

Original Research Article**HISTOPATHOLOGICAL ANALYSIS OF POST COVID MUCORMYCOSIS CASES IN CORRELATION WITH KOH MOUNT AND CULTURE – A PROSPECTIVE STUDY IN TERTIARY CARE CENTER**

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ABSTRACT

INTRODUCTION : Fungi belonging to the Mucoracea family are usually saprophytic. Mucormycosis disease is lately becoming prevalent since the pandemic of COVID –19. Immunosuppression and immunomodulation have long been postulated as potential risk factors for opportunistic systemic fungal infections. Examination of histopathological features, direct microscopy using potassium hydroxide (KOH) and fungal culture are considered to be the traditional diagnostic approaches for Mucormycosis disease.

MATERIALS AND METHODS : This was an observational study carried out at a tertiary care hospital for a period of one year. A total of 247 patients with biopsy proven diagnosis of rhino – maxillary – orbital mucormycosis were included in this study. Histopathological features were studied and correlation was made with direct microscopy by KOH and fungal culture.

RESULTS : Mucormycosis was found to be more prevalent in males (177 cases, 71.66 %) . Age groups mostly affected were between 41 – 50 years (84 cases, 34 %) followed closely by 51 – 60 years (77 cases, 31.17 %). Maxillary sinus (134 cases, 54.25 %) was the most commonly affected site. Non invasive fungal infection was frequently encountered (217 cases, 87.85 %). Direct Microscopy by KOH was positive in 79 cases (31.98 %), while fungal cultures were positive in 65 cases (26.32 %).

CONCLUSION : Histological evaluation remains the mainstay of diagnosis in Mucormycosis over the conventional Microbiological techniques. Identification of the disease at an incipient stage is crucial to prevent fatal outcomes.

KEY WORDS : Mucormycosis, COVID, Histopathology

INTRODUCTION :

Mucormycosis has been considered as one of the most lethal and rapidly progressive form of systemic fungal infections in humans. The ability of the Zygomycetes order of fungi to easily invade blood vessels and soft tissues has contributed to the adverse clinical outcomes associated with the disease. Hosts with immunosuppressive states like diabetes mellitus, hematological malignancies, renal failure and viral infections like HIV, COVID – 19 are susceptible.[1] The most frequently encountered clinical form is Rhino – Orbital – Cerebral Mucormycosis as the primary route of infection is via inhalation of fungal spores.[2] The current gold standard diagnostic methods of Mucormycosis include direct microscopy, culture studies and Histopathology.

Tissue biopsy provides morphological diagnosis of the fungi by observing pauciseptate or aseptate ribbon like hyphae with right angled branching.[3] It not only helps to gauge the extent of soft tissue invasion but also identifies vascular invasion. Special stains like Periodic Acid Schiff (PAS) and Grocott's - Gomori's Methenamine Silver (GMS) offer superior visualization of the fungal wall and its relation with surrounding tissues. However biopsy does not specifically identify the fungal species within the Zygomycetes order.[2]

Direct microscopy by Potassium Hydroxide (KOH) confers the advantages of prompt intraoperative diagnosis, delineate surgical margins and helps to institute antifungal agents early. However it cannot distinguish between pathogenic and colonisers, contaminant fungi.[4]

Fungal Culture is deemed the gold standard for speciation of most of the fungal pathogens. However Mucorales have a poor growth capacity in ordinary fungal culture media. Limitations also include homogenization of tissue, different sampling sites, nonviable organisms and use of preservatives containing anaesthetic agents.[5]

This study highlights the increased prevalence of Mucormycosis in biopsy specimens during COVID – 19. Associated tissue changes, concordance with KOH microscopy and fungal culture were duly studied.

MATERIALS AND METHODS :

This observational study was conducted in the Department of Histopathology of a tertiary care teaching hospital for a period of one year. Institutional ethics committee approval was obtained prior to the start of the research.

Analysis of biopsy samples of suspected Mucormycosis in post COVID – 19 patients was made. Biopsy samples pertained purely to Rhino – Maxillary – Orbital – Cerebral locations.

Clinical data was retrieved from hospital information system. Formalin Fixed Paraffin Embedded (FFPE) tissue sections OF 4 – 5 μm were studied following routine Hematoxylin and Eosin (H & E) staining by two independent Pathologists. Special stains with PAS and GMS were employed. Microscopy with KOH and fungal culture reports of the same patients were retrieved

from the archives of Microbiology department. Nonpigmented, wide (5 – 20 μm), ribbon like hyphae with pauci septations or aseptate, right angle branching were identified as fungi of Mucorales genera. Tissue changes such as necrosis, nature of inflammation, vascular and soft tissue invasion were studied. Coexistent fungi like *Aspergillus* was also noted.

RESULTS :

Biopsy samples from 247 patients with confirmed COVID – 19 positivity were studied in total. All the biopsy samples showed Mucormycosis disease (100 %). Non invasive form of the disease (confined to superficial epithelium) accounted for 217 cases (87.85 %). The rest showed deeper invasion.

Males were mostly affected by Mucormycosis. Wide age range was noted from as early as 20 years to 75 years. Majority of the patients belonged to the age group between 41 – 50 years (84 cases, 34.01 %) followed closely by 51 – 60 years (77 cases, 31.17 %). As shown in table 1.

Table 1 : Age wise Distribution of Mucormycosis Cases

S.No	Age in years	Number of Subjects	Percentage of cases
1.	1 - 20	01	0.4 %
2.	21 - 30	05	2.02 %
3.	31 - 40	28	11.34 %
4.	41 – 50	84	34.01 %
5.	51 - 60	77	31.17 %
6.	61 – 70	47	19.03 %
7.	71 - 80	05	2.02 %
	Total :	247	100 %

Maxillary sinus was mostly involved in 134 cases (54.25 %). Other locations were hard palate (52 cases, 21.05 %), nasal septa / turbinates (38 cases, 15.38 %) and orbit (23 cases, 9.3 %). No brain involvement was noted.

Macroscopic examination shows the tissue samples to be predominantly grey white to black in colour. Tissue necrosis was noted in 183 cases (74.09 %).

Predominant inflammatory response was mixed inflammatory (142 cases, 57.49 %) followed by neutrophilic pattern (83 cases, 33.60 %). Granulomas were noted in 4 cases (1.62 %). Mucorales along with *Aspergillus* species were identified in 4 samples. Angio invasion was noted in 24 subjects while bony invasion with necrotic bone was seen in 5 subjects as shown in **Figures 1 – 4**. On direct microscopy by KOH mount, 79 out of 247 cases (31.98 %) turned out to be positive, while 65 out of 247 (26.32 %) showed growth on fungal culture.

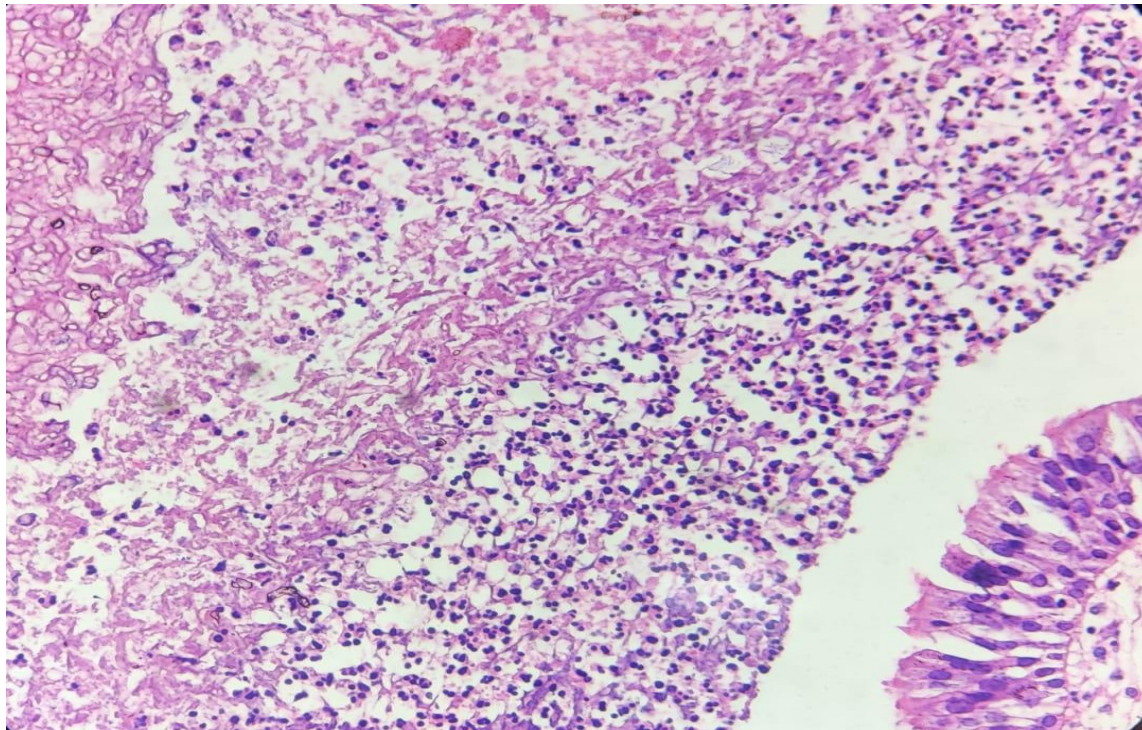


Figure 1 : Respiratory Epithelium (Right Lower Corner) with Inflammatory exudate and Fungal Hyphae (Left Upper Corner), PAS Stain, 400x

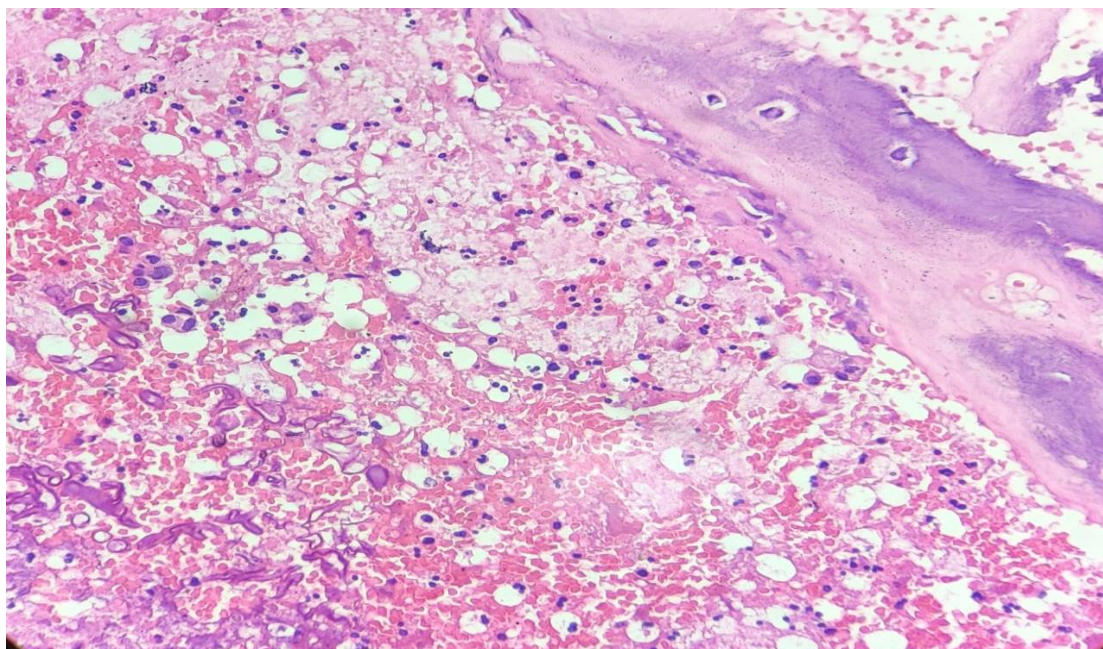


Figure 2 : Fungal Hyphae (Left lower corner) with Inflammatory exudate with adjacent bony trabeculae, PAS Stain, 400x

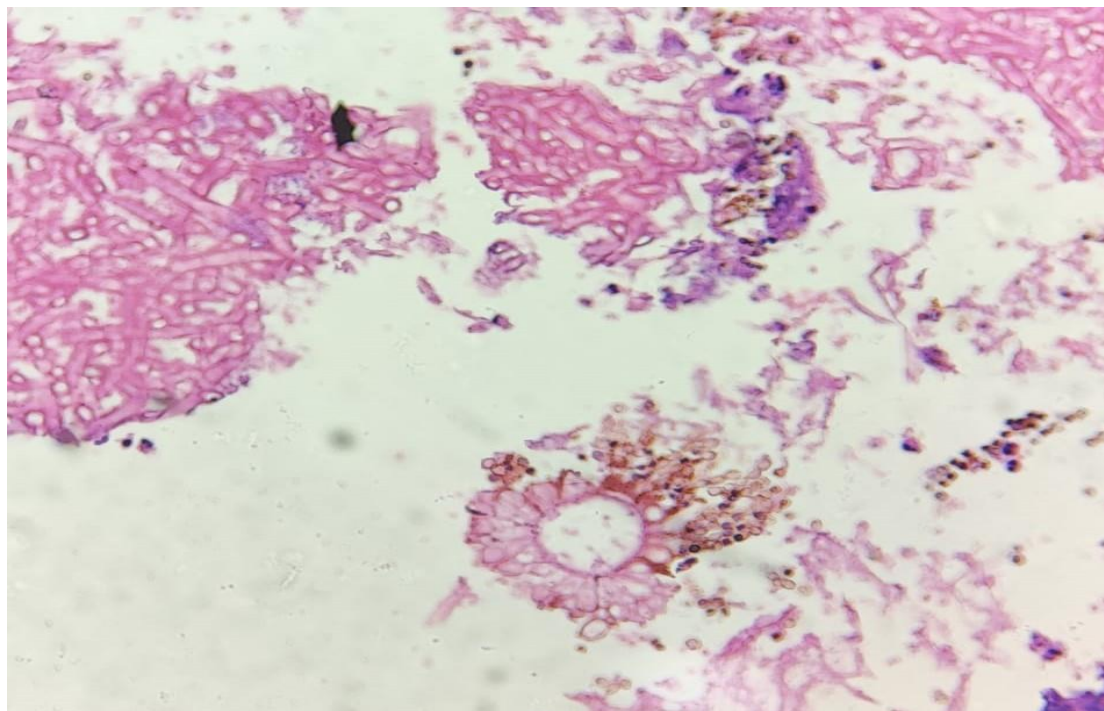


Figure 3 : Hyphae of Mucormycosis with Fruiting Body of Aspergillus, PAS Stain, 400x

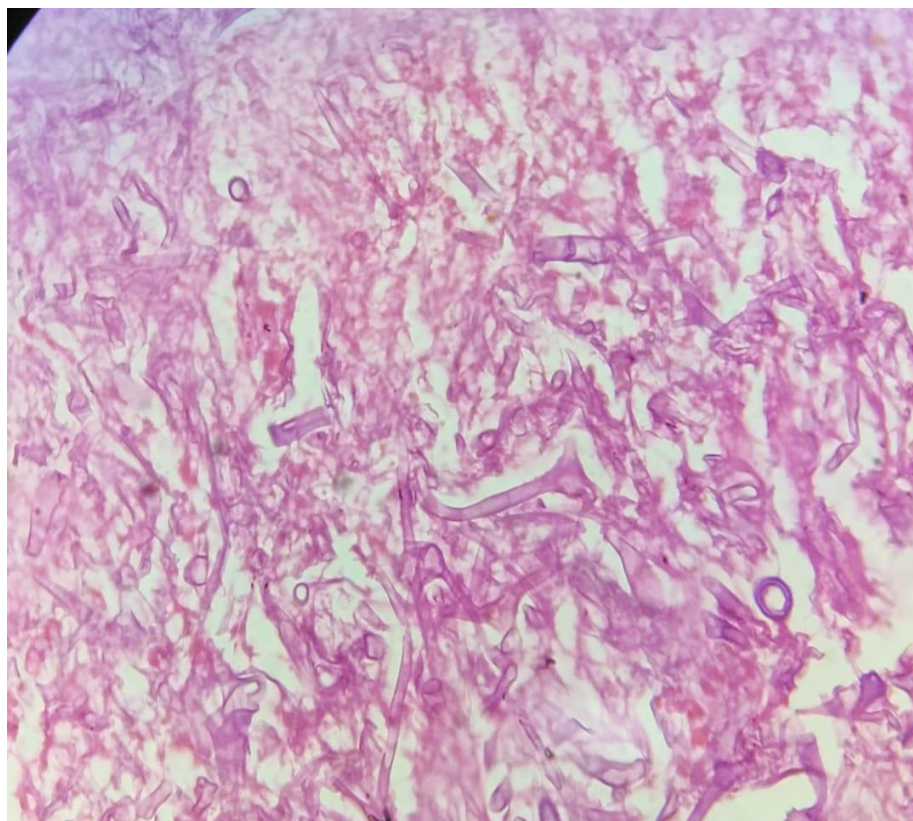


Figure 4 : Characteristic Broad, Pauciseptate or aseptate hyphae of Mucormycosis with right

angled branching, PAS Stain, 400x

DISCUSSION :

Mucormycosis is a reemerging fungal infection that has been in limelight since the outbreak of COVID – 19. Opportunistic systemic fungal infections often follow immunosuppression. In India, Mucormycosis was declared as an epidemic in the midst of an ongoing pandemic of COVID – 19.

Clinical presentation mostly noted was that of Rhino – Orbital – Cerebral Mucormycosis (CAROCM).[6] Various hypotheses proposed for the pathogenesis of CAROCM include COVID – 19 virus associated immune disruption, significant cytokine mediated inflammation, high iron levels and endothelial disruption.[7] Additional risk factors were co morbidities like diabetes, hyperglycemia and immune dysregulation by extensive use of steroids, broad spectrum antibiotics. Inhalation of steam advised to relieve nasal congestion in COVID was also found to facilitate Mucorale infection by mucosal alteration.[8]

In concordance with the studies of Bala et al and N. Ganeshan et al, our study showed higher preponderance of Mucormycosis infections in males.[1,9] Higher incidence in the 41 – 50 year age group correlates with the median age findings of Bala et al and Prakash et al.[9,10] Median age of the subjects was 57 years in a study by Arora et al which corresponds to the second highest age group in this study.[11]

Owing to the colour of the necrotic tissue, this disease was widely addressed to as “ Black Fungus ”.[12]

Pakdel et al in their study found Rhino – Maxillary – Orbital type to be the most common (47 %) followed by Sino – Orbital type (33 %) and Sino – Nasal type (7 %).[13] In the present research, there was a higher percentage of patients with Sino Nasal involvement. Histologically, acute lesions of Mucormycosis show hemorrhage, coagulative necrosis with neutrophilic infiltration while chronic lesions show pyogranulomatous inflammation with giant cells.[14] The current study documents similar classical features. With regards to soft tissue and angio invasion, our study showed significantly lesser percentages in contrast to the findings of Jain et al and Frater et al.[15,16] This may be due to the early presentation and superficial nature of the disease. Mixed infection with *Aspergillus* was also noted akin to the studies of Jain et al and Pal et al.[15,17]

Concordance was noted between Histopathology and direct microscopy by KOH mount in 31.98 % of cases. Histopathology and Fungal culture showed 26.32 % concordance. Different sampling sites, delay in culture inoculation, diversion of technical manpower towards COVID virus diagnosis leading to short staffing could be the reasons for the disparity of diagnosis between Histopathology and Microbiological diagnostic tests.

LIMITATIONS : This study was a laboratory based research. Clinical signs and symptoms of Mucormycosis, patient centric data concerning co morbidities, radiographic findings, treatment availed were not included.

CONCLUSION :

Mucormycosis is a life threatening infection, with a very high mortality. Histopathological examination is affirmed to be a gold standard in early identification of Mucormycosis. It is crucial to understand the interactions between the fungal pathogens and the host immune

mechanisms. This understanding will certainly aid in the development of newer point of care tests and biological therapies.

CONFLICT OF INTEREST : None

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