

CLINICOMYCOLOGICAL PROFILE OF MUCORMYCOSIS PATIENTS IN COVID-19 PANDEMIC IN A TERTIARY CARE CENTRE IN SOUTH INDIA- A RETROSPECTIVE STUDY

Priyadharshini M.S¹, Subathra N²

¹Assistant Professor, Department of Microbiology, Government Medical College Krishnagiri, Polupalli, Krishnagiri

²Associate Professor, Department of Microbiology, Government Medical College Namakkal, Siluvampatti, Namakkal

Received : 18/08/2024
Received in revised form : 08/10/2024
Accepted : 22/10/2024

Keywords:

Mucormycosis, Covid-19, Diabetes, Mucorales, Rhizopus sp.

Corresponding Author:

Dr. Priyadharshini M.S,
Email: saipriyamicro@gmail.com

DOI: 10.47009/jamp.2024.6.5.146

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

Int J Acad Med Pharm
2024; 6 (5); 767-771



Abstract

Background: Mucormycosis is a lethal infection caused by fungi belonging to the Order of Mucorales of Class Zygomycetes. During the early May 2021, when covid-19 cases were in its peak in India, the surge in Mucormycosis was attributed to the ideal environment in covid-19 patients that facilitated the mucorales spore to germinate. The objectives were to study the clinical profile of suspected Mucormycosis patients in covid 19 pandemic, to detect the presence of fungal agents causing mucormycosis by conventional KOH mount examination and to identify the causative fungi by performing Fungal culture of samples collected from clinically suspected cases of mucormycosis. **Materials and Methods:** This study included clinically suspected mucormycosis patients with COVID-19-like illness, between May 2021 and July 2021. Data regarding demography, underlying medical conditions, COVID-19 illness, diagnosis of clinical mucormycosis, site of involvement were collected. Samples of suspected patients were subjected to conventional KOH wet mount examination and fungal culture and the findings were recorded. **Result:** Among the 40 patients included in the study, 17 (42.5%) had covid-19 infection. The most common clinical presentation was Rhinomucormycosis accounting to 80%. 26 (65%) samples showed fungal elements in the KOH wet mount examination. In Fungal culture, 21 samples grew Rhizopus species, 3 samples grew Aspergillus species and 16 samples did not show any growth even after 2 weeks of incubation. 33(82.5%) patients had uncontrolled diabetes mellitus and 1(2.5%) patient had immunosuppressant drug intake as associated risk factors. **Conclusion:** This study had revealed the increased risks associated with covid infection and diabetes in Mucormycosis patients. Though labelled as difficult-to-treat infection with high mortality, Covid associated Mucormycosis can be managed better with increased suspicion, early diagnosis and control of Covid-19 infection.

INTRODUCTION

Mucormycosis is a lethal infection caused by fungi belonging to the Order of Mucorales of Class Zygomycetes. They often cause devastating angioinvasive fungal infections, primarily in patients with underlying risk factors.^[1] During the Covid 19 pandemic caused by Severe Acute Respiratory Syndrome –Coronavirus 2 (SARS-CoV2), a wide range of opportunistic bacterial and fungal infections have been reported.^[2] During the early May 2021, when covid-19 cases were in its peak in India,^[3] the surge in Mucormycosis was attributed to the ideal environment in covid-19 patients that facilitated the mucorales spore to germinate. This includes

conditions such as hypoxia, Diabetes, new onset hyperglycemia, steroid-induced hyperglycemia, metabolic acidosis, diabetic ketoacidosis [DKA], high iron levels (increased ferritin). Also, the decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated or background comorbidities) coupled with several other shared risk factors including prolonged hospitalization with or without mechanical ventilators contributed to the increased incidence.^[4] In reference with the above situation, we were intrigued to take over this study at our institutional level during the period from May 2021 to July 2021 to know the clinicomycological

profile of Mucormycosis patients during the ongoing covid 19 pandemic.

Objective of the study: The objectives of the study were to study the clinical profile of suspected Mucormycosis patients in covid 19 pandemic, to detect the presence of fungal elements causing mucormycosis by conventional KOH mount examination and to identify the causative fungi by performing Fungal culture of samples collected from individuals who were on clinical suspicion of mucormycosis.

MATERIALS AND METHODS

This study included patient diagnosed with mucormycosis clinically during their course of clinical COVID-19-like illness, between May 2021 and July 2021 in Government Medical College Krishnagiri. Data regarding demography, underlying medical conditions, COVID-19 illness, diagnosis of clinical mucormycosis, site of involvement were collected. Samples of wound debridement tissues of suspected Mucormycosis patients were collected and as per standard protocols. The biopsy or aspirated material was collected in sterile containers of normal saline. The microscopic examination was done using wet mount examination using 10-20% potassium hydroxide (KOH) to identify the fungal elements [Figure 1]. For culture, the tissue sample was cut into small pieces and inoculated into a Sabouraud dextrose agar (SDA) medium with antibiotics and incubated at 25°C and 37°C [Figure 2]. Following culture, the growth was subjected to macroscopic and microscopic examination using LPCB (Lactophenol Cotton Blue) mount to observe the morphology and identify the fungus grown [Figure 3]. The macroscopic and microscopic morphology is analysed and the fungus is identified.

RESULTS



Figure 1: KOH Mount of samples showing fungal elements



Figure 2: SDA tubes showing fungal growth

Rapidly growing cottony colonies with salt and pepper appearance resembling *Rhizopus* species seen inside the SDA tubes.

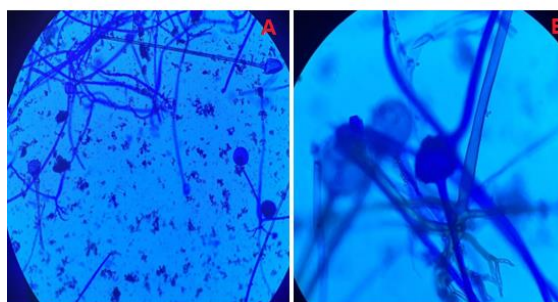


Figure 3: Microscopic identification of the fungal growth by LPCB mount. A: 10X magnification showing broad aseptate hyaline hyphae with rhizoids and unbranched sporangiophore and sporangia. B: 40X magnification showing rhizoids and unbranched sporangiophore with sporangia

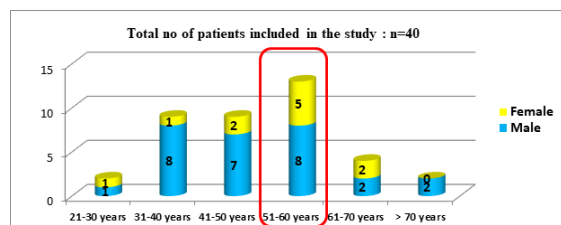


Figure 4: Age and Gender distribution of study patients

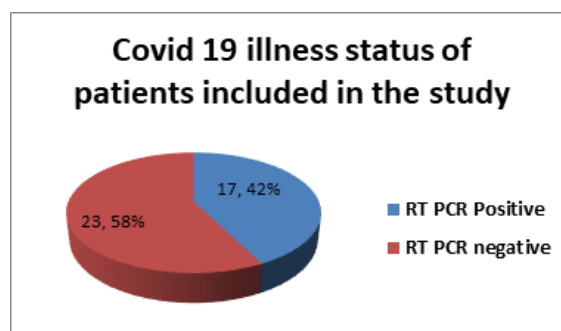


Figure 5: Covid 19 positivity in suspected mucormycosis patients

There were 40 cases of clinical mucormycosis during the study period of 3 months of covid-19 pandemic registered in the hospital. The 40 patients were also suspected of covid 19 illness along with mucormycosis. The mean age of the affected patients were 50.07 years. Out of the 40 patients, there were

29 male patients accounting to 72.5%, and 11 female patients accounting to 27.5%. The most common age group affected was 51-60 years of age with 13 patients falling under the category [Figure 4]. There were 17 patients who had covid-19 infection confirmed by RT PCR test for corona virus which is 42.5% of the total suspected patients [Figure 5]. 26(65%) out of 40 Samples from the suspected case of mucormycosis showed positive for fungal elements in the KOH wet mount examination [Figure 1]. Most of the isolates showed broad aseptate/pauciseptate hyaline hyphae. Samples included for fungal culture showed positive growth of Rhizopus in 21 samples, Aspergillus species in 3

samples and out of 40, and 16 samples did not show any growth. Of 40 patients suspected with mucormycosis, 33(82.5%) patients had uncontrolled diabetes mellitus and 1(2.5%) patient was on immunosuppressant drugs for both of which acted as associated risk factors. Out of the 21 culture confirmed Mucormycosis patients, 15(71.4%) of them were male and 6(28.6%) were females. The most common age group affected was 51-60 years of age. Out of 21 Mucormycosis patients, 13(61.90%) had confirmed covid 19 illness and 19 (90.5%) patients had underlying uncontrolled diabetes as risk factor.

Table 1: Clinical spectrum of study patients (n=40).

Type of Mucormycosis	No of patients
Rhinomucormycosis	32 (80%)
Rhinoorbital Mucormycosis	6 (15%)
Fungal Rhinosinusitis	1 (2.5%)
Oral Mucormycosis	1 (2.5%)

Table 2: Outcome of the samples tested

Total No of samples	KOH Positive	KOH negative	Fungal Culture positive	Fungal Culture negative
N= 40	26 (65%)	14(15%)	24(60%)	16(40%)

Table 3: KOH positivity and culture correlation (N=40).

Tests	Culture positive	Culture negative
KOH Positive	24 (60%)	2(5%)
KOH negative	0(0%)	14(35%)

Table 4: Fungi isolated in culture.

Culture isolates (n=24)	No of samples
Rhizopus species	21
Aspergillus fumigatus	2
Aspergillus niger	1

Table 5: Comparison of fungal culture positive and negative patients

Characteristics	Fungal culture positive (n=24)	Fungal culture negative (n=16)	Significance (p<0.05- significant)
Mean Age	54.19 years	42.81 years	P = 0.0024* significant at p<0.05
Gender preponderance	Male-n=18 (75%) Female – n=6(25%)	Male-n=11(68.75%) Female- n=5(31.25%)	p= 0.6638. Not significant at p < 0.05
Covid 19 status	Positive n=13(54.16%) Negative n=11(45.84%)	Positive n=4(25.0%) Negative n=12(75.0%)	p = 0.0675. Not significant at p < 0.05.
Association with Diabetes	DM-22(91.66%) Not diabetic- 2(8.34%)	DM-10(62.5%) Not diabetic- 6 (37.5%)	p= 0.0239* significant at p<0.05

Table 6: Characteristics of KOH wet mount as screening test

Test	KOH wet mount
Sensitivity	100%
Specificity	88.9%
Positive predictive value	92.31%
Negative predictive value	100%

DISCUSSION

The second wave of covid-19 pandemic in 2021, caused severe infections with high mortality⁵. During this period, there was also a surge in Mucormycosis in the world, especially in India⁴. In this study, there were 40 cases of clinically suspected covid associated Mucormycosis. The common age group that was affected was 51-60 years with mean age of 54.1 years which correlates with the study by Sharma R, Kumar

P et al⁵ and also a systematic review of such cases from 18 countries by Hoenigl M, Seidel D, et al.^[6] The older age is being more affected due to the presence of various comorbidities in this age group. There was a male preponderance accounting to 75% in culture confirmed cases in this study similar to many other studies.^[6-10] Though 54.1% of the culture positive cases had covid infection, the association between them was not significant which could be attributed to the smaller

sample size of the study. The most common predisposing risk factor in the study was uncontrolled Diabetes which was present in 32 cases of the total 40 patients included in the study. It was seen in 91.66% in the culture positive patients showing a significant association between them ($p < 0.05$). This coincided to a prospective multicentre study done in India by Prakash H, Ghosh AK, et al.^[11] This study authenticates the concept that presence of DM with or without DKA proportionately increase the chance of mucormycosis especially in covid illness. To support our study, there are several other studies which showed Diabetes as the predominant underlying risk factor for developing Mucormycosis.^[4,10-15] Presence of high amount of mucorale spore in the environment makes the people with DM to get infected.^[5,16] Normally our body defends the environmental mucorales by chemotaxis and oxidative/ non-oxidative fungicidal mechanism of phagocytic cells. This is hindered by the hyperglycemic states seen in the patients with uncontrolled diabetes.^[16] This explains the fact that Diabetes remains unchallenged among the predisposing factors in Covid associated Mucormycosis.

The most common clinical presentation of Mucormycosis in this study was Rhinomucormycosis accounting to 80% of the study cases. The orbital involvement was seen next common to nasal mucormycosis in 15% of the patients with suspected mucormycosis. Positivity percentage of KOH mount examination is similar to the various studies done by Singh AK et al, Hoenigl M, et al, Prakash H et al, Veisi A, et al.^[4,6,11,17,18] The screening of samples for fungal elements with KOH mount examination was positive in 26 patients (60%) of the suspected cases. This was closer to the multicentre study done in India by Patel A, Agarwal R, et al.^[19] The sensitivity of KOH wet mount was 100% and it is efficacious to use it as screening test for Mucormycosis.^[20] KOH may provide a rapid diagnosis and thereby help clinicians initiate antifungal treatment early in the disease course for a better and a favourable outcome.^[20] The fungal culture was positive in 24(60%) out of 40 suspected cases. The fungal culture positivity is influenced by the friable nature of the hyphae which is more prone to be destroyed while manipulated.^[21] Hence the positivity is only around 60% in this study which is similar to the study done by Hoenigl et al. *Rhizopus* species (87.5%) was the most common fungi that was isolated, followed by *Aspergillus* species in the study patients. There are many other studies showing similar results in the fungi isolated from Covid associated Mucormycosis (CAM) patients.^[19,21-23] Few patients grew *Aspergillus fumigatus* () and *Aspergillus niger* (). The identification of the fungal species causing Mucormycosis based on morphology was quite challenging due to lack of other diagnostic morphological features as mentioned in many studies.^[25,26] As per literature, *Rhizopus arrhizus* was the most common species to cause human

mucormycosis in India. This is followed by *Apophysomyces variabilis*, *Rhizopus microsporus*, and *R. homothallicus*.^[27,28] Other less common species were *Apophysomyces elegans*, *Lichtheimia ramosa*, *Mucor irregularis*, *Rhizomucor pusillus*, *Saksenaea erythrospora*, *Syncephalastrum racemosum*, etc. Few studies suggest that use of MALDI-TOF MS for consistent and faster identification of human pathogenic Mucorales.^[29] With frequent occurrence of morphologically cryptic fungal species, a DNA sequence-based species identification is the most reliable method. The universal barcode for fungi is the internal transcribed spacer region (ITS).^[30,31] Now the availability of several PCR such as nested PCR, real-time PCR (qPCR), and nested PCR combined with RFLP have made the diagnosis of human mucormycosis better.

CONCLUSION

Mucormycosis in covid-19 patients can be a fatal complication especially in patients with pre-existing Diabetes and those treated with steroids for covid infection. The complex relationship between covid and mucormycosis cannot be ignored and yet to be explored in detail emphasising the need for more studies. In spite of limitation of having a small sample size, this study revealed the increased risks associated with covid infection and diabetes in Mucormycosis patients. Though labelled as difficult-to-treat infection, owing to the associated high mortality, Covid associated Mucormycosis can be managed better with increased suspicion in covid patients, early diagnosis and control of Covid-19 infection.

REFERENCES

1. Chakrabarti A, Singh R. The emerging epidemiology of mould infections in developing countries. *Curr Opin Infect Dis* 2011; 24: 521–6.
2. Kubin CJ, McConville TH, Dietz D, et al. Characterization of bacterial and fungal infections in hospitalized patients with COVID-19 and factors associated with healthcare-associated infections. *Open Forum Infectious Diseases*; 2021. <https://doi.org/10.1093/ofid/ofab201.ofab20>.
3. Basis Covid-19 information India. [cited 2021 May 05]. <https://www.covid19india.org/>.
4. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2021 Jul 1;15(4):102146.
5. Sharma R, Kumar P, Rauf A, Chaudhary A, Prajapati PK, Emran TB, Gonçalves Lima CM, Conte-Junior CA. Mucormycosis in the COVID-19 environment: a multifaceted complication. *Frontiers in cellular and infection microbiology*. 2022 Jul 18;12:937481.
6. Hoenigl M, Seidel D, Carvalho A, Rudramurthy SM, Arastehfar A, Gangneux JP, Nasir N, Bonifaz A, Araiza J, Klimko N, Serris A. The emergence of COVID-19 associated mucormycosis: a review of cases from 18 countries. *The Lancet Microbe*. 2022 Jul 1;3(7):e543-52.
7. Dillirani V, Menaka R, Indumathi V, Balaji P. Prevalence of COVID Associated Mucormycosis with Antifungal Susceptibility Profiles of Isolates in a Tertiary Care Hospital. *Journal of Pure & Applied Microbiology*. 2023 Mar 1;17(1).

8. G Alshahawey M, S El-Housseiny G, S Elsayed N, Y Alshahrani M, Wakeel LM, M Aboshanab K. New insights on mucormycosis and its association with the COVID-19 pandemic. *Future science OA*. 2022 Feb 1;8(2):FSO772.
9. Mehta S, Pandey A. Rhino-orbital mucormycosis associated with COVID-19. *Cureus*. 2020 Sep;12(9).
10. Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A, Puri GD, Chakrabarti A, Agarwal R. Coronavirus disease (Covid-19) associated mucormycosis (CAM): case report and systematic review of literature. *Mycopathologia*. 2021 May;186:289-98.
11. Prakash H, Ghosh AK, Rudramurthy SM, Singh P, Xess I, Savio J, Pamidimukkala U, Jillwin J, Varma S, Das A, Panda NK. A prospective multicenter study on mucormycosis in India: Epidemiology, diagnosis, and treatment. *Medical mycology*. 2019 Jun 1;57(4):395-402.
12. Jadhav H, Vishwakarma P, Thamke S, Pundkar S, Takle T, Khaimar M. Correlation of Mucormycosis with Various Clinical Parameters Among COVID-19 Patients: A Cross-Sectional Study. *Journal of Maxillofacial and Oral Surgery*. 2023 May 16:1-5.
13. Ismaiel WF, Abdelazim MH, Eldsoky I, Ibrahim AA, Alsobky ME, Zafan E, Hasan A. The impact of COVID-19 outbreak on the incidence of acute invasive fungal rhinosinusitis. *American journal of otolaryngology*. 2021 Nov 1;42(6):103080.
14. Mishra Y, Prashar M, Sharma D, Kumar VP, Tilak TV. Diabetes, COVID 19 and mucormycosis: clinical spectrum and outcome in a tertiary care medical center in Western India. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2021 Jul 1;15(4):102196.
15. John TM, Jacob CN, Kontoyiannis DP (2021) When uncontrolled diabetes mellitus and severe COVID-19 converge: the perfect storm for mucormycosis. *J Fungi* 7(4):298. <https://doi.org/10.3390/jof7040298>.
16. Kapoor S, Saidha PK, Gupta A, Saini U, Satya S. COVID-19 associated mucormycosis with newly diagnosed diabetes mellitus in young males-a tertiary care experience. *International Archives of Otorhinolaryngology*. 2022 Nov 4;26:470-7.
17. Veisi A, Bagheri A, Eshaghi M, Rikhtehgar MH, Rezaei Kanavi M, Farjad R. Rhino-orbital mucormycosis during steroid therapy in COVID-19 patients: A case report. *European journal of ophthalmology*. 2022 Jul;32(4):NP11-6.
18. Pandit AK, Tangri P, Misra S, Srivastava MV, Bhatnagar S, Thakar A, Sikka K, Panda S, Vishnu VY, Singh RK, Das A. Mucormycosis in COVID-19 patients: a case-control study. *Microorganisms*. 2022 Jun 13;10(6):1209.
19. Patel A, Agarwal R, Rudramurthy SM, Shevkani M, Xess I, Sharma R, Savio J, Sethuraman N, Madan S, Shastri P, Thangaraju D. Multicenter epidemiologic study of coronavirus disease-associated mucormycosis, India. *Emerging infectious diseases*. 2021 Sep;27(9):2349.
20. Mohanty A, Gupta P, Arathi K, Rao S, Rohilla R, Meena S, Singh A, Kaistha N, Rath RS, Varshney S. Evaluation of direct examination, culture, and histopathology in the diagnosis of mucormycosis: reiterating the role of KOH mount for early diagnosis. *Cureus*. 2021 Nov;13(11).
21. Lackner M, Caramalho R, Lass-Flörl C. Laboratory diagnosis of mucormycosis: current status and future perspectives. *Future microbiology*. 2014 May 1;9(5):683-95.
22. Ismaiel WF, Abdelazim MH, Eldsoky I, Ibrahim AA, Alsobky ME, Zafan E, Hasan A. The impact of COVID-19 outbreak on the incidence of acute invasive fungal rhinosinusitis. *American journal of otolaryngology*. 2021 Nov 1;42(6):103080.
23. Khatri A, Chang KM, Berlinut I, Wallach F. Mucormycosis after Coronavirus disease 2019 infection in a heart transplant recipient—case report and review of literature. *Journal of Medical Mycology*. 2021 Jun 1;31(2):101125.
24. Seidel D, Simon M, Sprute R, Lubnow M, Evert K, Speer C, Seeßle J, Khatamzas E, Merle U, Behrens C, Blau IW. Results from a national survey on COVID-19-associated mucormycosis in Germany: 13 patients from six tertiary hospitals. *Mycoses*. 2022 Jan;65(1):103-9.
25. Cornely, O.A., Alastruey-Izquierdo, A., Arenz, D., Chen, S.C., Dannaoui, E., Hochhegger, B., Hoenigl, M., Jensen, H.E., Lagrou, K., Lewis, R.E. and Mellingshoff, S.C., 2019. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *The Lancet infectious diseases*, 19(12), pp.e405-e421.
26. Alvarez E, Stchigel AM, Cano J, Sutton DA, Fothergill AW, Jagdish Chander JC, Salas V, Rinaldi MG, Guarro J. Molecular phylogenetic diversity of the emerging mucoralean fungus *Apophysomyces*: proposal of three new species.
27. Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. *Journal of Fungi*. 2019 Mar 21;5(1):26.
28. Chander J, Kaur M, Singla N, Punia RP, Singhal SK, Attri AK, Alastruey-Izquierdo A, Stchigel AM, Cano-Lira JF, Guarro J. Mucormycosis: battle with the deadly enemy over a five-year period in India. *Journal of fungi*. 2018 Apr 6;4(2):46.
29. Schwarz P, Guedouar H, Laouiti F, Grenouillet F, Dannaoui E. Identification of *Mucorales* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Journal of fungi*. 2019 Jul 2;5(3):56.
30. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium, Fungal Barcoding Consortium Author List, Bolchacova E, Voigt K. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the national academy of Sciences*. 2012 Apr 17;109(16):6241-6.
31. Cornely, O.A., Alastruey-Izquierdo, A., Arenz, D., Chen, S.C., Dannaoui, E., Hochhegger, B., Hoenigl, M., Jensen, H.E., Lagrou, K., Lewis, R.E. and Mellingshoff, S.C., 2019. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *The Lancet infectious diseases*, 19(12), pp.e405-e421.