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ORIGINAL RESEARCH

Multidisciplanary Approach And Diagnostic Challenges In Covid-19 Cases Associated With Mucormycosis

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ABSTRACT

Background: Mucormycosis is an opportunistic disease caused by Mucormycetes, a group of fungus. Inimmunocompromised individuals such as in diabetes mellitus, neutropenia and organ transplant recipients the fungal spores break through defence mechanism and causes severe systemic infection. During COVID-19 pandemic patients were on glucocorticoids- an immunosuppressive drug which led to high risk of Mucormycosis. Mortality rate is very high due to delay in diagnosis and treatment.

Materials and methods: This study was a retrospective study conducted at Raichur Institute of medical sciences(RIMS), Raichur, Karnataka. Medical records of all the COVID-19 positive cases associated with Mucormycosis were retrieved. Clinical details along with radiological investigations were correlated with microbiology and histopathological diagnosis. A total of 109 cases were included in the study.

Results: Out of the 109 cases included in the study, 86(78.89%) were male and 23(21.1%) were female patients. Majority of the cases were noted in 4th and 5th decade with 29(26.6%) patients in each category. Radiology reports were available in 29 cases which stated as acute on chronic pan sinusitis possibly fungal etiology. 6(5.5%) cases were both fungal culture and histopathology positive for mucor species and 22(20.18%) cases were negative for fungal culture and positive in histopathology diagnosis for mucor species. The reason maybe because of sparsely septate fungi and while handling/processing of biopsy entire cytoplasm oozes out and hence the organism may be lost from the tissue biopsy.

Conclusion: Histopathology when compared to other techniques has advantage as it helps to identify morphology and determine host response in fungal infections. Hence histopathology can be considered as the gold standard for diagnosing mucormycosis.

Key words: Mucormycosis, COVID-19, Histopathology.

Introduction:

Mucormycosis is an opportunistic fungal infection caused by group of molds called Mucormycetes. Most common route of transmission of these fungi is inhalation of spores from the environment. Fungal spores also can gain entry through the damaged skin, gastrointestinal tract and respiratory system which affect lungs and paranasal sinus(PNS). [1]

India was amongst one of the nations to be hit by the global outbreak of COVID-19 pandemic. There was an upsurge of mucormycosis cases among COVID-19 patients in the second wave of COVID-19 pandemic. [2] COVID-19 associated mucormycosis(CAM) was prevalent among diabetes mellitus patients which was the convergence of the two storms. Most common clinical presentation seen were rhino-orbital and rhino-orbital-cerebral.[3]

High incidence of CAM in India was due to various causes which maybe due to immunosuppression resulting from poor glycemic control, steroid use, use of industrial grade oxygen, improper humidification and specific strains of the virus. CAM is a complication of COVID-19 associated with high morbidity and mortality. CAM also delays the recovery of COVID-19 patients resulting in longer hospital stay. Hence early diagnosis and management of Mucormycosis is very essential.[4]

Subjects and Methods:

This was a retrospective observational study conducted at a tertiary hospital in Raichur, Karnataka.

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All the COVID-19 positive patients with mucormycosis were included in the study. Ethical clearance was obtained from the Institutional Ethics committee. The clinical details and radiology reports were obtained from the Medical records section of RIMS, Raichur. KOH and fungal culture reports were obtained from Microbiology Department.

Types of specimens suspected of Mucormycosis obtained in Histopathology Department were maxillectomy, biopsy from nasal sinuses. Histopathology reports of the specimens were obtained from Histopathology section of Department of Pathology. All the Haematoxylin and Eosin stained slides were reviewed. Special stains like PAS and GMS done for the diagnostic purpose were also review

Results:

A total of 109 CAM cases were included in the study. Considering the age, majority cases(72) were above 40 years(66.05%). 86(78.89%) cases were male patients. All the biopsies of 109 cases were obtained from the nose and paranasal sinuses. Demographic details are shown in table1.

Table 1: Results of demographic data

	Number of cases	Percentage
AGE		
<40 years	37	33.94%
>40 years	72	66.05%
GENDER		
Male	86	78.89%
Female	23	21.1%
LOCALISATION		
Nose & paranasal sinuses	109	100%

KOH report was available in all cases amongst which 69(63.3%) cases were positive for fungal hyphae. Only 11(10.09%) cases were positive for Mucormycosis in culture. 37(33.94%) cases were positive for other miscellaneous species. Aspergillus flavus(AF) was reported in 18(48.64%) of the 37 miscellaneous cases. Other miscellaneous species and dual species reported are given in table 2. Radiology reports were available in 29(26.6%) cases. MRI of the nose and paranasal sinus in all the 29cases was reported as acute sinusitis of fungal etiology. Multidisciplinary approach to diagnosis of Mucormycosis is explained in table 3.

Culture positive for other species(n=37)	HP positive for respective species	HP positive for mucor	HP- Nonspecific inflammation	HP-not received
Aspergillus flavus(AF)	2	7	5	4
Aspergillus niger(AN)	0	3	2	0
Rhizopus	0	0	1	1
Candida	0	0	0	2
Microsporum	0	0	1	1

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Syncephalastrum	0	1	1	0
AF+AN	0	0	1	0
Mucor+AN	0	0	1	0
AF+Syncephalastrum	0	1	0	0

Table 2: Results-Miscellaneous cases(n=37)

	POSITIVE	NEGATIVE
KOH	69(63.3%)	40(36.69%)
CULTURE Mucor Miscellaneous	 11(10.09%) 37(33.94%)	61(55.96%)
HISTOPATHOLOGY Mucor Nonspecific inflammation Miscellaneous	41(37.61%) 30(27.52%) 6(5.5%)	32(29.35%)
RADIOLOGY Acute sinusitis of fungal etiology	29(26.6%)	

Table 3: Results: Multidisciplinary approach to diagnosis of Mucormycosis

Histopathology was positive for Mucormycosis species in 41(37.61%) and negative in 32(29.35%) of the cases. Broad pauciseptate or aseptate ribbon like fungal hyphae branching at 90 angle resembling Mucormycosis species(figure 1,2) were seen surrounded by granulation tissue, acute inflammation and granulomas along with foreign body giant cells in few of the cases. Special stains like periodic acid schiff(PAS) stains were done for confirmation of the species(figure 3). Nonspecific inflammation was noted in 30(27.52%) cases. Miscellaneous species were noted in 6(5.5%) cases. No fungal hyphae or inflammation was noted in 32(29.35%) cases.

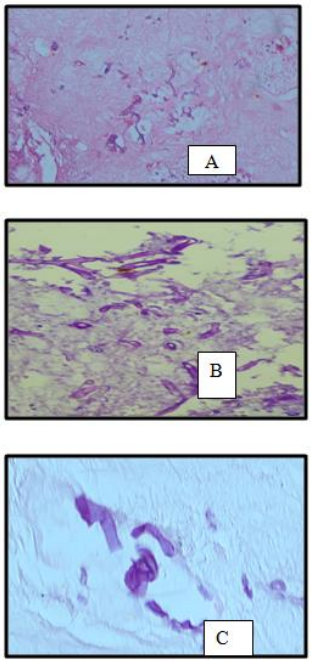

Figure 1A: Fungal hyphae of Mucormycosis species(10x magnification H & E). 1B: Fungal hyphae of Mucormycosis species(40x magnification H & E). 1C: Fungal hyphae of Mucormycosis species(40x magnification PAS stain).

Discussion:

Mucormycosis or "black fungus" infection is caused by a member of the order Mucorales including 11 genera and 27 species that can cause human infections. It is angioinvasive and can virtually affect any organ. In the second wave of COVID-19, it was observed that rate of Mucormycosis infections increased in COVID-19 patients and these cases had significant poor prognosis. Delayed diagnosis of CAM is mainly due to difficulty in taking invasive

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tissue biopsies and unease of aerosol-generating procedures in oral and maxillofacial surgery in COVID-19 patients.[5]

CAM was reported more commonly among age groups above 40 years. Similarly mean age of CAM patients was 44.5 years in a case series by Balushi et al.[6] Majority of the patients were male in our study which correlated with review studies done by Pal et al and Hoegniel et al which reported 78% male patients.Rhino-orbital Mucormycosis was the most common presentation in our study which can be correlated with study done by Pal et al and similarly rhino-orbito-cerebral was the most common presentation in study done by Hoegniel et al and Patel et al.[7,8,9]

Rapid diagnosis of clinically suspected cases is done by KOH mount which detects presence of fungal hyphae which are broad aseptate and branching at right angles. Reasons for KOH negativity may be due to contamination or the portion of tissue that was processed did not have fungal hyphae.[9] Culture is essential for diagnosis but it's time consuming. Few friable fungal hyphae may get damaged during tissue manipulation processes like homogenisation and grinding which may result in low positivity rate in culture. The portion of tissue with no fungal element can also lead to low positivity in culture.[5,10]

Advantages of HPE over culture helps in identification of morphology, true infection, tissue invasion and angioinvasion. Availability of special stains increases the sensitivity of histopathological identification of mucormycosis. However histopathological examination(HPE) can miss the fungal elements because of kinking, folding, fragmentation, necrosis and atypical morphological features.[11]

Histopathology is particularly valuable in distinguishing between contaminants, colonizers, and actual causative pathogens. Histopathology provides important information about tissue invasion and host response. Histopathology also identifies coinfections with other moulds, but it does not identify the genus/species of fungus. Fungal pathogens often exhibit similar morphologies in tissue sections, making it challenging to differentiate them based on histopathology alone. Correlation with microbiology findings is essential.[12,13]

Disease severity and prognosis also can be assessed by histopathology. Higher fungal load, extent and severity of inflammation, presence of granulomas and the degree of angioinvasion are the factors which help in assessment of severity and thus in need of extensive surgical intervention and debridement.[14,15]

Conclusion:

Though histopathology is gold standard, it is challenging to differentiate Mucor species with other fungi mimicking with similar morphology. Hence correlation with radiology and microbiology findings will help in definitive diagnosis. Multidisciplinary approach is essential for the definitive diagnosis thereby reducing the disease morbidity and mortality.

Limitations of the study:

Low positivity rate in culture may be due to sample site not taken from the same site for culture and histopathology which is not equally representative. Histopathology distinguishes the presence of fungal pathogen but cannot identify the genus/species of fungus. Culture can identify the species and anti fungal susceptibility but false positive results are more.

References:

1. Brown J. Zygomycosis: an emerging fungal infection.Am J Health Syst Pharm 2005;62(24):2593-6.
2. Pasquier G. COVID-19-associated mucormycosis in India: Why such an outbreak?. J Med Mycol 2023;33(101393):1-5.
3. John TM, Jacob CN, Kontoyiannis DP. When uncontrolled diabetes mellitus and severe COVID-19 converge: the perfect storm for mucormycosis. J Fungi 2021;7:298.
4. Chaganti PD, Katta R, Yerukalapudi SH, Karri MD, Firdous A. Mucormycosis in Post Novel Corona Virus Patients:An Institutional Experience. Indian J Otolaryngol Head Neck Surg 2023;75:617–23.
5. Osman NA, Anwar MM, Singh B, Gupta GK, Rabie AM. A peek behind the curtain in the diagnosis and management of COVID-19-Associated Mucormycosis (CAM). J Egypt Public Health Assoc 2023;98(4):1-9.
6. Balushi A, Ajmi A, Sinani Q, Menon V, Berieki Z, Shezawi A et al. COVID-19 associated Mucormycosis: An opportunistic fungal infection. A case series and review. Int J Infect Dis 2022;121:203-10.
7. Pal R, Singh B, Bhadada SK, Banerjee M, Bhopal RS, Hage N et al. COVID-19 associated mucormycosis: An updated systematic review of literature. Mycoses 2021;64:1452-9.
8. Hoenigl M, Seidel D, Carvalho A, Rudramurthy SM, Arastehfar A, Gangneux J et al. The emergence of COVID-19 associated mucormycosis:a review of cases from 18 countries. Lancet Microbe 2022;3: e543–52.
9. A. Patel, H. Kaur, I. Xess, J.S. Michael, J. Savio, S. Rudramurthy et al. A multicentre observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India. Clin Microbial Info 2020;26:944.
10. Hasan S, Gupta P, Shukla D, et al. A comparison between Potassium Hydroxide (KOH) microscopy and culture for the detection of post-COVID-19 rhino-orbital-cerebral Mucormycosis. Cureus 2023;15(10):1-6.
11. Sangoi AR, Rogers WM, Longacre TA, Montoya JG, Baron EJ, Banaei N. Challenges and Pitfalls of Morphologic Identification of Fungal Infections in Histologic and Cytologic Specimens: A Ten-Year Retrospective Review at a Single Institution. Am J Clin Pathol 2009;131:364-75.
12. Nielsen M, Cerqueira FM, Kavuri SB, Raymond C, Muneeb A, Kudlicki A et al. Diverse Clinical Manifestations and Challenges of Mucromycosis: Insights from serial cases. Open Forum Infect Dis 2023;1-6.
13. Ahmed, S. M., Divyarani, M. N., Roopa, A. N., Sowmya, S. M., Mohammed, R., Baig R et al.

DOI: 10.69605/ijlbpr_14.2.2025.97

Histomorphological study of mucormycosis in COVID-19 patients. Int J Health Sci 2023;7(S1):2875–2886.

14. Shanmugasundaram S, Ramasamy V, Shiguru S. Role of histopathology in severity assessments of post-COVID-19 rhino-orbital cerebral mucormycosis - A case-control study. Ann Diagn Pathol 2023;67(152183):1-7.
15. 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 Med 2022;10(1-8).