Journal of Infectious Diseases & Microbiology

Gade N, et al., 2022-J Infect Dis Microbiol

Research Article

Study of Molds in Post COVID -19 Patients: An experience from Tertiary Care Centre

Neeta Gade[1*], Soumyabrata Nag[2], Vishal Shete[3] and Meena Mishra[4]

[1]Associate Department of Microbiology, All India Institute of Medical Sciences, Nagpur, India

[2]Assistant Professor, Department of Microbiology, All India Institute of Medical Sciences, Nagpur, India

[3]Assistant Professor, Department of Microbiology, All India Institute of Medical Sciences, Nagpur, India

[4]Professor, Department of Microbiology, All India Institute of Medical Sciences, Nagpur, India

*Corresponding Author: **Neeta Gade**, Associate Professor, Department of Microbiology, All India Institute of Medical Sciences, Nagpur, India.

Received Date: 06-30-2022
Accepted Date: 07-04-2022
Published Date: 08-12-2022

Abstract

Introduction: Coronavirus disease 2019 (COVID-19) pandemic has emerged as a global health crisis in the century with a heavy toll on human life. The second wave of COVID-19 was lethal for the patients as they had to combat the virus along with the opportunistic fungal and bacterial infections triggered by it. Mucormycosis gained attention for its widespread existence during this period of the second wave of COVID-19.

Material & Methods: Various clinical samples like biopsy tissues from paranasal sinuses, deep nasal swabs, bronchoalveolar lavage, sputum, etc. were studied for the presence of fungal elements by KOH examination and culture on Sabouraud's dextrose agar. Identification of fungal isolate was done by growth characteristics, LPCB wet mount, and slide culture.

Result: During the study period, a total of 214 samples were received of which 17 (7.9%) were positive for fungal growth. *Rhizopus arrhizus* was most frequently isolated. Diabetes mellitus and the use of steroid during COVID-19 hospitalization was observed to be common risk factors. Rhino-cerebral mucormycosis was found to be the commonest type.

Conclusion: Invasive mould infections can lead to fatal outcomes, if not detected timely. Accurate identification of the fungus is important for the administration of appropriate antifungal therapy.

Keywords: COVID-19; *Chaetomium globosum*; fungal infections; Mucormycosis.

Abbreviations: COVID: Coronavirus Disease; KOH: Potassium hydroxide; LPCB: Lacto-Phenol Cotton Blue; ICU: Intensive Care Unit; CD: Cluster of Differentiation; T cells: Thymus cells; CDC: Centers for

Disease Control and Prevention; GOI: Government of India; SDA: Sabouraud's Dextrose Agar; SARS CoV-2-Severe Acute Respiratory Syndrome Coronavirus 2; ET: Endo-Tracheal; Spp: species; A terreus: Aspergillus terreus; A flavus: Aspergillus flavus.

Introduction

In the current COVID-19 pandemic several COVID-19-associated complications are being reported worldwide. COVID-19 patients, mostly those with severe illness or with a compromised immune status, were reported to have a higher risk of suffering from invasive fungal infections such as *Aspergillus* and *Candida* infections and other secondary fungal infections, particularly in patients with prolonged ICU stay or requiring mechanical ventilation [1,2]. Coronavirus itself causes immunosuppression with a decrease in CD4+T and CD8+T cells, and overexpression of inflammatory cytokines indicating its likelihood to develop fungal coinfections [3].

These fungal infections can be fatal for COVID-19 patients, especially in those with high-risk factors. Most COVID-19 patients did not have any assessment for fungal infections at the beginning which led to its neglect. Mucormycosis, a decade-old disease, is one of the dreaded complications that were reported during the second wave of COVID 19 in particular from India. Mucormycosis is a rare and most feared angio-invasive fungal infection caused by a ubiquitous group of fungi called mucormycetes.

Various risk factors for mucormycosis are immunocompromised host, uncontrolled diabetes mellitus especially diabetic ketoacidosis, long-term systemic corticosteroid use, haematological malignancies, treatment with iron-chelating therapies, etc. [1]. According to the 2019-2020 CDC report, the prevalence of mucormycosis ranges from 0.005 to 1.7 per million population globally. It is 80-fold higher (0.14 per 1000) in India as compared to developed countries [4,5]. Following the surge of COVID-19-associated mucormycosis, several states in India made mucormycosis a notifiable disease in May 2021. According to the press release dated 28th June 2021 by the Ministry of Health and Family Welfare, GOI, a total of 40,845 cases of mucormycosis have been reported of which 34,940 patients had COVID (85.5%) [6]. If not diagnosed and treated promptly, fungal infections affect the outcome of the disease and may lead to a poor prognosis. The present study reports COVID-associated mould infection from central India.

Material and methods

Samples like biopsy tissues from paranasal sinuses, deep nasal swabs, bronchoalveolar lavage, sputum, tracheal aspirates, endotracheal secretions, etc. were received in the Diagnostic Microbiology laboratory, from suspected cases of mucormycosis, admitted at our tertiary care hospitals, during the period from May to August 2021. Information such as age, gender, underlying comorbid conditions, COVID-19 status, use of oxygen and/or ventilator support, and steroid medication was recorded. The samples were subjected to KOH examination and cultured on two sets of Sabouraud's dextrose agar (SDA). SDA slopes were incubated at 25° C and 37° C separately and observed for fungal growth.

Identification of fungal isolate was done by growth characteristics, LPCB wet mount, and slide culture. Species identification was confirmed by the Centre for Advanced Research in Medical Mycology at the Post graduate institute of medical education and research, Chandigarh.

Results

During the study period, a total of 214 samples were received [Nasal swabs-122 (57%), Sputum-33 (15.4%)] depicted in Figure 1, of which 17 (7.9%) were positive for fungal growth. All the patients had a recent SARS CoV-2 infection. A total of 19 fungal isolates were obtained from suspected cases of mucormycosis. *Rhizopus arrhizus* was most frequently isolated as shown in Figure 2. Two mucormycosis patients were identified with dual infection with Aspergillus flavus as described in Figure 3. Males between the age of 41-60 years (n=09) were found to be commonly affected by mucormycosis. Diabetes mellitus (n=07) and use of steroid during COVID-19 hospitalization (n=08) were observed to be common risk factors among the cases who were positive for fungal growth. All the patients received broad-spectrum antibiotic therapy during SARS CoV-2 infection. Rhino-cerebral mucormycosis was found to be the commonest type.

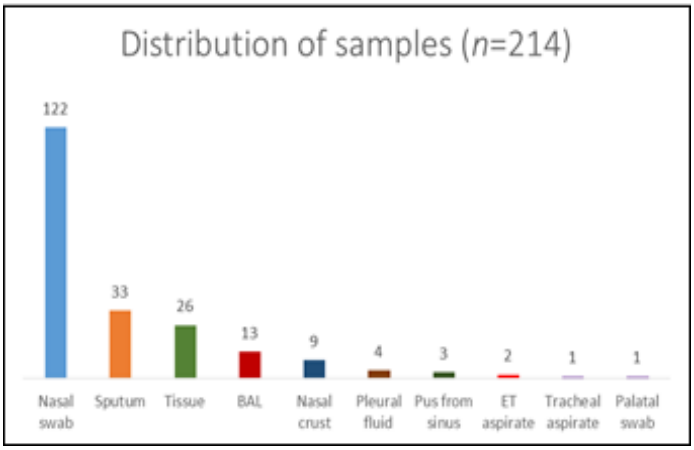

Figure 1: Distribution of samples received for fungal examination.

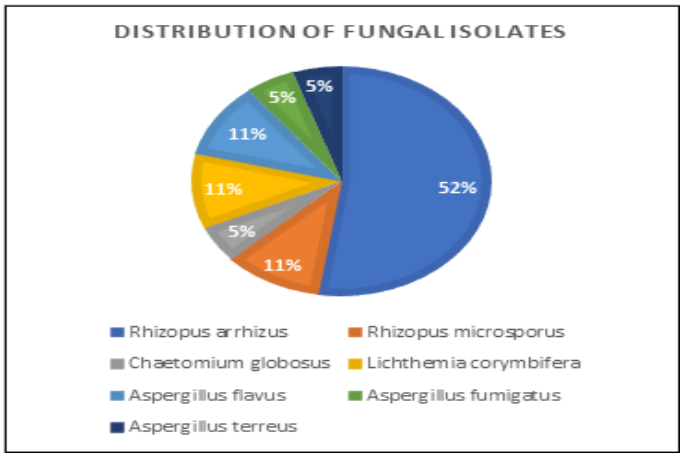

Figure 2: Distribution of fungal isolates.

Citation: Gade N, Nag S, Shete V, Mishra M. Study of Molds in Post COVID -19 Patients: An experience from Tertiary Care Centre. J Infect Dis Microbiol. 2022;1(2):1-7.
DOI: https://doi.org/10.37191/Mapsci-JIDM-1(2)-007

Discussion

Mucormycosis is a potentially fatal infection owing to its invasive nature resulting in angioinvasion, ischemic necrosis of tissues, and mycotic thrombosis. It has various clinical manifestations like rhino-orbito-cerebral mucormycosis, pulmonary mucormycosis, cutaneous mucormycosis, gastrointestinal mucormycosis, etc. Fungi of the genus *Rhizopus*, *Mucor*, *Lichtheimia*, *Rhizomucor*, *Syncephalastrum*, *Apophysomyces*, *Saksenaea*, *Cunninghumella*, etc. are most commonly associated with mucormycosis [7-9].

Rhino-orbito-cerebral mucormycosis was the most common presentation in our study followed by pulmonary mucormycosis. The incidence rate of mucormycosis in post-COVID patients was 7.9% in the present study, which is significantly higher than the global data. So, an increased association of mucormycosis is seen with post-COVID cases. However, because of our study's small sample size (17 positive cases), these results may be skewed.

The reason for an increasing number of cases of mucormycosis in COVID-19 patients may be attributed to several factors. An environment of low oxygen, high glucose (diabetes, steroid-induced hyperglycaemia), high iron levels, acidic medium (metabolic acidosis) and immunosuppression in combination with associated risk factors like prolonged hospitalization, mechanical ventilators provide a favourable condition for the germination of spores of Mucorales [10].

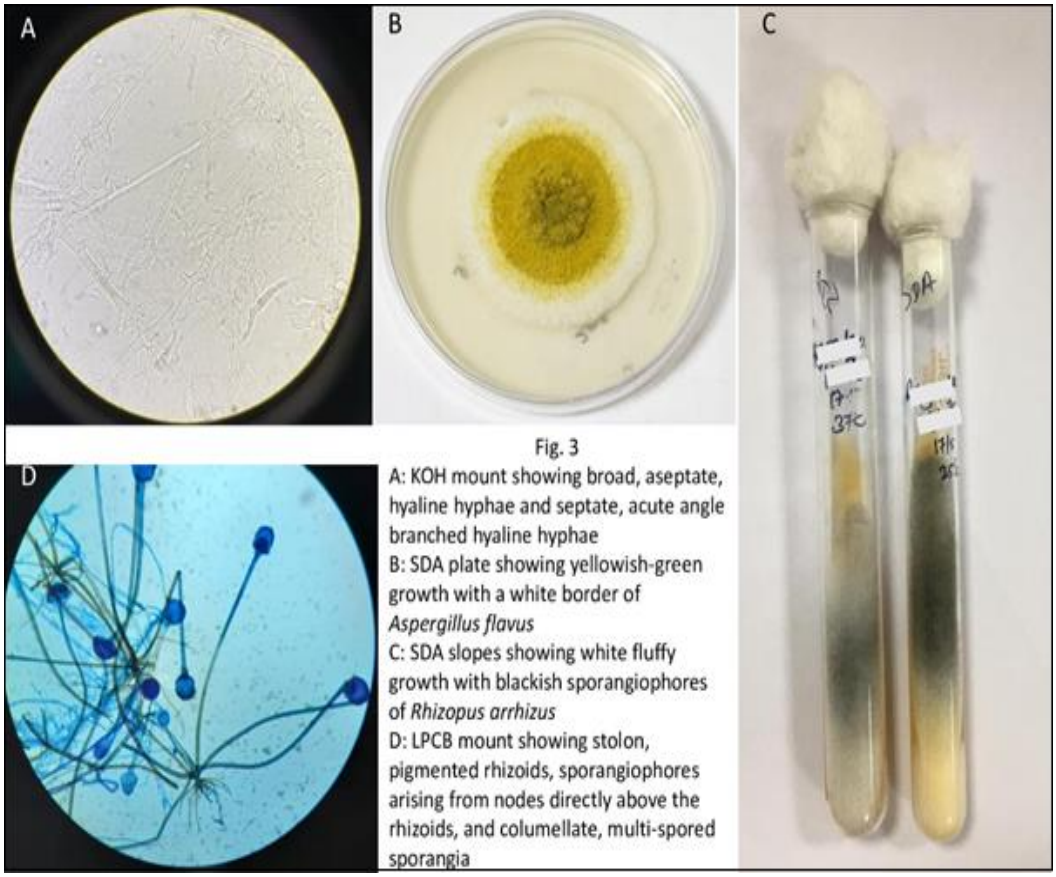

Fig. 3
A: KOH mount showing broad, aseptate, hyaline hyphae and septate, acute angle branched hyaline hyphae
B: SDA plate showing yellowish-green growth with a white border of *Aspergillus flavus*
C: SDA slopes showing white fluffy growth with blackish sporangiophores of *Rhizopus arrhizus*
D: LPCB mount showing stolon, pigmented rhizoids, sporangiophores arising from nodes directly above the rhizoids, and columellate, multi-spored sporangia

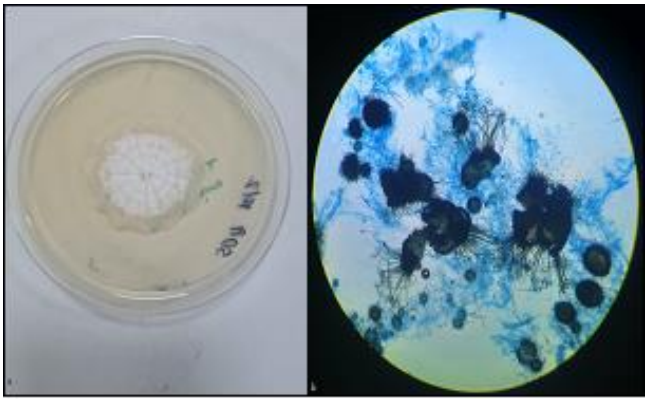

Figure 4: (a) SDA plate showing irregular, corrugated, white cottony colony with radial striations of *Chaetomium globosum*, (b) LPCB mount showing hyaline septate hyphae and dark brown spherical ascoma (perithecia) with numerous hairs.

Researchers found that most men belonging to the age group of 41-60 years were affected which was strikingly similar to the findings of other studies [10,11]. Diabetes mellitus (47%) and steroid use (41%) were found to be the two most common risk factors in our study. Other researchers also recorded similar findings, though the percentage varied depending on the study population and sample size [11,12].

All the fungal isolates were identified up to species level as displayed in Figure 2 and *Rhizopus arrhizus* was found to be the most common causative agent accounting for more than half of the cases. In a case series reported from north-western part of India, only 20% of the isolates were Rhizopus arrhizus, and the rest were identified up to genus level only [13].

Similar studies from different parts of India identified the fungal isolates up to genus only [14-16]. It is also important that people do not ignore the other fungi associated with COVID-19. 05 out of the 19 fungal isolates (>25%) were *Aspergillus* spp. (n=4) and another rare fungal isolate *Chaetomium globosum* (n=1) was isolated from one sample as described in Figure 4. Other studies across the globe have reported co-infection with *Aspergillus* spp. and *Candida* spp. In COVID-19 patients [17,18]. Accurate identification of the fungus is important for the administration of appropriate antifungal therapy.

As per CDC guidelines, mucormycosis is to be treated with lipid amphotericin formulations or Posaconazole. Voriconazole does not act against Mucorales. On the contrary, voriconazole or lipid amphotericin formulations, posaconazole, or isavuconazole are to be given to patients with invasive aspergillosis. [19,20]. However, species identification may not necessitate a different treatment in the case of mucormycosis but is important for epidemiological purposes.

In the case of invasive aspergillosis, however, species identification can dictate antifungal therapy. Intrinsic resistance to amphotericin B has been observed in *A. terreus*, and other species like *A. flavus* are reported to have reduced susceptibility to it [21,22]. Many Aspergillus species are also resistant to azoles and echinocandins.

Conclusion

Invasive mould infections can lead to fatal outcomes, if not detected timely. Genus and sometimes species identification are crucial for starting appropriate antifungal therapy.

One must bear in mind the possibility of mould infection in post-COVID patients with associated risk factors and co-morbidities, who are more prone to develop such deadly infections.

Conflict of interest

The authors have no conflict of interest.

Funding

No funding.

Ethical approval

Ethical approval for the study was obtained from Institutional Ethical Committee.

Acknowledgement

Authors thankfully acknowledges Centre for Advance Research in Medical Mycology, WHO collaborating Centre for Reference and Research on fungi of Medical Importance for the support provided for species confirmation of isolated fungi.

References

1. Song G, Liang G, Liu W. Fungal co-infections associated with global COVID-19 pandemic: a clinical and diagnostic perspective from China. Mycopathologia. 2020;185(4):599-606. PubMed | CrossRef
2. Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F, et al. COVID-19 associated pulmonary aspergillosis. Mycoses. 2020;63(6):528-34. PubMed | CrossRef
3. Yang W, Cao Q, Qin LE, Wang X, Cheng Z, Pan A, et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): a multi-center study in Wenzhou city, Zhejiang, China. J Infect. 2020;80(4):388-93. PubMed | CrossRef
4. Werthman-Ehrenreich A. Mucormycosis with orbital compartment syndrome in a patient with COVID-19. Am J Emerg Med. 2021;42:264-e5. PubMed | CrossRef
5. Fox A, Janson B, Stiff H, Chung A, Benage M, Van Heukelom J, et al. A multidisciplinary educational curriculum for the management of orbital compartment syndrome. Am J Emerg Med. 2020;38(6):1278-80. PubMed | CrossRef
6. https://pib.gov.in/PressReleseDetailm.aspx PRID=1718501
7. Shah K, Dave V, Bradoo R, Shinde C, Prathibha M. Orbital exenteration in rhino-orbito-cerebral mucormycosis: a prospective analytical study with scoring system. Indian J Otolaryngol Head Neck Surg. 2019;71(2):259-65. PubMed | CrossRef
8. Lee AS, Lee PW, Allworth A, Smith T, Sullivan TJ. Orbital mycoses in an adult subtropical population. Eye. 2020;34(9):1640-7. PubMed | CrossRef
9. Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Kong DC, et al. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clin Microbiol Infect. 2019;25(1):26-34. PubMed | CrossRef
10. 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. PubMed | CrossRef
11. Bhanuprasad K, Varghese GM. Risk Factors Associated with the Mucormycosis Epidemic During the COVID-19 Pandemic. Int J Infect Dis. 2022;116:S55-6.2021;111:267-70. PubMed | CrossRef
12. Rao VU, Arakeri G, Madikeri G, Shah A, Oeppen RS, Brennan PA. COVID-19 associated mucormycosis (CAM) in India: a formidable challenge. Br J Oral Maxillofac Surg. 2021;59(9):1095-8. PubMed | CrossRef
13. Nehara HR, Puri I, Singhal V, Sunil IH, Bishnoi BR, Sirohi P. Rhinocerebral mucormycosis in COVID-19 patient with diabetes a deadly trio: Case series from the north-western part of India. Indian J Med Microbiol. 2021;39(3):380-3. PubMed | CrossRef

14. Arjun R, Felix V, Niyas VK, Kumar MA, Krishnan RB, Mohan V, et al. COVID-19-associated rhino-orbital mucormycosis: a single-centre experience of 10 cases. QJM. 2021;114(11):831-4. PubMed | CrossRef
15. Sarkar S, Gokhale T, Choudhury SS, Deb AK. COVID-19 and orbital mucormycosis. Indian J Ophthalmol. 2021;69(4):1002. PubMed | CrossRef
16. Moorthy A, Gaikwad R, Krishna S, Hegde R, Tripathi KK, Kale PG, 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. J Maxillofac Oral Surg. 2021;20(3):418-25. PubMed | CrossRef
17. Verweij PE, Gangneux JP, Bassetti M, Brüggemann RJ, Cornely OA, Koehler P, et al. Diagnosing COVID-19-associated pulmonary aspergillosis. Lancet. 2020;1(2):e53-5. CrossRef
18. Zhang G, Hu C, Luo L, Fang F, Chen Y, Li J, et al. Clinical features and outcomes of 221 patients with COVID-19 in Wuhan, China. J Clin Virol. 2020;127:104364. PubMed | CrossRef
19. https://www.cdc.gov/fungal/diseases/aspergillosis/treatment.html
20. https://www.cdc.gov/fungal/diseases/mucormycosis/treatment.html
21. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW. European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST). EUCAST technical note on Aspergillus and amphotericin B, itraconazole, and posaconazole. Clin Microbiol Infect. 2012;18(7):E248-50. PubMed | CrossRef
22. Arendrup MC, Rodriguez-Tudela JL, Lass-Flörl C, Cuenca-Estrella M, Donnelly JP, Hope W. European committee on antimicrobial susceptibility testing-subcommittee on antifungal susceptibility testing (EUCAST-AFST). EUCAST technical note on anidulafungin. Clin Microbiol Infect. 2011;17(11):E18-20. PubMed | CrossRef