Section: Pathology

Original Research Article

CLINICOPATHOLOGICAL AND MICROBIAL CULTURE EVALUATION OF FUNGAL INFECTIONS RELATED TO COVID-19: AN INSTITUTIONAL EXPERIENCE

Alekhya M[1], Rukmangadha N [2], Amarnath S.B [3], Jayaprada R [4], Mohan A[5]

[1]*Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, India.*
[2]*Professor and HOD, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, India.*
[3]*Professor and HOD, Department of ENT, Sri Venkateswara Institute of Medical Sciences, Tirupati, India.*
[4] *Professor, Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati, India.*
[5] *Senior Professor and HOD, Department of Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati, India.*

Received *: 03/11/2025*
Received in revised form *: 12/12/2025*
Accepted *: 02/01/2026*

Corresponding Author:
Dr. Alekhya. M,
Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, India.
Email: alekhya.maduri@gmail.com

DOI: 10.70034/ijmedph.2026.1.21

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (1); 109-115

ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) has emerged as a major global health crisis and has been associated with a rise in secondary fungal infections, particularly in patients with uncontrolled diabetes and steroid exposure. These infections are difficult to diagnose and manage, often leading to increased morbidity and mortality. Early identification of disease patterns, laboratory abnormalities, and outcome predictors is essential to improve patient management. **Objectives:** To evaluate the demographic profile, clinical characteristics, histopathological and microbiological findings, management strategies, and outcomes of patients with fungal infections secondary to COVID-19, and to assess associations between diagnostic findings and clinical outcomes.

Materials and Methods: This observational study included 413 patients presenting with fungal infections secondary to COVID-19 at a tertiary care centre between January 2021 and December 2022. Clinical details, laboratory investigations, histopathology, and KOH/culture results were analysed. Patients were classified as early or late fungal infections. Management modalities and outcomes were recorded. Statistical analysis was performed using IBM SPSS version 25, with $p < 0.05$ considered statistically significant.

Results: The mean age was 49.8 ± 12.4 years, with a male predominance (72.4%). Late fungal infections constituted 97.8% of cases. Histopathological positivity was observed in 49.9%, while KOH/culture positivity was seen in 37.5%. Laboratory findings indicated systemic inflammation and poor glycaemic control. Combined medical and surgical management was required in 63.7% of patients. Overall survival was 94.7%. Histopathological positivity was significantly associated with mortality ($p < 0.001$), and KOH positivity was significantly associated with the need for combined management ($p < 0.001$).

Conclusion: Fungal infections secondary to COVID-19 are associated with significant systemic inflammation and metabolic derangements. Histopathological and microbiological findings are important predictors of disease severity, management requirements, and outcomes. Early diagnosis and aggressive multidisciplinary management are crucial to reduce morbidity and mortality.

Keywords: COVID-19; Secondary fungal infections; Mucormycosis; Histopathology; KOH mount; Diabetes mellitus; Systemic inflammation; Medical and surgical management; Patient outcomes.

INTRODUCTION

Coronaviruses are a diverse group of viruses infecting many different animals, and they can cause mild to severe respiratory infections in humans. In 2002 and 2012 respectively, two highly pathogenic corona viruses with zoonotic origin, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in humans and caused fatal respiratory illness, making emerging coronaviruses a new public health concern in the twenty-first century.[1] At the end of 2019, a novel coronavirus designated as SARS-CoV-2 emerged in the city of Wuhan, China, and caused an outbreak of unusual viral pneumonia. Being highly transmissible, this novel coronavirus disease, also known as coronavirus disease 2019 (COVID-19), has spread fast all over the world. [2,3]

COVID-19 has overwhelmingly surpassed SARS and MERS in terms of both the number of infected people and the spatial range of epidemic areas. The ongoing outbreak of COVID-19 has posed an extraordinary threat to global public health. [4,5] This pandemic is unprecedented with many challenges to the health community. Rise of these cases in India reflects local climate conditions and large numbers of susceptible people. One of the major challenges is secondary fungal infections due to overuse of steroids in infected patients which is an immunosuppressant. Places having decaying vegetation which is more common in tropical regions like India and rotting wood are places for lot of fungal spores. In weaker health systems, poorly filtered air in hospitals can also encourage spores to spread.

Amid this pandemic, fungal infections are a major calamity because they are so hard to be diagnosed and treated. Because of shortage in drugs due to covid crisis, prolonged treatment required and uncontrollable disease with existing drugs it is very difficult to control and treat fungal infections. Because of extremely limited ability to diagnose with invasive biopsies and examination under microscope for diagnosis, the cases are probably the tip of the iceberg. Patients who survived COVID can have invasive fungal infections that will kill them down the line.

Globally over 1 billion people are afflicted with a fungal infection and 25 million are at high risk of dying or losing their eyesight. [6,7] Medical mycology is a science that has markedly increased in significance over recent years. Fungal infections progress rapidly and are difficult to diagnose – especially at early stages, and in resource limited settings. [8,9]

In December, 2019, COVID-19 emerged from Wuhan, China, and has become pandemic.[10] In response to the needs of the rapidly evolving COVID-19 outbreak, the Clinical Characterization and Management Working Group of the WHO Research and Development Blueprint programme, the International Forum for Acute Care Trialists, and the International Severe Acute Respiratory and Emerging Infections Consortium [11] published a minimum set of common outcome measures for studies of COVID-19. It is surprising that only bacterial and viral secondary infections are considered in the proposed set, without mention of fungal coinfections.

The burden of COVID-19-associated pulmonary aspergillosis fungal co-infection is still unknown and probably underestimated, particularly in patients with COVID-19 with critical clinical course. [12] Despite the proven importance of co-infections in the severity of respiratory diseases, they are understudied during large outbreaks of respiratory infections. In India, until august, 2021 total number of confirmed cases of COVID-19 are 32424234 with deaths of 434367. In a retrospective observational study involving 16 healthcare centers across India, 187 cases of COVID-19 associated mucor mycosis were detected between September and December, 2020. [13] They found hypoxemia due to COVID-19 and inappropriate glucocorticoid administration were associated with development of late covid-19 associated mucormycosis. Here we evaluate clinical, pathological, and microbiological features of fungal infections related to COVID-19 patients.

MATERIALS AND METHODS

Study Design and Setting

This observational study was conducted at Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, a tertiary care teaching hospital. All patients with a history of COVID-19 infection or those recovered from COVID-19 who developed secondary fungal infections between January 2021 and December 2022 were included.

Sample Size

Sample size was calculated based on the reported prevalence of mucormycosis, the most common secondary fungal infection associated with COVID-19 (0.005–1.7 per million population).[14,15,16] Using a prevalence-based formula with finite population correction (N = 1,000,000), hypothesized prevalence of 2%, absolute precision of 5%, design effect of 1, and a confidence level of 95%, a minimum sample size of 31. (Open Epi, Version 3, open source calculator).

Case Definitions

COVID-19 was diagnosed by detection of SARS-CoV-2 using reverse transcription polymerase chain reaction (RT-PCR) or rapid antigen testing. Fungal infection was defined by compatible clinical and radiological findings with demonstration of fungal elements in tissue or sterile body fluids by direct microscopy or culture.

Based on the timing of onset, fungal infections were categorized as early fungal infections (EFI), occurring within one week of COVID-19 diagnosis, and late fungal infections (LFI), occurring after

recovery from COVID-19 (confirmed by negative RT-PCR or rapid antigen test) and within three weeks of hospital discharge.

Outcome Assessment

Clinical outcome was categorized as survival (discharged) or death. Patients who were discharged against medical advice (DAMA) were considered deceased for outcome analysis.

Inclusion and Exclusion Criteria

All patients with fungal infections secondary to COVID-19 were included. Patients with primary fungal infections or fungal infections unrelated to COVID-19 were excluded.

Ethical Approval

The study was approved by the Institutional Research Approval Committee and Institutional Ethics Committee. Written informed consent was obtained from all participants or from legally authorized representatives where applicable.

Histopathological Evaluation

All tissue specimens received in 10% buffered formalin at the Department of Pathology, SVIMS, were processed by an automated tissue processor. Paraffin-embedded blocks were prepared, and 3–4 µm sections were stained with hematoxylin and eosin (H&E). Special stains, including Periodic Acid–Schiff (PAS) and Grocott Methenamine Silver (GMS), were used for confirmation of fungal organisms and assessment of tissue invasion.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 25 (Armonk, NY: IBM Corp). Continuous variables were expressed as mean ± standard deviation or median with interquartile range (IQR), and categorical variables as percentages. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

A detailed clinical history, investigations and management were tabulated as follows.

Table 1: Demographic characteristics of the study population (N = 413)

Variable	Value
Age (years), Mean ± SD	**49.8 ± 12.4**
Sex	
• Male	**299 (72.4%)**
• Female	**114 (27.6%)**

The study included 413 patients, with a mean age of 49.8 ± 12.4 years and a male predominance (72.4%). [Table 1]

Table 2: Disease classification

Disease type	Number of patients (N)	Percent (%)
LFI	378	97.8
EFI	9	2.2
Total	**413**	**100**

The most common disease type was LFI (97.8 %), while EFI constituted 2.2% of cases [Table 2].

Table 3: Histopathological (HPE) findings

HPE result	Number of patients (N)	Percent (%)
Positive for fungal elements	206	49.9
Negative	207	50.1
Total	**413**	**100**

Histopathological examination demonstrated fungal elements in 49.9% of patients, whereas KOH/culture positivity was observed in 37.5%. [Tables 3&4]

Table 4: KOH mount / culture report

KOH / Culture result	Number of patients (N)	Percent (%)
Positive	155	37.5
Negative	258	62.5
Total	**413**	**100**

KOH/culture positivity was observed in 37.5% [Table 4].

Table 5: Laboratory investigations

Variable	Observation
Hemoglobin (g/dL)	**11.8 ± 2.6**
Total leukocyte count (cells/mm³)	**10,449 ± 5,485**
Neutrophils	**365.2 ± 1,940.0**
Lymphocytes (%)	**21.5 ± 13.6**
Packed cell volume (%)	**35.3 ± 7.3**
Mean corpuscular volume (fL)	**85.6 ± 10.8**
Mean corpuscular hemoglobin (pg)	**30.0 ± 10.1**
Platelet count	**7.2 ± 17.2**

ESR (mm/hr)	**64.5 ± 32.7**
Biochemical Parameters	
Serum ferritin (ng/mL)	**582.8 ± 426.4**
HbA1c (%)	**9.3 ± 3.2**
C-reactive protein (mg/L)	**73.3 ± 79.2**

*Data are expressed as mean± standard deviation

The investigative parameters indicate the presence of systemic inflammation, poor glycemic control, and hematological abnormalities in the study population. Mild to moderate anemia, elevated total leukocyte count with neutrophil predominance, raised ESR, and markedly increased CRP and serum ferritin levels reflect an active inflammatory state. Elevated HbA1c values suggest suboptimal long-term glycemic control, a known contributor to increased perioperative risk.

Table 6: Management details

Treatment modality	Number of patients (N)	Percent (%)
Medical management alone	136	32.9
Surgical management alone	14	3.4
Combined medical + surgical management	263	63.7
Total	**413**	**100**

Most patients (63.7%) required combined medical and surgical management, while 32.9% were managed medically alone (Table 6). The mean duration of hospital stay was 22.7 ± 17.9 days.

Table 7: Outcome of patients

Outcome	Number of patients (N)	Percent (%)
Survived / Discharged	391	94.7
Death	22	5.3
Total	**413**	**100**

Overall survival was 94.7%, with a mortality rate of 5.3%. [Table 7]

Table 8: Association between HPE positivity and outcome*

HPE result	Number of patients survived (%)	Number of patients died (%)	Total
Positive	186 (90.3)	20 (9.7)	206
Negative	205 (99.0)	2 (1.0)	207
Total	**391**	**22**	**413**

****p < 0.001***

Statistically significant association was observed between HPE positivity and mortality, with higher deaths among HPE-positive patients ($p < 0.001$). [Table 8]

Table 9: Association between KOH positivity and management modality*

KOH result	Medical N (%)	Combined N (%)	Total
Positive	28 (18.1)	127 (81.9)	155
Negative	108 (41.9)	150 (58.1)	258

****p < 0.001***

KOH positivity was significantly associated with the need for combined medical and surgical treatment ($p < 0.001$). [Table 9]

Figure 1: clinical photograph showing necrotic black eschar involving the hard palate and maxillary alveolar region, with surrounding mucosal discoloration and dental involvement, characteristic of rhino-maxillary mucormycosis

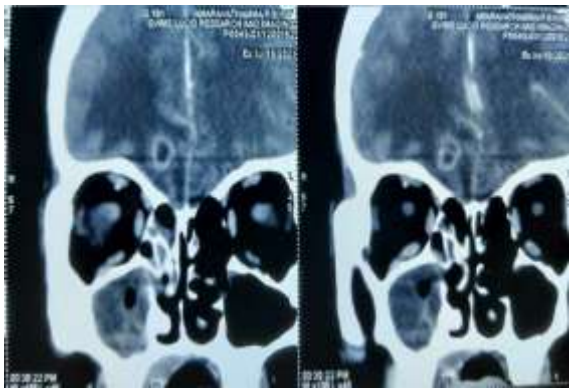

Figure 2: Coronal CT scan of the paranasal sinuses demonstrating features suggestive of rhino-orbito-maxillary mucormycosis, including soft tissue density within the maxillary and ethmoid sinuses with mucosal thickening, sinus opacification, and evidence of bony erosion suggesting invasive fungal sinusitis

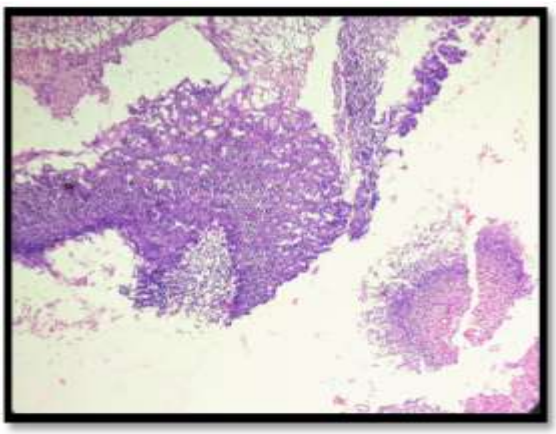

Figure 3: photomicrograph showing necrotic tissue with extensive inflammatory infiltrate and areas of fungal colonisation consistent with invasive fungal infection (Haematoxylin and eosin (H&E) stain, ×100)

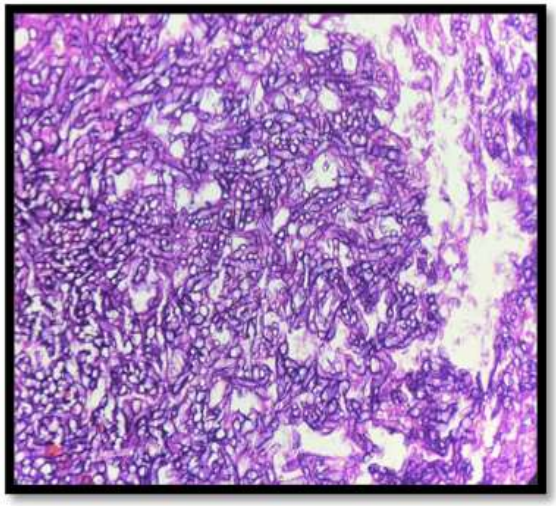

Figure 4: photomicrograph showing broad, pauciseptate fungal hyphae with right-angle branching staining magenta suggestive of mucormycosis (Periodic acid–Schiff (PAS) stain, ×400)

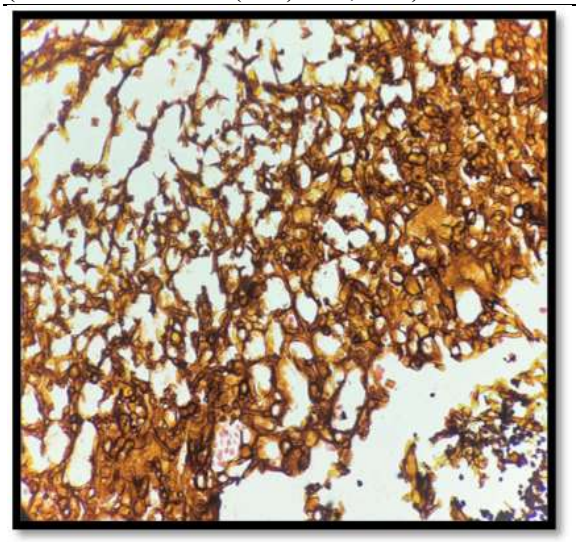

Figure 5: photomicrograph showing broad, irregular, aseptate hyphae with right-angle branching, highlighted in black against a pale green background suggestive of mucormycosis (Grocott's methenamine silver (GMS) stain, ×400)

DISCUSSION

The present study evaluated the demographic profile, disease characteristics, investigative parameters, management strategies, and outcomes in a cohort of 413 patients, with a particular focus on the association between histopathological and microbiological findings and clinical outcomes. The findings are broadly consistent with recent national and international literature published over the last five years.

Demographic and Disease Profile

The study population demonstrated a mean age of 49.8 ± 12.4 years with a clear male predominance (72.4%), findings that are comparable to recent Indian and international studies by Skiada et al. (2020),[14] and Patel et al. (2021),[13] which reported higher disease prevalence among middle-aged males. The predominance of LFI (97.8%) over EFI in the present study is similar to observations by Cornely et al. (2021),[17] and Prakash et al. (2021),[18] who reported that localized forms constitute the majority of cases at initial presentation, especially in patients presenting early or with limited dissemination.

Association with histopathological and microbiological findings

Histopathological positivity for fungal elements was observed in 49.9% of patients, while KOH/culture positivity was noted in 37.5%. These findings are consistent with studies by Challa et al. (2020),[19] and Muthu et al. (2022),[20] which reported variable sensitivity of microbiological tests compared to histopathology. Recent literature emphasises that histopathology remains the gold standard for diagnosis, while KOH and culture are complementary but with less sensitive confirmation, particularly in patients already receiving antifungal therapy.

Laboratory Parameters and Systemic Inflammation

The investigative profile in the present study revealed mild to moderate anemia, leukocytosis with neutrophil predominance, elevated ESR, markedly raised CRP and serum ferritin, and poor glycemic control (HbA1c 9.3 ± 3.2%). Similar laboratory derangements have been reported in recent studies by Sharma et al. (2021),[21] Singh et al. (2021),[22] and Raut et al. (2023),[23] who highlighted the role of systemic inflammation and uncontrolled diabetes in disease progression and adverse outcomes. Elevated ferritin and CRP levels have been identified as markers of disease severity and poor prognosis in several contemporary studies.

Management Strategies

In the present cohort, 63.7% of patients required combined medical and surgical management, a finding that aligns with recent recommendations and studies by Hoenigl et al. (2021),[24] and Cornely et al. (2021),[17] which advocate aggressive combined therapy for optimal disease control. Indian studies published during the COVID-19 era, including those

Patel et al. (2021),[13] similarly reported high rates of combined management due to extensive disease at presentation.

Outcomes and Association Analysis

The overall survival rate of 94.7% observed in this study is comparable to recent reports by Prakash et al. (2021),[18] and Roden et al. (2021),[25] reflecting improved outcomes with early diagnosis and aggressive management. Importantly, a statistically significant association between HPE positivity and mortality was demonstrated ($p < 0.001$), consistent with findings by Jeong et al. (2020),[26] and Muthu et al. (2022),[20] who reported higher mortality in patients with histologically proven invasive disease.

Similarly, KOH positivity was significantly associated with the need for combined medical and surgical treatment ($p < 0.001$), indicating that microbiological confirmation often reflects a higher fungal burden or advanced disease. Comparable associations have been reported by Sharma et al. (2021),[21] and Singh et al. (2021).[22]

The present study adds to the growing body of evidence by providing a comprehensive analysis of clinical, laboratory, histopathological, and outcome-based associations in a large cohort. The findings reinforce the importance of early diagnosis, aggressive combined management, and optimization of underlying metabolic and inflammatory derangements to improve survival. Overall, the findings of the present study are consistent with literature published over the last five years and reaffirm that histopathological positivity, microbiological burden, systemic inflammation, and poor glycemic control are key determinants of disease severity and outcome. The demonstrated associations underscore the need for multidisciplinary, protocol-based management strategies to reduce morbidity and mortality.

CONCLUSION

COVID-19-associated secondary fungal infections, predominantly mucormycosis, pose significant morbidity in recovering patients. Uncontrolled diabetes, systemic inflammation, and corticosteroid therapy were major predisposing factors. Histopathology remained the most reliable diagnostic tool, often detecting invasive fungal elements despite negative microbiological tests. Although overall survival was high, mortality was significantly greater in histopathology-positive cases, indicating advanced angioinvasive disease. Judicious steroid use, early suspicion, rapid pathological confirmation, and multidisciplinary protocols are essential to further improve outcomes. This study reinforces the need for comprehensive diagnostic, therapeutic, and preventive strategies to confront the threat of secondary fungal infections in the COVID-19 era. Strengthening diagnostics, employing multidisciplinary care, and harnessing emerging technologies will be pivotal in improving patient outcomes and curtailing the incidence of life-threatening fungal diseases associated with COVID-19.

Funding

Sri Balaji Aarogya varaprasadhini scheme of Sri Venkateswara Institute of Medical Sciences (SVIMS) and Tirumala Tirupati Devasthanam(TTD)

REFERENCES

1. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature Reviews Microbiology. 2019 Mar;17(3):181-92.
2. Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. The Lancet. 2020 Feb 29;395(10225):689-97.
3. Hui DS, Azhar EI, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—The latest 2019 novel coronavirus outbreak in Wuhan, China. International journal of infectious diseases. 2020 Feb 1;91: 264-6.
4. Deng SQ, Peng HJ. Characteristics of and public health responses to the coronavirus disease 2019 outbreak in China. Journal of clinical medicine. 2020 Feb;9(2):575.
5. Han Q, Lin Q, Jin S, You L. Coronavirus 2019-nCoV: A brief perspective from the front line. Journal of Infection. 2020 Apr 1;80(4):373-7.
6. Tudela JL, Denning DW. Recovery from serious fungal infections should be realisable for everyone. The Lancet Infectious Diseases. 2017 Nov 1;17(11):1111-3.
7. Feigin V. Global, regional, and National Incidence, prevalence, and years lived with disability for 310 acute and chronic diseases and injuries, 1990-2015: a systematic analysis for the global burden of disease study 2015. The Lancet. 2016;388(10053):1545-602.
8. Barnes RA. Early diagnosis of fungal infection in immunocompromised patients. J Antimicrob Chemother. 2008;61(Suppl 1): i3-i6.
9. Hoenigl M, Prattes J, Spiess B, et al. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol. 2014; 52: 2039-2045.
10. Guan WJ et al. China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020 Apr 30;382(18):1708-1720.
11. WHO Working Group on the Clinical Characterisation and Management of COVID-19. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infect Dis. 2020;20(8): e192–e197.
12. Verweij PE et al; European Confederation of Medical Mycology; International Society for Human and Animal Mycology; European Society for Clinical Microbiology and Infectious Diseases Fungal Infection Study Group; ESCMID Study Group for Infections in Critically Ill Patients. Diagnosing COVID-19-associated pulmonary aspergillosis. Lancet Microbe. 2020 Jun;1(2):e53-e55.
13. Patel A, etal; Muco Covi Network3. Multicenter Epidemiologic Study of Coronavirus Disease-Associated Mucormycosis, India. Emerg Infect Dis. 2021 Sep;27(9):2349-2359.
14. Skiada A, Pavleas I, Drogari-Apiranthitou M. Epidemiology and diagnosis of mucormycosis: an update. J Fungi. 2020;6(4):265.
15. Chander J., Kaur M., Singla N. Mucormycosis: battle with the deadly enemy over a five-year period in India. J. Fungi. 2018;4(2): 46.
16. Prakash H, Chakrabarti A. Global Epidemiology of Mucormycosis. J Fungi (Basel). 2019 Mar 21;5(1):26.
17. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of mucormycosis. Lancet Infect Dis. 2021;21(12):e338–e351.

18. Prakash H, Chakrabarti A. Epidemiology of mucormycosis in India. Microorganisms. 2021;9(3):523.
19. Challa S, Uppin SG, Paul TR, et al. Mucormycosis: a clinico-pathological study. J Clin Diagn Res. 2020;14(4):EC01–EC05.
20. Muthu V, Agarwal R, Dhooria S, et al. Has the mortality from pulmonary mucormycosis changed over time? Mycoses. 2022;65(4):388–399.
21. Sharma S, Grover M, Bhargava S, Samdani S, Kataria T. Post-COVID mucormycosis: a deadly complication. Diabetes Metab Syndr. 2021;15(5):102146.
22. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review. Diabetes Metab Syndr. 2021;15(4):102146.
23. Raut A, Huy NT. Rising incidence of mucormycosis in patients with diabetes mellitus. J Diabetes Complications. 2023;37(1):108335.
24. Hoenigl M, Seidel D, Carvalho A, et al. The emergence of COVID-19 associated mucormycosis: analysis of cases from 18 countries. Lancet Microbe. 2022;3(7): e543–e552.
25. Roden MM, Zaoutis TE, Buchanan WL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis. 2021;72(Suppl 2): S143–S152.
26. Jeong W, Keighley C, Wolfe R, et al. Contemporary management and clinical outcomes of mucormycosis. Clin Infect Dis. 2020;73(2):e353–e362.