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Original Research Article

# Histopathological spectrum of fungal infections in COVID 19 cases – A retrospective observational study

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ABSTRACT

**Background:** A wide range of fungal and bacterial infections are associated with Corona virus disease (COVID19). There was a surge of mucormycosis during COVID19 pandemic in 2021. This study elaborates the histopathological findings observed in fungal infections of head and neck in COVID 19 patients.
**Materials and Methods:** A retrospective observational study of histopathological findings of invasive fungal infection in COVID 19 case was conducted in our laboratory. All the tissue samples diagnosed as post COVID fungal infections during January 2021 to June 2021 were analyzed. Representative sections were taken and slides were stained with Hematoxylin & Eosin, Periodic Acidic Schiff (PAS) and Gomori Methenamine silver (GMS) stains. Histopathological features were studied.
**Results:** There were total 32 cases which had invasive fungal infections associated with COVID 19. The most affected age group was 31 to 50 years of age (50%), with male preponderance (87.5%). Diabetes was present in 62.5% cases, and steroid use during the treatment was present in 31.3% of the cases. The most common site was maxillary sinus (53.1%) followed by nasal cavity (34.4%). Isolated infection by mucor was seen in 68.8% cases, there were cases of secondary bacterial infection and candida with aspergillus infection. Angioinvasion was present in 56.3% cases and bone destruction in 15.6% cases.
**Conclusion:** COVID 19 infection results in immune dysregulation and background of diabetes and over use of steroids increases risk of mucormycosis. An early histopathological diagnosis provides early diagnosis.



## 1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or COVID19 created an unprecedented crisis 2020-2021 in India and other countries.[1] Mucormycosis was earlier considered a rare life threatening invasive fungal infection.[2] However, there is a rise in COVID19 associated Mucormycosis (CAM) due to immune dysregulation, comorbidities like diabetes, transplants and cancers.[3] In COVID19 cases there is damage in airway epithelium. The angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) expressing cells are targeted by the virus which causes expression of integrin.[4] These integrins mediate interactions with proteins on the surface of the Mucorales, Aspergillus, or Candida cell wall, namely the spore-coating (CotH) proteins, the thaumatin-like protein CalA, and mannoproteins, to promote the adhesion and invasion of fungi.[4]

The order Mucorales has predominantly Rhizopus species including R. delemar and R. oryzae. Other common causative organisms for mucormycosis are include species of Mucor, Lichtheimia (previously Absidia), Rhizomucor, Apophysomyces, and Cunninghamella. During the month of May 2021 to July 2021 India saw an unexpectedly

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large number of reports of mucormycosis; approximately 50,000 cases.[5] Western region of India showed the highest number of reported cases followed by the South India.[5]The significant higher numbers in India can be attributed to uncontrolled diabetes, non-judicious use of steroids and humid weather conditions.[3]As per previous reports, mucormycosis may develop in one fourth of the COVID19 patients, anywhere between 15 to 30 days after COVID19 infection and there was a lot media attention given to this entity due the color of the necrotic tissue as 'Black fungus'.[5,6]On histopathological examination there is mixed inflammation, fungal elements, necrosis, bone and angioinvasion.[5] The objective of this study is to understand the histopathological spectrum of these fungal infections.

## 2. Materials and Methods

This was a retrospective observational study conducted in single laboratory in western India. The diagnosed cases of CAM were included in the study. There were 32 tissue samples diagnosed as CAM during the period of January 2021 to June 2021. These samples were received in our laboratory from the different centres. Gross examination was done, representative sections were taken and slides were stained with Hematoxylin & Eosin (HE), Periodic Acidic Schiff (PAS) and Gomori Methenamine silver (GMS) stains. The slides were reviewed and clinical details were collected from Tissue Requisition Form (TRF) received in the laboratory. The TRF provided the demographic details like, patients age, gender, diabetic status, use of steroids during treatment and COVID 19 status. Samples received from non-COVID patients were excluded from the study. On gross examination the colour of the tissue, necrosis and bone destruction were noted. On microscopy following parameters were analysed such as fungal elements revealing presence of aseptate broad hyphae, septate narrow hyphae, pseudohyphae and yeast forms. Type of inflammation was assessed as acute, mixed, chronic inflammation and granulomatous inflammation with presence or absence of giant cells. Necrosis, bone invasion and angioinvasion were also noted. As the current study is retrospective histopathological analysis of tissue samples, the follow up details of the patients were not available.

## 3. Results

The tissue samples diagnosed as CAM during 6-month period were analysed. There were 32 cases, with a wide range of age, from 15 years to 74 years of age [Figure 1]. There were 28 males (87.5%) and 4 (12.5%) females [Figure 2]. Half of the patients were between 31 to 50 years of age.

There were 20 patients (62.5%) with history of diabetes mellitus and history steroid administration was present in 10 (31.5%) patients. The most site involved was

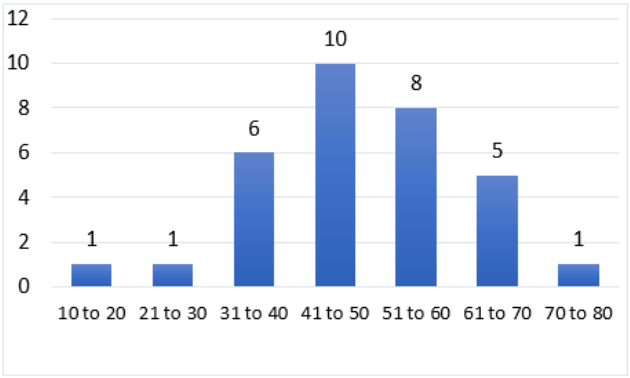


**Figure 1:** Graph showing age in years in x axis and y axis shows number of cases

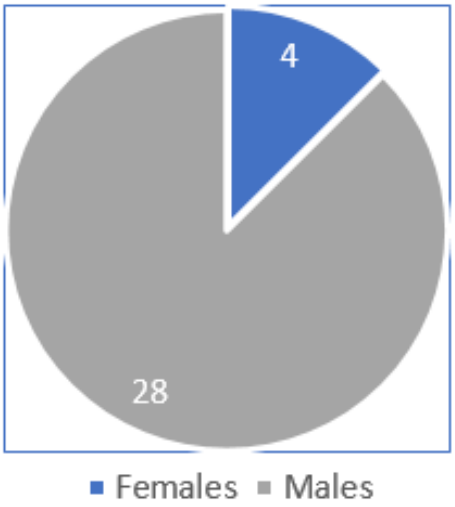


**Figure 2:** Pie diagram showing male preponderance

maxillary sinus 53.1% [Table 1]. The tissue was removed by debridement or excision depending upon the extent of invasion and the sample was sent for histopathology. On gross examination the affected site revealed black discolouration, congestion and necrosis in all the cases [Figure 3a]. On microscopic examination there was dense inflammation and necrosis in all the cases. Most cases (68.8%) showed isolated CAM [Table 2]. Mixed inflammation comprising lymphocytes, plasma cells and neutrophils with necrosis was the most common finding [Table 3]. Increased eosinophils were noted in 15.6% cases and granuloma with multinucleated giant cells were noted in 9.4% cases. The cases of mucormycosis showed broad aseptate hyphae in the necrotic debris along with inflammation [Figure 3b], these hyphae were highlighted and showed black colour with GMS stain [Figure 3c]. Bone necrosis and destruction was noted in 15.6% cases [Figure 3d]. Angioinvasion was seen in 56.3% cases, it was highlighted by GMS stain [Figure 4a,c,d]. One of the cases

showed narrow hyphae and pseudo-hyphae with yeast like structures [Figure 5]. This case had mixed fungal infection and fruiting bodies of aspergillus were noted occasionally [Figure 6]. Perineural invasion was not identified in any case in the current study. After the debridement patients were treated with systemic Amphotericin B for 2 – 8 weeks.

**Table 1:** Sites of involvement of mucormycosis.

| Sites | Number of cases | Percentage |
|---|---|---|
| Maxillary sinus | 17 | 53.1% |
| Maxillary sinus and oral cavity | 1 | 3.1% |
| Maxillary sinus and ocular | 1 | 3.1% |
| Nasal cavity | 11 | 34.4% |
| Palate | 2 | 6.2% |

**Table 2:** Inavsive fungi identified on histopathology

| Infectious agent | Number of cases | Percentage |
|---|---|---|
| Mucormycosis | 22 | 68.8% |
| Mucormycosis with bacterial infection | 9 | 28.1% |
| Mucormycosis with aspergillus and candida infection | 1 | 3.1% |

**Table 3:** Histopathological findings in cases of fungal infections associated with COVID19

| Pattern of inflammation | Case | Percentage |
|---|---|---|
| Acute inflammation | 1 | 3.1% |
| Chronic inflammation | 6 | 18.8% |
| Mixed Inflammation | 17 | 53.1% |
| Mixed Inflammation with increased eosinophils | 5 | 15.6% |
| Granuloma and giant cells | 3 | 9.4% |
| Tissue involvement by the disease | | |
| Soft tissue involvement | 27 | 84.4% |
| Bone invasion | 5 | 15.6% |
| Angioinvasion | 18 | 56.3% |
| Necrosis | 32 | 100% |

## 4. Discussion

Fungi of order Mucorales are ubiquitous in the nature and a healthy host with intact skin, mucosa and innate immunity usually does not develop invasive disease.[7] The conditions that interfere with or reduce immunity like uncontrolled diabetes mellitus, haematological and other malignancies, neutropenia, organ transplantation, neutropenia, and corticosteroid or immunosuppressive therapy, acquired immunodeficiency syndrome (AIDS), and COVID-19 predispose to mucomycosis.[8] Mucormycosis

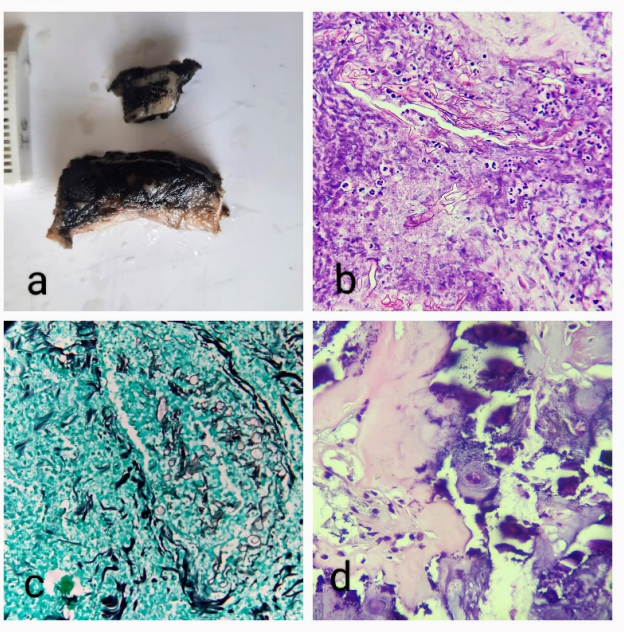


**Figure 3: a:** Gross tissue showing blackish discoloration, **b:** Necrosis, neutrophils and broad aseptate fungal hyphae (400x HE), **c:** GMS stain highlighting the fungal hyphae (100x GMS, **d:** Bone necrosis (100x HE).

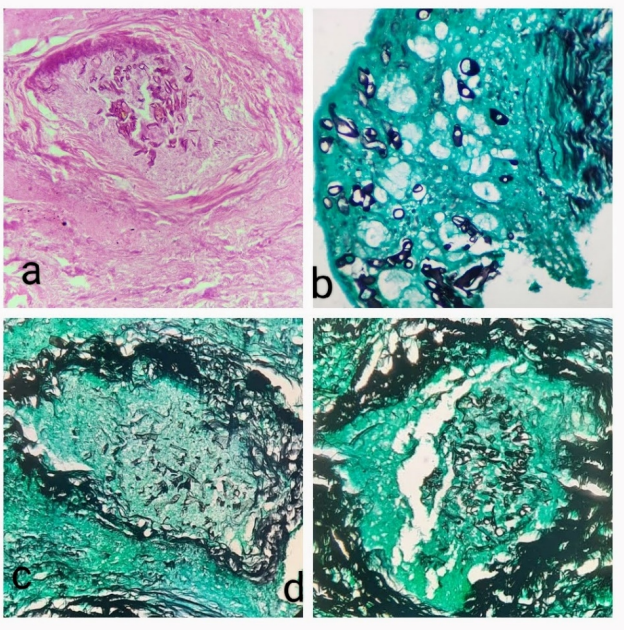


**Figure 4: a:** Angioinvasion by fungal hyphae (100x HE), **b:** GMS stain showing broad black coloured hyphae (100x GMS), **c** & **d:** Angioinvasion highlighted by GMS stain (100x GMS).

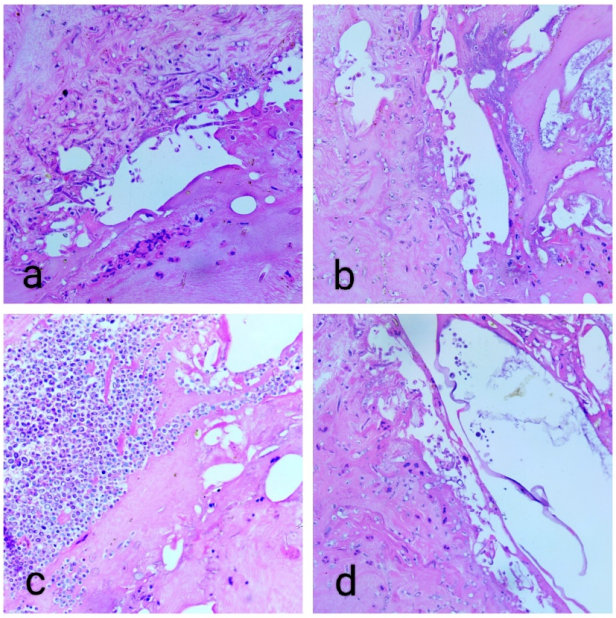


**Figure 5: a** & **b**: Thin septate fungal hyphae mixed with pseudohyphae (100x HE), **c**: Yeast forms of fungus, likely to be candida (400x HE), **d**: Fungal elements with necrosis (100x HE).

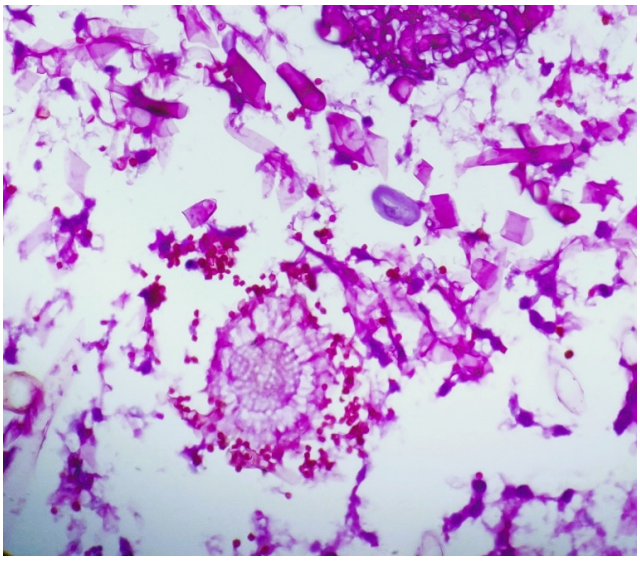

**Figure 6:** Broad fungal hyphae mixed with aspergillus fruiting body (400x PAS)

was first described in 1876 in Germany by Fürbinger in a cancer patient's lung; which revealed a haemorrhagic infarct with fungal hyphae and a few sporangia.[9] In 1885, the description of mucormycosis was first published by Paltauf; it is described the most lethal and rapidly progressing form of fungal infection, initiated by saprophytic fungi such as Rhizopus and Mucor.[10] The Mucorales species are thermotolerant, and have capacity of utilizing high carbohydrate sources and iron.[7] They have many virulence factors such as dormant spore formation, angio-invasive behaviour.[7] As per Ganesan et al India was one of the worst hit countries with COVID19 and towards the end of July 2021 there were about 31 million reported cases.[5] Critically ill COVID-19 patients who are admitted to the ICU are highly susceptible to secondary infections like CAM.[4]

On the basis of its anatomic location, mucormycosis can be classified as isolated nasal, rhino-orbital or rhino-orbital cerebral, pulmonary, gastrointestinal, cutaneous, disseminated and miscellaneous.[8] In a study by Shree Laxmi et al the age of patients ranged from 21 to 80 years with a male preponderance; similar demographic details were noted in the present study.[8] The maxillary sinus was the most common affected site. The fungus gains entry into through nose and sinuses directly or blood vessels as it can penetrate vascular system.[8] Thereby causing thrombosis, ischemia and necrosis; rapid spread to adjacent tissue.[10] Soft tissue necrosis was noted all cases in current study.

The following risk factors are considered in the pathogenesis of CAM. Uncontrolled diabetes with hyperglycaemia, COVID19 causes destruction of cells islet cells of pancreas with ACE2 receptors. Other causes of hyperglycaemia could be corticosteroids and cytokine storm.[11,12] COVID 19 can worsen acidosis due to direct renal tropism, renal infection and diabetic ketoacidosis, thrombosis and ischemia etc.[2] There is also an increase in free iron due to cytokine storm, glycosylation of ferritin and transferrin induced by hyperglycaemia. Increased reactive oxygen species are attributable to increase ferritin and hepcidin.[13] Acidosis, hyperglycemia and free iron increase the expression of GRP78 (glucose-regulated protein-78) and promotes its interaction with CotH of the hyphae of mucor, thereby leading to invasive fungal infection.[12]

Diabetes-related neutrophilic dysfunction and neutropenia are considered as predisposing factors for mucormycosis as neutrophils are very important in forming NETs (neutrophil extracellular trap) to eliminate fungal hyphae.[7] These NETS were noted in all of our cases; with presence of nuclear debris or chromatin material creating extracellular network surrounding necrosis and fungal elements. In a study by Ganesan et al[5] and Sahu et al[1] diabetes was present in 88% of the cases; in the present study it was 62.5%. Corticosteroid administration was present in 31.5% of patients in the current study however it was 63% in a study by Chaganti et al.[14] In a review by

Hoenigl et al the systemic corticosteroids were given in 79% of the cases and among these cases 81% patients received systemic corticosteroids before the confirmation of diagnosis of mucormycosis.[15] As per Singh et al diabetes was present in 80% case and steroid use was present in 76.3% cases.[16]

On histopathology higher fungal load correlates with rapid progression of the disease and immunocompromised state.[8] Most of these fungal hyphae are seen in necrotic areas. In the study by Sree Lakshmi et al[8] invasion of blood vessel by fungal hyphae was seen in 24% cases and carries a worse prognosis, similar finding was noted in study by Goel et al.[17] Angioinvasion was also described in mucor by Balushi et al.[18] Baldin et al suggested that GRP78 and PDGF (Platelet derived growth factor) receptor act independently or act as coreceptors in facilitating the invasion of endothelial cells by Mucorales.[12] Angioinvasion can be highlighted by GMS stain. GMS stain detects the carbohydrate (glucan) component of the cells wall of the fungi, which appears dark and black coloured.[19] PAS stain also detects the carbohydrate component of the fungal cell wall which is rich in β 1-3, β 1-4 D-glucan.[19] The carbohydrate component is oxidized into aldehyde by periodic acid, which is detected by the addition of Schiff reagent, where the fungal elements appear pink or magenta.[19] In the present study all cases showed GMS and PAS positive fungal elements.

In the present study there was a case of mixed fungal infection showing broad hyphae of mucor and fruiting bodies of aspergillus with yeast forms of candida. Such co-infections are rare; Paul et al have reported a series of 12 cases of mucormycosis and aspergillus infection.[20]

In the present study predominantly, the histopathological spectrum was analysed, however there are other tests which can used for diagnosis of mucormycosis. Microbiological tests such as KOH (Potassium Hydroxide) wet mount reveals fungal hyphae, Calcofluor white (CFW) stain (a non-specific fluorochrome dye), culture using Sabouraud's Dextrose agar (SDA) is most common. The causative fungal pathogen is also identified by understanding colony characteristics, morphological features on lactophenol cotton blue mount (LPCB).[19]

## 5. Conclusions

The etiopathogenesis of CAM is complex and multi-factorial. Higher prevalence of uncontrolled and undetected diabetes were the most common comorbidities in during the pandemic. The poor resource setting, poor hygiene and rampant use of corticosteroid were also important risk factors and COVID-19 itself causes cytokine storm, lymphopenia, and endothelial damage and all these factors promote invasive fungal infection. The current retrospective analysis shows that efforts should be taken to maintain optimal glycaemic state and only judicious evidence-based use of corticosteroids in patients with COVID19 is recommended in order to reduce the morbidity of fungal infections in COVID19.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

## References

1. Sahu M, Shah M, Mallela VR, Kola VR, Boorugu HK, Punjani AAR, et al. COVID-19 associated multisystemic mucormycosis from India: a multicentric retrospective study on clinical profile, predisposing factors, cumulative mortality and factors affecting outcome. *Infection*. 2023;51(2):407–16.
2. Radotra B, Challa S. Pathogenesis and Pathology of COVID-Associated Mucormycosis: What Is New and Why. *Curr Fungal Infect Rep*. 2022;16(4):206–20. doi:10.1007/s12281-022-00443-z.
3. Zirpe K, Pote P, Deshmukh A. 2021) A Retrospective Analysis of Risk Factors of COVID-19 Associated Mucormycosis and Mortality Predictors: A Single-Center Study. *Cureus*. 2021;13(10):e18718. doi:10.7759/cureus.18718.
4. Negm EM, Mohamed MS, Rabie RA, Fouad WS, Beniamen A, Mosallem A, et al. Fungal infection profile in critically ill COVID-19 patients: a prospective study at a large teaching hospital in a middle-income country. *BMC Infect Dis*. 2023;23(1):246. doi:10.1186/s12879-023-08226-8.
5. Ganesan N, Sivanandam S. Histomorphological features of mucormycosis with rise and fall of COVID-19 pandemic. *Pathol Res Pract*. 2022;236:153981. doi:10.1016/j.prp.2022.153981.
6. Baldin C, Ibrahim AS. Molecular mechanisms of mucormycosis-The bitter and the sweet. *PLoS Pathog*. 2017;13(8):1006408. doi:10.1371/journal.ppat.1006408.
7. Mani S, Thirunavukkarasu A. A clinico-pathological study of COVID-19 associated rhino-orbital-cerebral mucormycosis. *Indian J Ophthalmol*. 2022;70(3):1013–8.
8. Lakshmi IS, Kumari BS, Jyothi C, Devojee M, Malini P, Sunethri K, et al. Histopathological Study of Mucormycosis in Post COVID-19 Patients and Factors Affecting it in a Tertiary Care Hospital. *Int J Surg Pathol*. 2023;31(1):56–63.
9. Skiada A, Pavleas I, Drogari-Apiranthitou M. Epidemiology and Diagnosis of Mucormycosis: An Update. *J Fungi (Basel)*. 2020;6(4):265. doi:10.3390/jof6040265.
10. Ponnaiyan D, Anitha CM, Prakash PSG, Subramanian S, Rughwani RR, Kumar G, et al. Mucormycosis diagnosis revisited: Current and emerging diagnostic methodologies for the invasive fungal infection (Review). *Exp Ther Med*. 2022;25(1):47. doi:10.3892/etm.2022.11746.
11. Radotra B, Challa S. Pathogenesis and Pathology of COVID-Associated Mucormycosis: What Is New and Why. *Curr Fungal Infect Rep*. 2022;16:206–20. doi:10.1007/s12281-022-00443-z.
12. Baldin C, Ibrahim AS. Molecular mechanisms of mucormycosis-The bitter and the sweet. *PLoS Pathog*. 2017;13(8):1006408. doi:10.1371/journal.ppat.1006408.
13. Tabassum T, Araf Y, Moin AT, Rahaman TI, Hosen MJ. COVID-19-associated-mucormycosis: possible role of free iron uptake and immunosuppression. *Mol Biol Rep*. 2022;49(1):747–54. doi:10.1007/s11033-021-06862-.
14. Chaganti PD, Katta R, Yerukalapudi SH. Mucormycosis in Post Novel Corona Virus Patients: An Institutional Experience. *Indian J Otolaryngol Head Neck Surg*. 2023;75:617–23. doi:10.1007/s12070-022-03305-9.

15. Hoenigl M, Seidel D, Carvalho A, Rudramurthy SM, Arastehfar A, Gangneux JP, et al. The emergence of COVID-19 associated mucormycosis: a review of cases from 18 countries. *Lancet Microbe*. 2022;3(7):543–52.
16. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: A systematic review of cases reported worldwide and in India. *Diabetes Metab Syndr*. 2021;15(4):102146. doi:10.1016/j.dsx.2021.05.019.
17. Goel A, Kini U, Shetty S. Role of histopathology as an aid to prognosis in rhino-orbito-cerebral zygomycosis. *Indian J Pathol Microbiol*. 2010;53(2):253–7.
18. Balushi AA, Ajmi AA, Sinani QA, Menon V, Berieki ZA, Shezawi AA, et al. COVID-19-Associated Mucormycosis: An Opportunistic Fungal Infection. A Case Series and Review. *Int J Infect Dis*. 2022;121:203–10. doi:10.1016/j.ijid.2022.05.005.
19. Gupta MK, Kumar N, Dhameja N, Sharma A, Tilak R. Laboratory diagnosis of mucormycosis: Present perspective. *J Family Med Prim Care*. 2022;11(5):1664–71.
20. Paul M, Sasidharan J, Taneja J, Chatterjee K, Abbas SZ, Chowdhury V, et al. Invasive Mucormycosis and Aspergillosis Coinfection Associated with Post-COVID-19 Pneumonia in a Tertiary Care Hospital. *Med Mycol J*. 2022;63(3):59–64.

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