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and above 0.81 almost perfect agreement.

The analysis revealed that RTPCR demonstrated higher positivity (87.1%) compared to KOH (72.9%) and Culture (44.3%).

However, the agreement between RTPCR and the conventional diagnostic methods was poor to slight, indicating that while RTPCR is more sensitive, it may detect additional cases not identified by microscopy or culture. These results

emphasize the importance of integrating molecular diagnostics with conventional methods for accurate and early detection of fungal infections.

Of the 107 clinically suspected CAM cases, 23 (21.5%) patients succumbed to the disease. Baseline demographic and clinical characteristics were compared between survivors and non survivors using Fisher's exact test, and odds ratios (OR) with 95% confidence intervals (CI) were calculated to identify predictors of mortality. Table 2. Uncontrolled diabetes with diabetic ketoacidosis (DKA), chronic liver disease (CLD), and chronic kidney disease (CKD) were the most significant predictors of mortality, each with a p value < 0.001 . Patients with these comorbidities were 9–17 times more likely to die compared with those without these conditions. A shorter interval (< 2 weeks) between COVID-19 recovery and onset of mucormycosis was also strongly associated with mortality ($p < 0.001$, OR = 13.5). Severe COVID infection and ICU admission showed significant associations ($p < 0.05$), indicating the role of overall disease severity in determining outcomes. Gender, steroid therapy, ventilator support, malignancy, asthma, and High Flow Nasal Oxygen (HFNO) use did not show statistically significant associations with mortality. Thus, metabolic control and management of systemic diseases appear to be crucial determinants of patient survival. Fig 3

Table 1: Classification of CAM cases according to The EORTC/MSGERC criteria

Diagnostic Test	Positive (n = 107)	Positivity (%)
Possible CAM	37 / 107	34.6%
Probable CAM	70/ 107	65.4%
Proven CAM	51/107	47.7%

Table 2: Comparison of Diagnostic Modalities:

Diagnostic Test	Positive (n / 70)	Positivity (%)
KOH finding	51 / 70	72.9%
Culture finding	31 / 70	44.3%
RT-PCR finding	61 / 70	87.1%

Table 3. Comparison of Clinical and Co-morbidity Variables between Survivors and Non survivors:

Variable	Survivors (n=84)	Non survivors (n=23)	P value	Odds Ratio	95% CI	Interpretation
Male gender	58	18	0.82	0.75	0.29–1.90	Not significant
Diabetes mellitus	68	21	0.39	1.63	0.53–5.01	Not significant
Uncontrolled DM & DKA	18	19	0.0001	17.4	5.7–53.4	Highly significant
Hypertension	50	17	0.49	1.49	0.48–4.64	Not significant
Coronary artery disease	28	15	0.04	3.75	1.04–13.5	Significant
Chronic liver disease	23	18	0.0002	9.6	3.3–27.7	Highly significant
Chronic kidney disease	9	12	0.0003	11.1	3.1–39.5	Highly significant

Steroid therapy	79	23	0.60	1.84	0.22–15.5	Not significant
HFNO use	82	21	0.99	0.95	0.14–6.2	Not significant
Ventilator support	36	8	0.69	0.76	0.28–2.07	Not significant
Malignancy	28	4	0.39	0.61	0.19–2.02	Not significant
Asthma	57	11	0.48	0.74	0.28–1.99	Not significant
Severe COVID	72	22	0.03	5.7	1.05–30.7	Significant
ICU admission	71	23	0.02	6.35	1.21–33.4	Significant
Interval <2 weeks (COVID–Mucor)	32	20	0.0001	13.5	4.4–41.8	Highly significant

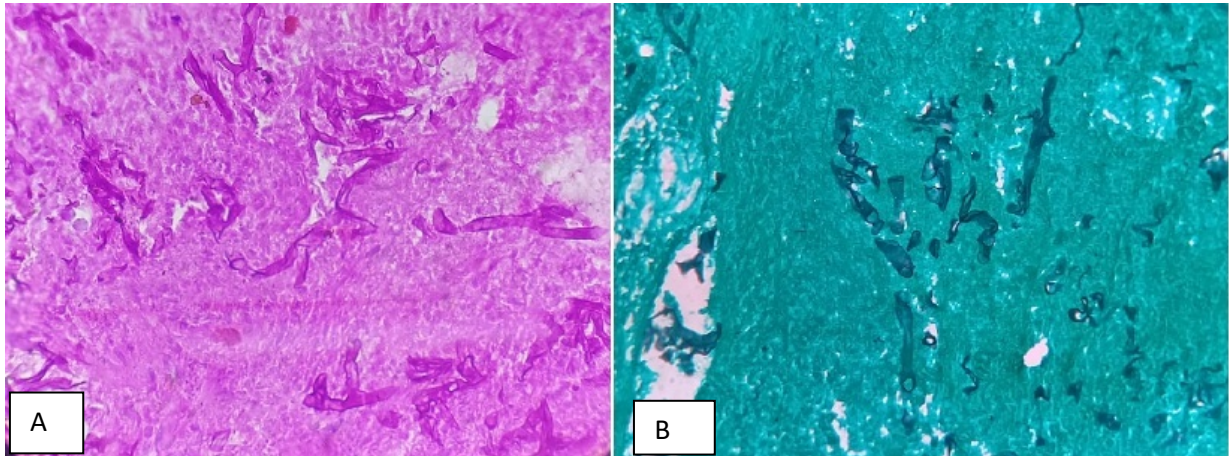


Figure 1: Mucorales aseptate ribbon like in tissue sections, A. PAS staining, B. GMS staining

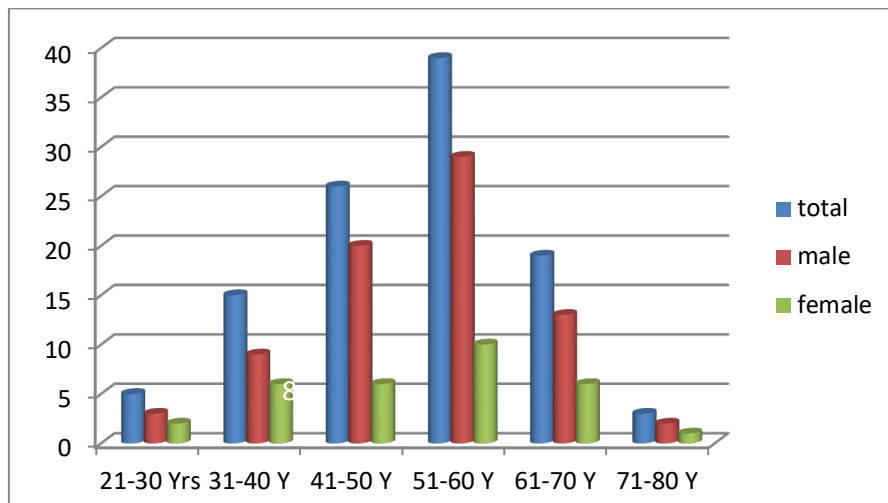


Figure 2: Age distribution

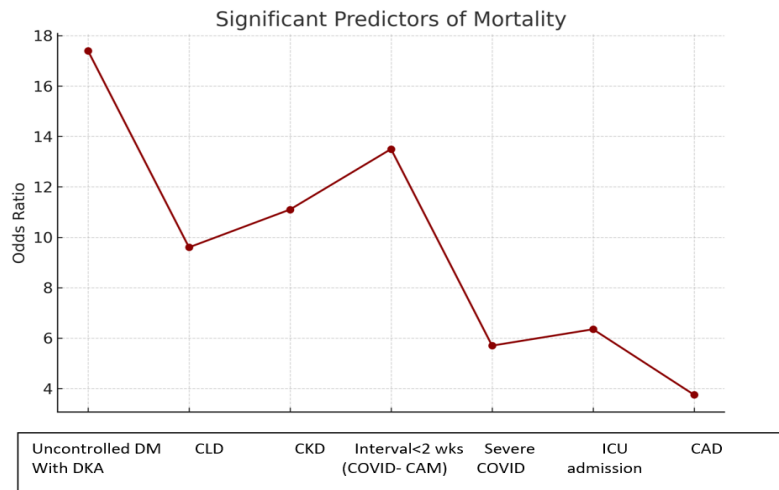


Figure 3: The magnitude of odds ratios among variables significantly associated with mortality.

Discussion

Post-COVID mucormycosis (CAM) emerged as a major epidemic in India during the second wave of SARS-CoV-2 infection in 2021. Large multicentre datasets, such as those from the MucoCovi Network and the COSMIC registry, documented thousands of cases across the country and confirmed India as the global epicenter of CAM during this period.[1,2] The MucoCovi study reported CAM in 187 of 287 mucormycosis cases across 16 centres, with an estimated prevalence of 0.27% among hospitalized COVID-19 patients.[1] The COSMIC study, involving 2826 patients, demonstrated that most cases occurred during or shortly after COVID-19 recovery, particularly in patients treated with corticosteroids.[2] Smaller regional studies from Jaipur, Ahmadabad, and southern India supported these national findings which can be well corroborated with our study revealing clustering of cases within weeks to months following COVID-19 infection.[11,18,20]

Across almost all Indian studies, diabetes mellitus and corticosteroid exposure were the dominant risk factors. Over 80% of CAM patients had diabetes, and 60–90% had received corticosteroids for COVID-19 management.[1,2,11,17,21] A multicentre case-control investigation across 25 hospitals further established these associations quantitatively: diabetes and corticosteroid use were each strongly linked to CAM, while other factors—such as oxygen therapy, zinc supplementation, or environmental exposure—showed inconsistent associations.[21] In the present study, strongest association was found with Corticosteroid therapy (102/107; 95.32%) followed by Diabetes mellitus (89/107; 83.17%) [Table 5].

Several studies also reported new-onset or stress-induced hyperglycaemia during COVID-19 treatment as a contributing factor.[5,10] The

combination of steroid-induced immunosuppressant and uncontrolled diabetes created an ideal environment for Mucorales proliferation. [3,4,22] In our study also uncontrolled diabetes with DKA, CKD, CLD, and shorter post-COVID intervals were the strongest predictors of mortality.

Microbiological analyses revealed *Rhizopus arrhizus* (syn. *R. oryzae*) as the predominant etiologic species across India.[1,3,23] However, emerging species such as *Rhizopus homothallicus* were increasingly identified in tertiary mycology centres, indicating potential shifts in epidemiology.[23,24] Rudramurthy et al.[23] found that *R. homothallicus* accounted for a growing proportion of cases in recent years, suggesting environmental or ecological adaptation. However in our study, *Rhizomucor pusillus* was found to be the predominant etiology. These findings highlight the need for regional surveillance of Mucorales species, as species-specific antifungal susceptibility and virulence may influence clinical outcomes.

Most studies reported rhino-orbital-cerebral mucormycosis (ROCM) as the most frequent presentation of CAM, followed by pulmonary and disseminated forms[1,2,11,18] which is also in agreement with our studies. ROCM accounted for >80% of cases in most Indian cohorts. Typical symptoms—facial pain, nasal blockage, orbital swelling, and Ophthalmoplegia—developed within 2–3 weeks after COVID-19 infection.[2,14,19] Pulmonary mucormycosis was less common but carried a higher mortality.[1,23]

Combined surgical and medical therapy remained the cornerstone of CAM management. Early and aggressive debridement, coupled with systemic antifungal therapy (primarily liposomal amphotericin B) was the mainstay of treatment in our study which also similar with other studies and was associated with improved survival.[1,2,11,14]

Several tertiary care studies highlighted challenges in drug availability during India's second COVID-19 wave, leading to delays or substitution with conventional amphotericin B, which may have contributed to mortality variability.[11,19] Step-down therapy with posaconazole or isavuconazole was used widely after disease stabilization which was not followed in our study population.[2,14,22]

Mortality rates varied significantly across studies, ranging from 14% to 49% depending on disease extent, organ involvement, and timeliness of intervention.[1,2,11,14,21] In our study, the mortality rate among post-COVID mucormycosis patients was 21.5%. Studies consistently identified cerebral extension, delayed presentation, and uncontrolled diabetes as predictors of poor outcome.[2,14,19,21] However in our study uncontrolled diabetes with DKA, CKD, CLD, and shorter post-COVID intervals were the strongest predictors. The COSMIC registry emphasized that early surgical intervention and strict glycaemic control improved survival, particularly in limited-stage ROCM.[2]

Differences in reported incidence and outcomes stem from heterogeneity in study design, case definitions, diagnostic capacity, and regional healthcare infrastructure. Multicentre networks such as MucoCovi and COSMIC provided comprehensive overviews but may have under-represented smaller centres with limited diagnostic access.[1,2] Conversely, single-centre studies offered detailed clinical descriptions but lacked population-level generalizability.[18,19,25] Although our study is a single-centre study, the depth of clinical information, uniformity of diagnostic protocols, and continuous follow-up enhance the reliability of the findings and support their relevance to comparable tertiary-care environments.

Conclusion

Post-COVID mucormycosis emerged as a major public health concern during the pandemic, particularly in India, due to the convergence of COVID-19-induced immune suppression, diabetes, and irrational corticosteroid use. Among post-COVID mucormycosis patients, mortality was significantly associated with uncontrolled diabetes with DKA, CKD, CLD, short interval between COVID recovery and mucormycosis onset, and severe COVID infection requiring ICU admission.

Strict glycemic control, early diagnosis, and comprehensive management of co-morbidities are essential to improve survival outcomes. Early clinical suspicion, rapid microbiological and molecular diagnosis, and multidisciplinary management are crucial for favorable outcomes [27,29,30]. Strengthening awareness among

clinicians, ensuring judicious steroid use, and optimizing diabetic control can significantly reduce morbidity and mortality associated with this infection [31,33,34].

Limitations and Future directions: Despite the strength of systematic data collection and uniform diagnostic methodologies, this study has certain limitations. Our study is a single-centre investigation that provides in-depth clinical, microbiological and epidemiological insights; the finding should be interpreted with caution, as they may not fully reflect the heterogeneity of disease burden, healthcare practices or patient characteristics across different regions or institutions.

Additionally, variations in referral patterns, resource availability and local epidemiology may limit the external applicability of the results. Future multi-centric studies with larger or more diverse populations are recommended to strengthen the evidence base and validate these observations.

Despite extensive Indian data, most studies were retrospective, conducted during a healthcare crisis, and lacked uniform follow-up. Microbiological confirmation was incomplete in some series due to laboratory limitations. Prospective surveillance and controlled studies are needed to evaluate environmental contributors and long-term sequel.

Key public health lessons include the need for rational corticosteroid use, diabetes control, early diagnosis, and multidisciplinary management to prevent future outbreaks [4, 11, 23]. Future research should focus on understanding host-pathogen interactions, resistance patterns, and developing rapid diagnostic assays to enable timely intervention in high-risk patients.

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