

ORIGINAL ARTICLE

Post COVID Mucormycosis: A Cross Sectional Study at a Designated COVID Hospital in Central India

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Abstract:

Background: The second wave of Covid19 beginning in March 2021 proved to be more devastating with more infections and mortality. With this wave another crisis of Post Covid mucormycosis caused by mold fungi emerged as a matter of great concern. **Aims:** 1. To carry out preliminary observational study of post COVID Mucormycosis with regards to clinical, radiological findings. 2. To know association of post covid mucormycosis with risk factors/comorbidities. 3. To study histopathology findings in post covid mucormycosis cases. 4. To report association of other fungal infections with mucormycosis (mixed infections) on histopathology. **Material and Methods:** This is retrospective cross sectional study of post Covid Mucormycosis conducted in a tertiary care hospital from May 2021 to August 2021 including 132 cases. Analysis of data pertaining to Covid status, demographics, clinical features, co-morbidities, laboratory investigations, surgical procedure and histopathology was done in clinically suspected patients of Covid 19. **Results:** All cases had antecedent risk factors like diabetes mellitus (32), hypertension (12) and systemic corticosteroids administration (65) with some overlap. In 95 cases, infection was limited to paranasal sinuses. Extension beyond paranasal sinuses was seen in 14 cases. Surgical debridement was the mainstay of treatment and was done in 109 cases. Histopathological examination revealed necro-inflammatory reaction in 86 cases, giant cell reaction in 21 cases, angioinvasion in 4 cases, neuroinvasion in 1 case and mixed infection with other fungi in 6 cases. **Conclusion:** High index of suspicion is very essential for the diagnosis of mucormycosis in post-COVID-19 patients especially patients with high risk factors to reduce the morbidity and mortality. Histopathologic examination can confirm the diagnosis and can help to prognosticate the condition.

Keywords: Covid 19, Mucormycosis, Mold Fungii, Histopathology, Clinical, Necro-inflammatory reaction.

Introduction:

The year 2020 brought a pandemic of unprecedented proportions that startled the entire world. A novel coronavirus infection designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible coronavirus and was responsible for this pandemic. Coronavirus 2 which emerged in 2019 in the wet markets city of Wuhan, China spread rapidly across continents, and affected India and caused a pandemic of acute respiratory disease, named coronavirus disease 2019 (COVID-19). The first SARS-CoV-2 positive case in India was reported in Kerala on January 30th, 2020. Subsequently, the number of Covid cases drastically rose peaking in mid-September with over 90,000 cases reported per-day, dropping to below 15,000 in January 2021.⁽¹⁾ The second wave of Covid 19 beginning in March 2021 proved to be more devastating with overall 34 million infections and around 4 lakh deaths globally since its inception. As India was about to achieve stability over the second wave, another crisis of post Covid mucormycosis emerged as a matter of great concern. Mucormycosis is caused by mold fungi of the genus *Rhizopus*, *Mucor*, *Rhizomucor*, *Cunninghamella* and *Absidia* of order- *Mucorales*, Class- *Zygomycetes*.⁽²⁾ 60% of human mucormycosis is caused by *Rhizopus Oryzae* which also accounts for 90% of the Rhino-orbital-cerebral form.⁽³⁾ Incidence of this black fungus rose rapidly during the second wave compared to the first wave in India, with at least 14,512 infections till May 2021.⁽⁴⁾ In India the prevalence of mucormycosis was 80 times higher than rest of the world.⁽⁵⁾ The Global prevalence ranged from 0.005 to 1.7 per million populations as against 0.14 per 1000 in India.⁽⁶⁾ During the second wave of the COVID-19 pandemic there was a huge surge in the number of cases of mucormycosis. India reported 41,512 cases and 3,554 deaths due to this life-threatening fungal infection from May to July 2021, leading India to declare mucormycosis an epidemic on May 10, 2021, with Maharashtra alone reporting 90

deaths due to mucormycosis.⁽⁴⁾ Various factors may have contributed to the increased incidence of mucormycosis in Covid 19 affected patients. The widespread use of steroids which was found to be effective in improving the survival in COVID -19 patients was a major contributing factor in the rise of Mucormycosis and pulmonary aspergillosis.⁽⁷⁾ Use of immunomodulatory drugs such as Tocilizumab adds to the risk of infection.⁽⁷⁾ Other factors may be low oxygen levels, pre-existing Diabetes mellitus (steroid-induced or recent onset), and an acidic medium due to metabolic or diabetic ketoacidosis, decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated or steroid-mediated). Prolonged hospitalization and mechanical ventilation may have also promoted the spread within the hospital environment.⁽⁵⁾

Aims & Objectives:

This study of post Covid mucormycosis was carried out with the following objectives 1) To carry out preliminary observational study of post COVID Mucormycosis with regards to clinical, radiological findings. 2) To know association of post covid mucormycosis with risk factors/comorbidities. 3) To study histopathology findings in post covid mucormycosis cases. 4) To report association of other fungal infections with mucormycosis (mixed infections) on histopathology.

Material & Methods:

This is a retrospective cross-sectional study of post Covid Mucormycosis conducted in a tertiary care hospital central India over a period of 4 months extending from May 2021 to August 2021. In this period majority of cases of post covid Mucormycosis were recorded and one hundred thirty two cases were included in our study. All histopathology samples received from ENT & Ophthalmology departments with suspected diagnosis of Covid associated mucormycosis were included in the study. Poorly fixed/unfixed specimens, inadequate biopsies were excluded. Analysis of data pertaining to Covid status, demographics, clinical features, co-morbidities, laboratory investigations, surgical procedure and histopathology was done in clinically suspected patients of Covid 19. Diagnosis of Covid 19 was established by real-time polymerase chain reaction (RT-PCR) test from nasopharyngeal or oropharyngeal samples.

Radiological investigations like computed tomography (CT) and/or magnetic resonance imaging (MRI) of the orbit, brain and/or paranasal sinuses were performed to determine the extent of involvement from mucormycosis. The types of samples received included endoscopic debridement from nasal mucosa, sinuses, maxillectomy specimens, orbital exenteration to Functional Endoscopic Sinus Surgery (FESS). Gross examination of samples mostly revealed extensively necrotic tissue. All samples were processed for histopathology and slides were stained with Haematoxylin and eosin (H&E). On microscopic examination broad pauciseptate ribbon like hyphae showing irregular branching are seen on H & E only. Special stain Periodic acid Schiff's (PAS) and GMS were done wherever necessary. Additional tissue reaction seen on microscopic examination included 1. Necroinflammatory reaction, 2. Giant cell reaction, 3. Granulation tissue, 4. Vascular invasion and neural invasion, 5. Mixed infection with overlapping features in some cases. All cases which demonstrated mucor hyphae on histopathology were included in the study.

Result:

During the study period we received a total of 132 histopathological specimens with a diagnosis of suspected mucormycosis. One hundred- nine cases of mucormycosis were confirmed on histopathology while remaining 23 did not reveal any fungus on microscopy. We included these 109 cases in our study which demonstrated mucor hyphae on histopathology. About 98 of these patients were hospitalized during their Covid illness with moderate to severe disease. All these 98 patients needed oxygen support during their Covid illness. Males constituted a majority with 64% of the cases whereas 36% were females (figure 1), and the mean age was 43 years.

Table no. 1: Antecedent risk factor

Sr. No.	Antecedent risk factor	No. of cases
1	Diabetes mellitus	32
2	Hypertension	12
3	Systemic corticosteroids administration	65

Risk factors like diabetes mellitus, hypertension and systemic corticosteroids administration were present in all 109 cases (Table 1). There was overlap of risk

factors like some patients had all the three risk factors and some had two. All patients presented with headache, fever, and pain, facial swelling (all 109 patients). Additional symptoms included cough, rhinorrhoea, myalgia and breathlessness upon exertion or at rest (45 patients). Fifteen patients presented with visual complaints like diminution of vision, blurring, watering of eyes.

Figure 1. Orbital exenteration done for Mucormycosis



Table 2: Extent of involvement based on CT PNS or MRI findings

Extent of involvement	No of cases
Sinus involvement	109
Orbit	11
CNS	3

Based on radiological evaluation (CT and MRI) involvement of paranasal sinuses was the most common feature. (Table 1) Extension of infection beyond paranasal sinuses was seen in 14 cases (11 orbital involvements and 3 CNS involvements). (Table 2)

Table 3: Sinus involvement based on CT PNS or MRI findings

Pan-sinusitis	86
Maxillary	100
Frontal	56
Ethmoid	78
Sphenoid	73

Table 3 shows that among all sinuses, maxillary sinus involvement was most common. Here also there was overlap, some patients had pan sinusitis, some had only 2 or 3 sinuses involvements. The various surgical procedures carried out for debridement and removal of necrotic tissue are given in Table 4. Table 5 shows the key histopathological findings necro-inflammatory tissue reaction was seen in most cases (86). invasion in 1 case. Mixed infection with other fungi was seen in 5 cases and actinomycosis was seen in 1 case.

seen in 6 cases. Candida coinfection was seen in 5 cases and actinomycosis was seen in 1 case.

Table 4: Surgical intervention

Surgical intervention	No of cases
Debridement	83
Maxillectomy (Partial or complete)	10
FESS	5
Debridement and maxillectomy	8
Debridement and orbital exenteration	3

Table 5: Histopathology findings

Histopathology findings	No of cases
Necro-inflammatory reaction	86
Giant cell reaction	21
Angioinvasion	4
Neuroinvasion	1
Mixed infection with other fungi	6
Intraorbital spread	1

Table 5 shows the key histopathological findings necro-inflammatory tissue reaction was seen in most cases (86). Angioinvasion was seen in 4 cases and per neural invasion in 1 case. Mixed infection with other fungi was seen in 6 cases. Candida coinfection was seen in 5 cases and actinomycosis was seen in 1 case.

Figure 2. Broad ribbon shaped Mucor fungal hyphae (H&E stain x10)

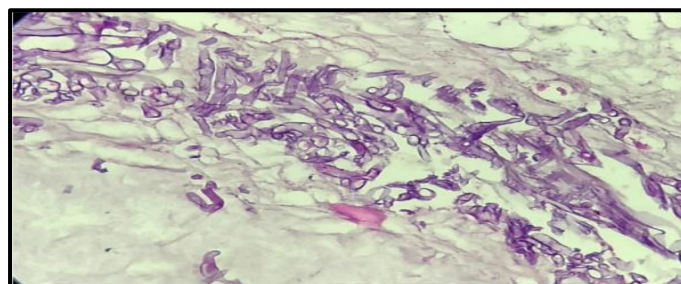


Figure 3. Broad ribbon shaped Mucor fungal hyphae (PAS stain x40)

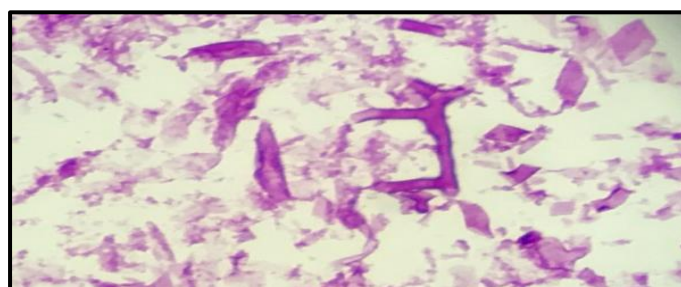
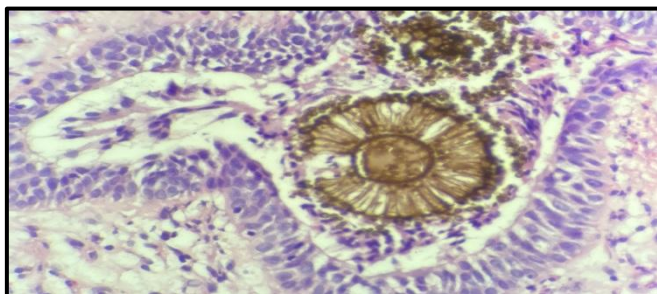
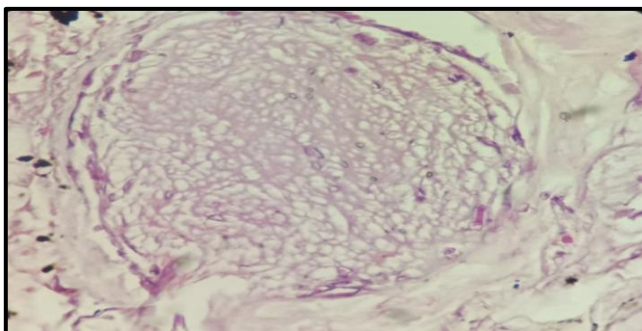
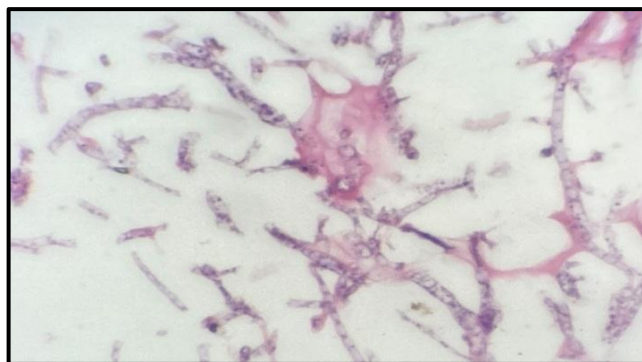
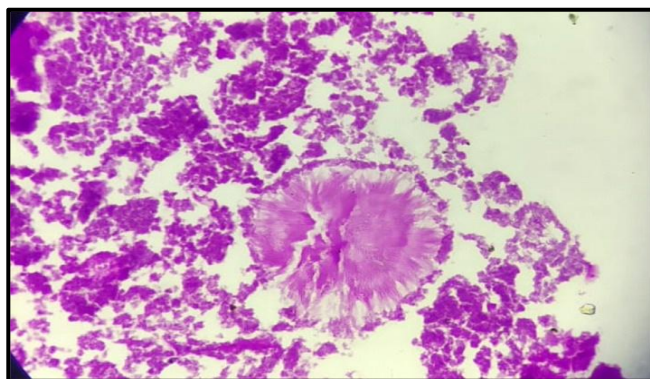


Figure 4. Conidia of mucormycosis (H& E stainx10)**Figure 5. Perineural invasion by Mucor fungi (H&E stain X40)****Figure 6. Mixed inflammation mucormycosis and aspergillosis (H& E stainx40)****Figure 7. Mixed inflammation mucormycosis and actinomycosis (H&E stainx40)****Discussion :**

The second wave of COVID-19 which swept across India from middle of March 2021 to June 2021 proved to be the most severe with high morbidity and mortality. Across India many patients required hospitalization with or without ventilator support and left many patients with residual problems like breathlessness, cough, weakness, easy fatigue ability, joint pain, insomnia, anosmia etc. One particularly distressing fallout of COVID-19 pandemic was the sudden spurt in cases of mucormycosis. The cases began rising from late April peaked around May and showed a sharp decline in the month of December. Mucormycosis is a potentially fatal fungal infection, caused by ubiquitous, environmental moulds and gains entry via inhalation to enter the paranasal sinuses. If it remains untreated contiguous spread to adjacent structures occur.⁽⁸⁾ Different immunodeficiencies predispose invasion by the inhaled spores. Mucor fungi invade blood vessels leading to thrombosis, ischaemic infarction and necrosis of tissues leading to fatal fungal infection.⁽⁹⁾ The previously infected respiratory tract cells with SARS- COVID-2 respiratory virus are an easy target for Mucor. The long term use of steroids is known to cause opportunistic fungal infection like aspergillosis & mucormycosis. In diabetics, or immunocompromised patients even a short course of steroids is reported to cause mucormycosis.⁽¹⁰⁾ This was a definite contributing factor in COVID 19 pandemic where steroids were used to treat moderate to severe infections particularly in the second wave due to delta variant which was a cause of greater morbidity and mortality than the first wave. Our study reported a spike in mucormycosis cases during month of May and sharp decline with July reporting only 11 cases and no cases in the month of September. (figure 2) The peak of Mucormycosis cases post COVID was reported in much higher numbers in India.⁽¹¹⁾ The rise of mucormycosis significantly contributes to the morbidity and mortality particularly those cases showing intracranial extension, where mortality can be as high as 85%.⁽¹²⁾ Orbital involvement occurs through the ethmoid bone via the lamina papyracea, infratemporal fossa, inferior orbital fissure or orbital apex. Intracranial extension occurs through the ethmoid cribriform plate leading to cavernous sinus or sagittal sinus thrombosis, carotid occlusion, cerebral infarction. Mucormycosis can be sino-orbital⁽¹³⁾, rhino-cerebral⁽¹⁴⁾, pulmonary⁽⁷⁾, cutaneous, gastrointestinal⁽¹⁵⁾ and disseminated^(16, 17) depending on the affected organ. In our study, depending on the radiological findings most cases were limited to nose and paranasal sinuses (95%) with a minority showing orbital & intracranial involvement. This is in concordance with the study of Selarka et al⁽¹⁸⁾ who also found features

of pan-sinusitis in 95.7% cases. They found extension beyond paranasal sinuses in 78.7% cases which is higher than our study. Symptoms of mucormycosis include facial swelling, pain, headache, fever, blindness and black necrotic lesions. Proptosis of eye, ophthalmoplegia and cranial nerve palsies can be seen in intracranial extension. The Mucorales have a capability of angio-invasion leading to vasculitis and thrombosis of vessels, causing large areas of infarction and necrosis.^(19,20,21) In our study we got histopathological evidence of characteristic broad, aseptate, ribbon-patterned, thin-walled, and irregular 90° branching fungal hyphae in H&E staining in all cases which were highlighted by PAS stain. The underlying necroinflammatory tissue reaction was seen in 86 (78.8%) cases and giant cell reaction in 21 (19.26%) cases. Rarely granulomatous response can be seen which is due to immune dysregulation caused by steroid therapy. Jain et al.⁽²²⁾ also found similar results with inflammatory reaction in all cases and giant cell reaction in 33.3% cases. Histopathological confirmation is very important as it distinguishes the fungus as a pathogen in the specimen from a contaminant presence in culture. Patients with immunocompromised status, with uncontrolled diabetes, and who are treated for long periods with high doses of steroids have increased risk of post covid mucormycosis.⁽²³⁾ Diabetes may cause quantitative and functional alterations in cell-mediated immunity reduces natural killer (NK) cell activity.⁽²⁴⁾ Moreover diabetes reduces natural killer cell activity, attenuate IFN- γ response and extends hyper inflammatory state. Patient having uncontrolled diabetes with keto acidosis are at a higher risk because of further dysregulation of immune system affecting phagocytosis and intracellular killing of organism. Corticosteroids treatment enhances these effects by inducing corticosteroid induced hyperglycaemias. Corticosteroids also lead to impairment in the neutrophil migration, ingestion, and phagolysosome fusion. So diabetic covid patient receiving corticosteroids are extremely vulnerable to the development of mucormycosis.⁽¹¹⁾ In our study more

than 90% patients had antecedent history of diabetes mellitus along with a poor glycaemic control at presentation. This finding is in concordance with other studies stating diabetes as an independent risk factor for mucormycosis^(11,25,26). To the various predisposing conditions in mucormycosis, the COVID-19 infection is a new addition. The Mucorales have a capability of angio-invasion leading to vasculitis and thrombosis of vessels, causing large areas of infarction and necrosis.^(19,20,21) In our study we got histopathological evidence of angioinvasion in 4 cases. Mucormycosis is a rapidly fatal disease and mandates aggressive interventions, like antifungals and debridement of dead tissue (which is disfiguring and invasive).⁽²⁷⁾ Surgical debridement sometimes becomes necessary because of poor drug penetration in devitalized tissue. Most of our subjects were presented with classical symptoms of sinusitis pain and headache. In 14 cases, there was extension beyond paranasal sinuses. These patients developed additional symptoms of diplopia, diminution of vision. So it is very crucial to keep a close watch on patient symptoms.^(28, 29) Radiological investigation like CT, MRI gives clue to extent and progression of disease.⁽³⁰⁾ Final diagnosis depends on demonstration of fungal hyphae by KOH mount, culture or by demonstration of broad aseptate branching filaments. On histopathology, special stains for fungus can be done to demonstrate fungal hyphae. Post Covid mucormycosis is managed by surgical debridement of affected tissue and Amphotericin B. However, good glycaemic control is necessary.

Conclusion:

High index of suspicion is very essential for the diagnosis of mucormycosis. In post-COVID-19 patients especially patients with high risk factors. Early diagnosis and management can help to reduce the mortality and morbidity associated with it. Histopathologic examination can confirm the diagnosis and can help to prognosticate the condition based on presence of tissue necrosis, fungal load and angioinvasion.

References

1. Safi Michael. "India's shocking surge in Covid cases follows baffling decline" [Jan 11, 2022]; *The Guardian*. 2021 Apr 21; Available from: <https://www.theguardian.com/world/2021/apr/21/india-shocking-surge-in-covid-cases-follows-baffling-decline>. [Google Scholar]
2. Eucker J, Sezer O, Graf B, Possinger K. Mucormycoses. *Mycoses* 2001;44(7):253e60.
3. Sugar AM, Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. fifth ed. New York, USA: Churchill Livingstone; 2000.
4. Raut A, Huy N. (2021). Rising incidence of mucormycosis in patients with COVID-19: another challenge for India amidst the second

- wave?. *The Lancet Respiratory Medicine* 2021; 9(8): E-77.
5. Chander J, Kaur M, Singla N, et al. Mucormycosis: battle with the deadly enemy over a five-year period in India. *Journal Fungi* 2018;4:46.
 6. Jeong W, Keighley C, Wolfe R, et al. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. *Clinical Microbiology and Infection* 2019;25:26-34.
 7. Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A, et al. Coronavirus Disease (Covid-19) Associated Mucormycosis (CAM): Case Report and Systematic Review of Literature. *Mycopathologia* 2021;186:289-298.
 8. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. *Clinical Microbiology Reviews* 2005;18(3):556-569.
 9. Afroze SN, Korlepara R, Rao GV, Madala J. Mucormycosis in a Diabetic Patient: A Case Report with an Insight into Its Pathophysiology. *Contemporary Clinical Dentistry* 2017;8:662-666.
 10. Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. *Lancet* 2003; 362:1828–1838.
 11. 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* 2021;15(4):102146.
 12. Palejwala SK, Zangeneh TT, Goldstein SA, Lemole GM. An aggressive multidisciplinary approach reduces mortality in rhinocerebral mucormycosis. *Surgical Neurology International* 2016;7:61.
 13. Mekonnen ZK, Ashraf DC, Jankowski T, et al. Acute invasive rhino-orbital mucormycosis in a patient with COVID-19-Associated acute respiratory distress syndrome. *Ophthalmic Plastic and Reconstructive Surgery* 2021;37(2):e40-e80.
 14. Revannavar SM, Supriya PS, Samaga L, V k V. COVID-19 triggering mucormycosis in a susceptible patient: a new phenomenon in the developing world? *BMJ Case Reports* 2021;14(4):e241663.
 15. Monte Junior ESD, Santos MELD, Ribeiro IB, et al. Rare and fatal gastrointestinal mucormycosis (zygomycosis) in a COVID-19 patient: a case report. *Clinical Endoscopy* 2020;53(6):746-749.
 16. De Pauw B, Walsh T, Donnelly J, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clinical Infectious Diseases* 2008;46(12):1813-1821.
 17. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of ucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *Lancet Infectious Diseases* 2019;19(12):e405-e421.
 18. Selarka L, Sharma S, Saini D, Sharma S, Batra A, Waghmare VT, et al. Mucormycosis and COVID-19: An epidemic within a pandemic in India. *Mycoses* 2021 ;64:1253-60.
 19. Farmakiotis D, Kontoyiannis DP. Mucormycoses. *Infectious Disease Clinics of North America* 2016;30:143e63.
 20. Ibrahim AS, Spellberg B, Walsh TJ, Kontoyiannis DP. Pathogenesis of mucormycosis. *Clinical Infectious Diseases* 2012;54:S16e22.
 21. Rammaert B, Lanternier F, Poiree S, Kania R, Lortholary O. Diabetes and mucormycosis: a complex interplay. *Diabetes Metabolism* 2012;38:193-204.
 22. Jain K, Surana A, Choudhary TS, Vaidya S, Nandedkar S, Purohit M. Clinical and histology features as predictor of severity of mucormycosis in post-COVID-19 patients: An experience from a rural tertiary setting in Central India. *SAGE Open Medicine* 2022;10:20503121221074785.
 23. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clinical Microbiology Reviews* 2000;13(2):236.
 24. Erener S. Diabetes, infection risk and COVID-19. *Mol Metabolism* 2020;39:101044.

25. Werthman-Ehrenreich A. Mucormycosis with orbital compartmentsyndrome in a patient with COVID-19. *American Journal of Emergency Medicine* 2021;42:e265-e264.e268.
26. Moorthy A, Gaikwad R, Krishna S, et al. SARS-CoV- 2,uncontrolled diabetes and corticosteroids-an unholy trinity in invasive fungal infections of the maxillofacial region? a retrospective, multi-centric analysis. *Journal of Maxillofacial Oral Surgery* 2021;20(3): 418-425.
27. Binder U, Maurer E, Lass-Flörl C. Mucormycosis-from the pathogens to the disease. *Clinical Microbiology and Infection* 2014;20:60-66.
28. Sen M, Lahane S, Lahane TP, Parekh R, Honavar SG. Mucor in a viral land: a tale of two pathogens. *Indian Journal of Ophthalmology* 2021;69:244-252.
29. Badrah M, Riad A, Kassem I, Boccuzzi M, Klugar M. Craniofacial pain in COVID-19 patients with diabetes mellitus: clinical and laboratory description of 21 cases. *Journal of Medical Virology* 2021;93:2616-2619.
30. Papadogeorgakis N, Parara E, Petsinis V, Vourlakou C. A case of successfully treated rhino cerebral mucormycosis: dental implications. *International Journal of Dentistry* 2010;2010:273127.

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