ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(10): 6815-6819 (2026)
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BIOINFORMATION
Discovery at the interface of physical and biological sciences
BIOMEDICAL INFORMATICS
www.bioinformation.net
Volume 22(10)
Research Article

Received October 1, 2026; Revised October 31, 2026; Accepted October 31, 2026, Published October 31, 2026
DOI: 10.6026/973206300226815

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478
2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

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Edited by Vini Mehta
E-mail: vmehta@statsense.in
Citation: Saban *et al*. Bioinformation 22(10): 6815-6819 (2026)

Microbiological profile of rhino-orbito-cerebral mucormycosis in patients with COVID-19: A clinical laboratory-based study

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ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(10): 6815-6819 (2026)
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Abstract
Mucormycosis is a life-threatening opportunistic fungal infection commonly caused by *Rhizopus*, *Mucor* and *Lichtheimia* species. This prospective cross-sectional study evaluated 133 clinical specimens, including tissue samples and scrapings, from patients clinically suspected of mucormycosis. COVID-19 infection was confirmed in all patients using reverse transcription polymerase chain reaction (RT-PCR). Fungal growth was detected in 93 specimens (69.9%), including 87 cases following recovery from COVID-19 and six during active COVID-19 treatment. Thus, early microbiological diagnosis, timely antifungal therapy and surgical intervention when indicated are essential to improving patient outcomes.

Keywords: COVID-19, Mucormycosis, fungal hyphae, SDA, KOH

Background:
Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and presents with a wide range of clinical manifestations, varying from mild respiratory illness to severe pneumonia, multiorgan dysfunction and death **[1].** During the second wave of the COVID-19 pandemic, a sudden and alarming increase in secondary fungal infections was observed, particularly mucormycosis, leading to substantial morbidity and mortality **[2].** Mucormycosis is a life-threatening opportunistic fungal infection predominantly caused by species of *Rhizopus*, *Mucor* and *Lichtheimia* **[3].** The disease primarily affects individuals with impaired immunity, including patients with poorly controlled diabetes mellitus, prolonged corticosteroid therapy, malignancies, or organ transplantation **[4].** A hallmark feature of mucormycosis is angioinvasion, resulting in vascular thrombosis, tissue ischemia, necrosis and rapid disease progression **[5].** Several COVID-19 related factors increase susceptibility to mucormycosis, such as extended hospital or ICU stay, steroid-induced hyperglycemia, immune dysregulation caused by SARS-CoV-2 infection and indiscriminate use of broad-spectrum antimicrobial agents that disrupt normal microbial flora **[6-8].** The interaction of host-related risk factors and virus-induced immunosuppression creates a favorable environment for invasive fungal infections **[9].** India witnessed an unprecedented surge in COVID-19 associated mucormycosis (CAM), with the rhino-orbito-cerebral form being the most commonly reported presentation, posing a major public health challenge **[10].** Early diagnosis, prompt antifungal therapy and aggressive surgical management are crucial for improving survival and reducing disease-related complications **[11].** Rhino-cerebral mucormycosis commonly involves the paranasal sinuses, orbit, facial bones and cranial cavity. Early clinical features often include facial pain, headache, facial swelling and periorbital edema. Fungal invasion of bone and blood vessels leads to endothelial damage, osteomyelitis, bone necrosis and compromised blood supply **[12].** Increased angioinvasive behavior has been linked to platelet-derived growth factor signalling pathways. Mortality rates range from 30% to 80%, depending on disease extent and timeliness of treatment **[13].** Due to overlapping clinical features with COVID-19 and limited early awareness, diagnosis is frequently delayed. Management involves early initiation of antifungal treatment, preferably liposomal amphotericin B, surgical removal of necrotic tissue when indicated and effective control of underlying risk factors such as diabetes mellitus **[14, 15].** Therefore, it is of interest to evaluate the microbiological profile of rhino-orbito-cerebral mucormycosis in patients with COVID-19, emphasizing the role of microbiological techniques in its diagnosis.

Materials and Methods:
A prospective cross-sectional study was carried out in the Department of Mycology at Sri Aurobindo Institute of Medical Sciences and Postgraduate Institute, Indore, Madhya Pradesh, over a period of 10 months from March 2020 to December 2020. All samples were received on Consecutive basis during the study period. A total of 133 clinical specimens, including tissue samples and scrapings, received in Microbiology Department for fungal determination were included in this study. One tissue sample received in formalin was rejected and asked for repeat sample as per institutional rejection criteria. All received sample for fungal detection in department of microbiology were either active COVID-19 infection or post COVID-19 infection. All samples received in microbiology laboratory for COVID-19 detection was confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). The isolation or direct microscopic demonstration of fungal elements from clinical specimens exhibiting suitable clinical characteristics and/or radiological evidence suggestive of fungal infection was considered a fungal-positive case. Where relevant, cases deemed potential colonizers or contaminants based on recurring negative and lack of clinical connection were eliminated. All specimens were subjected to direct microscopic examination using potassium hydroxide (KOH) mount and India ink preparation. Fungal culture was

ISSN 0973-2063 (online) 0973-8894 (print)
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performed on Sabouraud dextrose agar (SDA). Identification of fungal isolates was based on colony morphology, slide culture techniques, lactophenol cotton blue (LPCB) staining and relevant biochemical tests such as urease test, germ tube test. Diagnosis is confirmed by presence of yeast or fungal hyphae in KOH mount followed by growth on Sabouraud dextrose agar (SDA), slide culture and lactophenol cotton blue (LPCB) staining **(Figure 1).**

Study variables:

The study population included suspected case of fungal infection with COVID-19 or without COVID-19 (recovered from COVID-19) admitted to a tertiary care hospital in Indore, Madhya Pradesh. The presence of associated comorbid conditions such as diabetes mellitus, renal disease and malignancy was also evaluated to determine their role in the development of fungal infections.

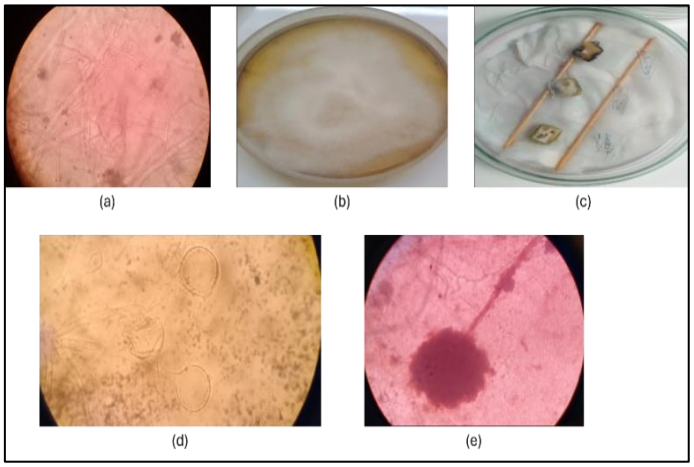


Figure 1: (a) fungal hyphae as seen in KOH mount, (b) growth of *mucor spp*. as seen on SDA agar, (c) slide culture, (d) *mucor spp.* as seen in LPCB mount, (e) *Aspergillus spp.* as seen in LPCB mount

Results:

Among the 133 clinical samples evaluated in this study, 92 samples were male, whereas 41 samples were female**. (Table 1).** Out of 93 positive cases of fungal infection, 87 (55 male and 32 female) cases developed fungal infection after recovered from COVID (post COVID fungal infection) and 6 cases (5 male and 1 female)developed fungal infection during active COVID treatment. **(Table 2).** One tissue sample received in formalin was rejected and asked for repeat sample then further processing for detection of fungus was done ,Of the 133 samples (tissue (70) 52.6% and scraping (63) 47.4%) processed, fungal infection was detected in 60 (85.7%) of the tissue and 33 (52.4%) scraping samples. Overall, 93 out of 133 samples (69.9%) were positive for fungal infection. **(Table 3)**Among the 93 fungal species, *Mucor species* isolated from 78 (58.64%) samples were the most frequently isolated fungi followed by *Candida species* from 10 cases (7.51%) and *Aspergillus species* were from 5 cases (3.75%) isolated from clinical samples **(Table 4)** among the all age group, highest number of positive cases was recorded in aged more than 70 years (27 cases), followed 20 cases, 13 cases, 12 cases, 11 cases and 10 cases were recorded in 61-70, 21-30, 41-50, 51-60 and 31-40 age groups respectively. *Mucor species* were the predominant isolates across all age categories, with the greatest frequency seen in older age group, particularly those above 70 years of age. *Candida species* were distributed across most age groups, showing a gradual increase with advancing age and peaking in the 61–70-year group. *Aspergillus species* were less commonly isolated and were mainly detected in patients older than 30 years, with the highest occurrence in those above 70 years. No fungal infections were observed in the 10–20-year age group **(Table 5).** Of all study variables, Diabetes mellitus was the most common underlying condition, with 8 out of 10 patients showing fungal positivity, accounting for 18% of the total study population. All patients who had undergone post-renal transplantation were positive for fungal infection, contributing 6% of the total cases. Among patients with acute myeloid leukaemia, fungal infection was detected in 4 out of 5 individuals, representing 9% of the study population. Overall, 15 positive cases were identified among 18 patients with these predisposing conditions **(Table 6).** Among all clinical feature associated with mucormycosis in COVID patients, facial pain was most common symptom observed in 35 patients followed by nasal congestion; orbital swelling, necrotic lesions and all clinical features were observed in 32, 10, 5 and 11 patients respectively **(Table 7).**

Table 1: Distribution of clinical samples according to gender of patients

Total sample	Male	Female	Total positive	Total negative
133	92	41	93 (69.9%)	40 (30.07%)

Table 2: Distribution of positive samples according to gender of patients

	COVID negative/post COVID (%= n/93)	COVID positive (%= n/93)	Total
Male	55 (59.13%)	5(5.37%)	60 (64.51%)
Female	32 (34.40%)	1(1.07%)	33 (35.48%)
Total	87 (93.54%)	6 (6.45%)	93

Table 3: Distribution of clinical samples

Name of samples	Total samples	Samples positive for fungal infection	%	P value
Tissue	70	60	85.7%	**< 0.001**
Scrapping	63	33	52.4%.	
Total	133	93	69.9%	
where α = "0.05"				

Table 4: Distribution of fungal spp in COVID patients

Species	No. of Isolates	Percentage (%)
Candida spp	10	7.51%
Aspergillus spp	5	3.75%
Mucor spp	78	58.64%
Total negative	40	30.07%
Total positive	93	69.92%
p = < 0.0001		

Table 5: Distribution of fungal species according to various age groups of patients

S NO	Species	10-20	21-30	31-40	41-50	51-60	61-70	>70	Total (n%= 93)
1	*Candida spp*	0	1	1	2	2	3	1	10 (10.75%)
2	*Aspergillus spp*	0	0	1	0	1	0	3	5 (5.37%)
3	*Mucor spp*	0	12	8	10	8	17	23	78 (83.87%)

4	Total	0	13	10	12	11	20	27	93
Mean age: 55.3 years (approximately)									

Table 6: Distribution of study variables among positive cases

	Total patients	Number of positive cases	Total	Percentage of patients (%)
Diabetes	10	8	18	55.6
Post renal transplant	3	3	6	6.7
Acute myeloid leukemia	5	4	9	27.8
Total	18	15	33	

Table 7: Distribution of clinical feature among the positive case of mucormycosis

Clinical feature	Number of patients (n%= 93)	P value
Facial pain	35(37.63%)	< 0.0001
Nasal congestion	32 (34.40 %)	
Orbital swelling	10(10.75%)	
Necrotic lesions.	5 (5.37%)	
All clinical feature	11 (11.82%)	
Total	93	
χ^2 = 41.14; df = 4		

Discussion:

In the present study a total of 133 clinical samples, 69.9% samples were positive for fungal infection of which higher number of positive cases reported from male patient 64.51% as compared to female 35.48% patients. The finding aligns with of Meshram *et al.* reported predominance of male 61.11% as compared to female 38.88% **[15].** The present study showed high burden of fungal infection in COVID patients with p value < 0.0001. However, study done by Manda *et al.* reported higher incidence of female among COVID patients. The variation in demographic may be due to local population or healthcare-seeking behaviour trends **[16].** The immune suppression in SARS-CoV-2 infection predisposes patients to opportunistic fungal infections, with *Mucor, Aspergillus* and *Candida species* being the most commonly implicated pathogens in COVID-19 patients **[17-18].** In this study fungal infection associated with COVID were statistically significant, among all fungal species *Mucor species* were dominated with 83.87% followed by *Candida* and *Aspergillus spp* were 7.51% and 3.75% respectively. In a study done by Mohite *et al.* showed *Mucor* (82.5%) were predominant fungal species isolated from COVID patients followed by *Aspergillus* were 18.75 % and *candida* were 52.6% **[19].** In contrast to study done by John *et al.* reported there were statistically insignificant association of fungal infection in COVID patients **[20].** The variation may be attributed to regional, healthcare-related issues and less sample size. Opportunistic fungal infection most commonly occurred in elderly patients due to decline in immune function that affect both innate and adaptive immunity **[20-21].** In our study higher incidence of fungal infection reported from elderly patients with mean age group of 55.3 years approximately that was consistent with study done by Hoenigl *et al.* **[21].** But a study done by Sharma *et al.* reported incidence of fungal infection associated with COVID patients in younger age group **[22].** Comorbidity factors, healthcare accessibility and COVID-19 management approaches could be the reason attributed for variation in age groups. In addition to elderly age group there are several others factors like comorbidity of patients, prolong use of antibiotics and immunosuppression agent also contributed for development of opportunistic fungal infections **[23].** In the present study higher incidence of fungal infection was seen in diabetic patients that are similar to study done by Prakash *et al.* **[24]** facial pain, nasal congestion, eye symptom (periorbital swelling, pain, ptosis, blurred or loss of vision), fever, Headache are the most common symptoms of mucormycosis. In present study facial pain (37.63%) was the most common symptom reported in patients with mucormycosis and association of all symptoms (reported in this study) found statistically significant with mucormycosis. These symptoms are consistent with mucormycosis, as reported by Balushi *et al.* **[25].** In contrast to study done by Kumar *et al.* reported a greater number of symptoms as compare to present study. The variation may be due to comorbidity and severity of disease **[26]**. The limitations of the present study include, small sample size, detailed characterization of the fungal species and antifungal susceptibility testing was not performed.

Conclusion:

Rhino-orbito-cerebral mucormycosis is a rapidly progressive fungal infection associated with substantial morbidity and mortality, particularly in immunocompromised patients. Early clinical suspicion, microbiological confirmation, prompt antifungal therapy and surgical debridement when indicated are essential for timely and effective management. Early intervention and control of underlying risk factors may improve clinical outcomes and reduce disease-related complications.

Advancement to knowledge:

Early diagnosis, prompt antifungal therapy and aggressive surgical management are crucial for improving survival and reducing disease-related complications.

Acknowledgements:

The corresponding author contributed to writing of entire research paper followed by 1st, 2nd and authors contributed to data collection. All authors express their sincere gratitude to institute and management for the continuous support and immense guidance.

Financial support: Nil

Conflict of interest: Nil

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