ORIGINAL ARTICLE

COVID-19 associated mucormycosis in Pakistan: Adding fuel to the fire?

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ABSTRACT

Background: The SARS-CoV-2 pandemic has introduced several public health challenges, including an increase in fungal infections. COVID-19-associated mucormycosis (CAM) has resulted in rising morbidity and mortality worldwide. This study aims to assess the demographics, associated risk factors, outcomes, and frequency of CAM.

Material and Methods: This retrospective observational study was conducted from June 1, 2021, to June 30, 2023, in the Histopathology and Microbiology section of the Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences, Hyderabad. Data from 102 suspected mucormycosis cases were analyzed for demographics, clinical history, vaccination status, radiological findings, and outcomes. Fisher's exact test was used to assess risk factors and outcomes in COVID-19 and non-COVID-19 patients, with significance set at $p<0.05$.

Results: Among the 102 samples analyzed for suspected mucormycosis, 35 (34.3%) were confirmed as proven cases. Of these, 22 (62.8%) had a history of COVID-19. 90.9% were male, 54.5% had diabetes, 22.7% were receiving chronic steroid treatment, and 10 (28.5%) patients died, primarily due to rhino cerebral involvement (2/10), demonstrating a high mortality rate in patients with CAM. Additionally, eight patients with CAM had received a single dose of the vaccine. The rate of CAM was more in unvaccinated population.

Conclusion: This study underscores mucormycosis as a growing post-COVID-19 threat, with high CAM fatality emphasizing the need for early diagnosis and strong healthcare systems. Integrating findings into the national database is vital to assess fungal infection prevalence in Pakistan.

Keywords: COVID-19, COVID-19 associated mucormycosis, Mucorales, COVID-19 vaccine

BACKGROUND

The recent outbreak of the SARS-CoV-2 pandemic, caused by a newly discovered coronavirus, is continuing to wreak havoc in numerous regions of the globe, including Pakistan. Symptoms vary from mild disease to more severe conditions characterized by extensive pneumonia and hypoxemia followed by various opportunistic bacterial and fungal infections.[1,2,3]

Each wave of COVID-19 infection has presented health care providers with newer challenges such as COVID-19 associated aspergillosis, mucormycosis and hospital acquired *Candida auris* infections,[4] which are associated with an increased morbidity and mortality rate.[5,6] Literature suggests that the risk of these opportunistic infections is much higher in individuals with compromised immune function, particularly decreased CD4+ T cells and CD8+ T cells.[7]

Mucormycosis is a rare but a life threatening angio-invasive disease that mainly affects people with compromised immune function. It is highly prevalent in individuals with uncontrolled diabetes mellitus (DM), DM associated ketoacidosis (DKA) and those on systemic corticosteroid treatment in Asia,[8,9] whereas the primary risk factors for the onset of this fungal infection are hematological malignancies and solid organ or bone marrow transplantations in Europe and the USA. Additionally, factors such as a history of surgeries, trauma, neutropenia, protein-calorie malnutrition, autoimmune diseases, chronic kidney disease or renal failure, HIV infection, deferoxamine therapy, and corticosteroid use have also been identified as global risk factors. Beyond individual health conditions, other contributing factors like seasonal variations and recovery from natural disasters also play a role in increasing susceptibility.[9] However, the COVID-19 era has seen a shift, with otherwise immunocompetent individuals, especially those with diabetes or those receiving steroids, developing CAM.[10]

Epidemiological survey of mucormycosis worldwide suggests a rise in Asian countries, with the estimated rate of 14/100000 in Pakistan.[11] The inapt national

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healthcare system, inadequate medical supplies and laboratory diagnostic systems, lack of a proper antimicrobial stewardship program and easy accessibility to over-the-counter drugs, has led to difficulty in assessing the actual burden of disease. [11,12,13]
A sharp rise in CAM cases was observed during the second wave of the pandemic in countries like India, where mucormycosis was declared an epidemic in several states. This increase has been attributed to the triad of COVID-19 infection, uncontrolled diabetes**,** and immunosuppressive therapy**,** particularly corticosteroids.[14] While several case reports and regional data have been published on CAM, there is still a paucity of large-scale, systematic analyses that assess the full clinical spectrum, prognostic indicators, and treatment outcomes. This study aims to fill that gap by providing a comprehensive evaluation of CAM cases in a tertiary care center during and after the pandemic surge by determining the frequency, risk factors and outcomes of patients with COVID-19 associated mucormycosis.

MATERIAL AND METHODS

This retrospective observational study was conducted in section of Histopathology and Microbiology, at Diagnostic and Research laboratory, Liaquat University of Medical and Health Sciences, Hyderabad, Pakistan from 1st of June 2021 till 30th of June 2023. Samples from 102 patients with suspected mucormycosis were included. Demographics, clinical history, vaccination status, radiological findings and outcome data were received as per laboratory protocol and retrieved from the laboratory database.
On the basis of recently updated criteria of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) criteria, cases with clinical or radiological evidence of invasive disease and histopathological findings of mucormycosis or/and isolation of Mucorales on culture, were considered as proven cases of mucormycosis. Cases that had a history of PCR proven SARS-CoV-2 infection with histopathological and microbiological diagnosis of mucorales were labeled as cases with COVID-Associated Mucormycosis (CAM). Due to the unavailability of pulmonary tissue for histopathological or culture-based confirmation, and with only sputum and bronchoalveolar lavage (BAL) samples submitted for microbiological analysis, cases could not fulfill the criteria for proven pulmonary COVID-19-associated mucormycosis (CAM). As a result, based on established diagnostic definitions, only probable cases of pulmonary CAM were identified and included in the final analysis.
Biopsy samples from maxilla, mandible, palate, nasolabial area, maxillary sinus and epiglottic vallecula with history of suspected mucormycosis were submitted in histopathology section. The samples were subjected to periodic acid Schiff stain. The samples that revealed acute and chronic inflammation with broad aseptate hyphae with invasion of blood vessels and bony trabeculae were diagnosed as mucormycosis.
10% potassium hydroxide and gram stain was performed on all the samples of sputum, broncho-alveolar lavage and nasal tissue sent from suspected cases of mucormycosis. Culture was inoculated on Chocolate agar, blood agar plate, MacConkey agar and Sabouraud's dextrose agar. Isolates were identified based on colony and microscopic morphology.
The data obtained were entered into statistical software IBM SPSS version 19.0 (SPSS. Inc, Chicago, IL). For descriptive variables analysis, mean and standard deviation were calculated. For categorical variables such frequencies and percentages were calculated. Significance of risk factors and outcomes associated with mucormycosis in patients with and without COVID-19 were calculated using fisher exact test. A *p*-value of less than 0.05 was considered significant.
This study was approved by the institutional ethical review committee (LUMHS/REC/-141)- Dated: 12th September 2023.

RESULTS

In this study, we analyzed a total of 102 samples obtained from suspected cases of mucormycosis for histopathologic and microbiological examination. Among these samples, 25 were confirmed positive through histopathologic evaluation, while 10 demonstrated positive results via microscopy and culture (Figure-I. Notably, 22 patients (21.5%) had a documented history of COVID-19 and presented with symptoms 30 to 150 days post-recovery, with none requiring hospitalization. The majority of these patients were male (90.9%), and their ages ranged from 17 to 80 years, with the mean age being 42.49 years (SD= 36.91). A significant portion of the cases involved necrosis of the maxilla and mandible (36.3%) attributed to

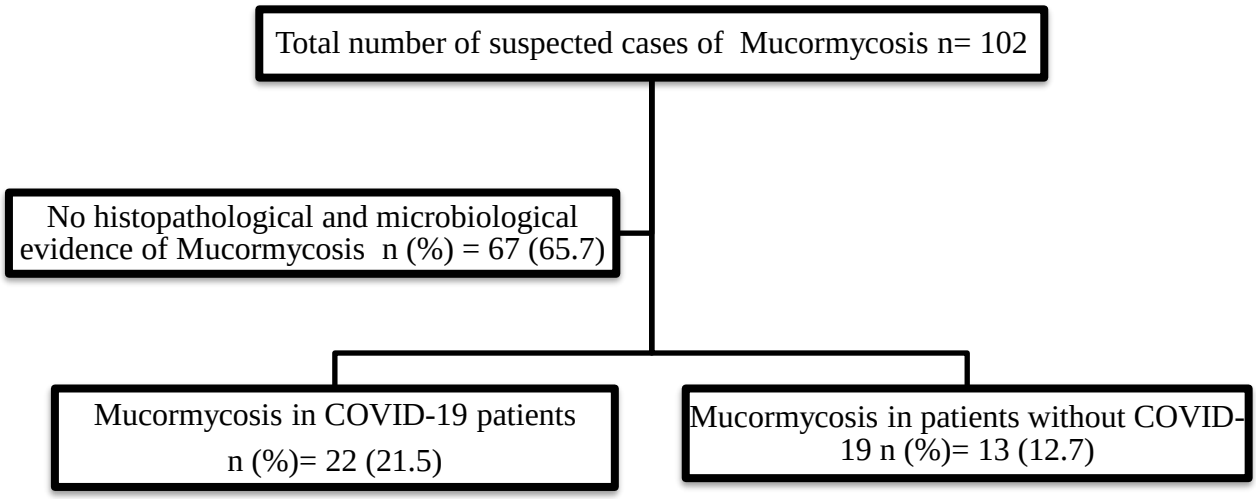
mucormycosis. The mortality rate among the patients was 45.4%, with ten individuals succumbing to the disease, primarily due to rhinocerebral involvement (2/10). Among the cohort with COVID-19-associated mucormycosis (CAM), eight patients (36.3%) had received vaccinations. Furthermore, uncontrolled diabetes and systemic corticosteroid therapy were prevalent among most individuals diagnosed with CAM.

In all cases where Mucorales were isolated from culture of sputum or BAL of COVID-19 patients, the identified species belonged to the genus *Rhizopus*. All five cases showed consolidation on chest radiography. Statistical analysis revealed a significant association between CAM and the unvaccinated population (*p*-value = 0.0125), as well as a higher incidence of mortality among patients with CAM (*p*-value = 0.005) (Table-I).

Table-I: Demographics, risk factors and outcomes in patients with mucormycosis with or without COVID-19 (n=35).

Variables	Mucormycosis in Covid-19 patients n (%)	Mucormycosis in patients without Covid-19 n (%)	*p*-value
Total number of cases	22 (62.8)	13 (37.1)	
Gender			
Male	20 (90.9%)	11(84.6)	0.61
Site of infection			
Maxilla and mandible	10 (45.5)	4 (30.7)	1.00
Palate	6 (27.3)	2 (15.3)	0.68
Nasal	1 (4.5)	3 (23.1)	0.13
Sinuses	1 (4.5)	1 (7.6%)	1.00
Eye	1 (4.5)	0	1.00
Epiglottic vallecula	1 (4.5)	0	1.00
Lungs (Sputum and BAL)	2 (9)	3 (23.1)	1.00
Risk factors			
Uncontrolled diabetes with > 6.0 g/dl glycated Hemoglobin A1C	12 (54.5)	10 (76.9)	0.28
Systemic corticosteroid treatment	5 (22.7)	0	0.13
Mycological evidence			
Histopathology	19 (86.4)	8 (61.5)	
Microbiology	3 (13.6)	5 (38.4)	
Vaccination status			
Unvaccinated	14 (63.6)	2 (15.4)	**0.0125**
Outcome			
Expired	10 (45.5)	0	**0.005**

Figure-I: Study design showing total number of included and excluded cases



DISCUSSION

Our study emphasizes the emergence of mucormycosis as a significant post-COVID complication, leading to

increased morbidity and mortality among individuals in Pakistan. Our findings reveal a notable association between COVID-19-associated mucormycosis (CAM) and the unvaccinated population, as well as a heightened mortality rate among CAM patients. Additionally, we compared the incidence and risk factors for mucormycosis in individuals with and without a history of COVID-19.

In 2021, ten cases of CAM were identified among patients admitted to the ICU with severe to critical COVID-19 in Pakistan, comprising four proven and six probable cases.[13] Given the rising trend of CAM in India, where 82 cases have been reported, our study similarly observed an emergence of CAM cases.[3] Notably, none of the patients in our study had been hospitalized for COVID-19, highlighting the focus on individuals with less severe disease.

In our study, 91% of cases were males, consistent with global observations. The underlying reasons for this gender disparity remain unclear, although it may be related to a higher prevalence of Mucorales infections in male diabetic patients, as suggested by findings in a mouse model.[15] Notably, this gender distribution was not limited to those with COVID-19.

The case fatality rate among patients with CAM in our study was 45.5%, comparable to rates reported in various countries.[5] However, mortality rates observed in India were lowest, where the majority of the cases were reported from. This could be explained by the high number of rhino-orbital mucormycosis cases reported in India, which are associated with better outcomes when compared with pulmonary, gastrointestinal, and disseminated mucormycosis.[16]

Additionally, 54.5% of cases exhibited uncontrolled diabetes, while 22.7% received systemic corticosteroid treatment, indicating that this population may be more susceptible to CAM. These findings align with data reported globally.[17] In environments characterized by high acidity and elevated glucose levels—common during uncontrolled diabetes, diabetic ketoacidosis, COVID-19, and corticosteroid therapy—*Rhizopus* hyphae are able to proliferate and flourish.[18] In our study mortality was associated with isolation of *Rhizopus* species. Our findings reinforce the existing recommendations to avoid glucocorticoid use in COVID-19 patients who are not experiencing hypoxemia.[19]

Our study indicates a high incidence of CAM among unvaccinated individuals, highlighting their increased susceptibility to the disease. These results align with findings from a study conducted in India.[20] Thus, our research underscores the importance of vaccination as a preventive measure against CAM.

It is important to note that our study is a retrospective analysis of laboratory data, lacking information on treatment regimens and their durations. Additionally, we did not include laboratory parameters such as leukocyte counts, nor did we assess whether survivors developed any subsequent complications. Our cohort did not consist of critically ill patients or those with a history of ICU admission; instead, it focused on individuals with less severe disease. The findings from this study contribute to the growing body of data on CAM cases in our national database and aim to facilitate the establishment of improved diagnostic and reporting systems.

Due to an inadequate healthcare system and limited diagnostic resources, the true burden of fungal infections in Pakistan remains difficult to assess.[10] Our study specifically highlights this challenge, as mucormycosis is known for its rapid progression, high fatality rate, and diagnostic difficulties, especially in the context of COVID-19. This situation underscores the urgent need to train laboratory personnel and establish well-equipped sentinel laboratories for the rapid diagnosis and reporting of Mucorales.

It is essential that all patients receive follow-up care at least 60 days post-COVID infection, as our findings indicate that CAM can occur in individuals with less severe disease and is not limited to critical care settings, thus emphasizing the importance of surveillance.

CONCLUSION

In summary, our study brings to light a pressing public health issue that must be prioritized to mitigate associated morbidity and mortality. The rise in CAM cases, particularly among the unvaccinated population, highlights the necessity for a competent laboratory and healthcare system, as well as skilled personnel, to prevent the progression of this condition.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE
Declared none

AUTHOR CONTRIBUTION

Yusra Shafquat: Concept and design of study, acquisition, analysis and interpretation of data, drafting and revising article critically for important intellectual content, final approval, agreement to be accountable for all aspects of the work

Muhammad Anique: design of study, acquisition, analysis and interpretation of data, drafting and revising article critically for important intellectual content, final approval, agreement to be accountable for all aspects of the work

Riyasat Ahmed Memon: Design of study, acquisition, analysis and interpretation of data, drafting and revising article critically for important intellectual content, final approval, agreement to be accountable for all aspects of the work

Muhammad Rahil Khan: Analysis and interpretation of data, drafting and revising article critically for important intellectual, content final approval, agreement to be accountable for all aspects of the work

Zahida Shaikh: Analysis and interpretation of data, drafting and revising article critically for important intellectual content, final approval, agreement to be accountable for all aspects of the work

Ikram Din Ujjan: analysis and interpretation of data, drafting and revising article critically for important intellectual content, final approval, agreement to be accountable for all aspects of the work.

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