



A RETROSPECTIVE ANALYSIS OF HISTOPATHOLOGICAL FINDINGS IN RHINO-ORBITAL FUNGAL INFECTION IN POST COVID 19 PATIENTS

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ABSTRACT

Introduction: Health care sector across India reported significant increase in cases of covid linked mucormycosis and other fungal infections in covid 2nd wave. Mucormycosis is an opportunistic fulminant fungal infection in immunocompromised host. Concomitant diabetes and use of high dose steroid, further add to already compromised immunity in covid patients. It may manifest as rhino-orbital or cerebral mucormycosis. Fungus invades the arterioles and causes tissue necrosis, which is the hallmark of mucormycosis. **Objectives:** To study frequency and histomorphology of various tissue invasive fungal infections and their correlation with other parameters. **Material and Methods:** This is retrospective observational study. Here we present histological and other findings in 236 cases of clinically suspected post covid fungal infection during 2-month periods. **Result:** Out of 236 specimens received, 211 showed presence of tissue invasive fungal infection with positivity rate of 89.4%, where male to female ratio was 2.2:1, mean age was 51 years. Most common organism detected was mucor species in 72%, Aspergillus in 4.3% and 23.7% had mixed infection. Angioinvasion was seen in 38.86%. The most common site of involvement was sinonasal (84.36%) followed by orbital (15.16%) and cerebral (0.47%). 68.18% patients were diabetics, 39% had history of moderate to severe covid requiring oxygen, 72.7% had received steroid. **Conclusion:** Diabetes mellitus and rampant use of corticosteroid in a background of Covid-19 appears to increase opportunistic fungal infection. Mucor and aspergillus were commonly observed tissue invasive and angioinvasive fungal species.

KEYWORDS : Mucormycosis, Covid 19, Tissue invasive fungal infection

INTRODUCTION

Coronavirus disease 2019 (COVID-19), a leading cause of an emergency global pandemic caused by novel SARS-CoV-2 has affected more than 635 million people worldwide, accounting for over 6.6 million deaths till date. ⁽¹⁾ The disease has been associated with varying degree of illness ranging from mild cough to life-threatening pneumonia. ⁽²⁾ In the second wave of COVID, India also witnessed an upsurge in cases of fungal rhinosinusitis with/ without associated orbital or cerebral involvement by Mucormycosis in post COVID patients. India is among the worst affected country by Mucormycosis, the prevalence of which is nearly 70 times higher than the global data ⁽³⁾.

Mucormycosis is a potentially life-threatening, opportunistic, invasive, fungal infection commonly called the "Black fungus". ⁽²⁾ Aspergillosis and candidiasis are other subtypes of fungal sinusitis observed in post covid patients in some studies. ^(4,5)

A complex spectrum of interrelated factors behind the rise in cases of fungal rhinosinusitis include systemic immune alteration by COVID-19, poor glycemic control in DM, poorly monitored/ rampant use of systemic corticosteroid therapy, pre-existing comorbidities, possible nosocomial sources due to prolonged hospitalization and O₂ need, overexpression of certain cytokines and inflammatory markers also called 'Cytokine Storm' and decreased T helper (CD4 and CD8) cells also makes individual vulnerable to a wide range of opportunistic bacterial and fungal co-infection. ⁽²⁾

Rhino-orbito-cerebral Mucormycosis causes high mortality

and morbidity due to the angioinvasive. properties of the fungus, causing vascular occlusion and consequently resulting in extensive tissue necrosis, which is a hallmark of Mucormycosis. ⁽⁶⁾ The most commonly employed technique for diagnosis and differentiating between different fungal organism is a histopathological study which shows the morphology of fungal hyphae, invasion of tissue by fungal hyphae, fungal angioinvasion, perineural invasion in the background of necrotic and inflamed tissue. ⁽⁵⁾ The primary aspects of effective care of this fatal infection include early identification, surgical debridement, appropriate antifungal medication, and control of risk factors such as diabetes mellitus ⁽⁴⁾

The intent of this research was to study various morphological patterns of tissue invasive fungal infection, appearance of fungal forms, frequency of various fungal subtypes and mixed infection and also to study associated factors like comorbidities, use of steroids and other COVID related parameters.

MATERIALS AND METHODS

This is retrospective cross-sectional study conducted at the Department of Pathology of a tertiary care hospital during the period of 2 months from May – June 2021. Institutional Ethics committee approval was obtained, prior to the start of the research. The study includes data of tissue specimens of all the suspected cases of rhino orbital Mucormycosis in post covid patients that were received during the study period at Histopathology section of Department of Pathology. All the study cases were those with known Covid positive status (

tested by RT PCR)

Clinical details of the patients including demographic detail, clinical features, site of infection, radiological investigation findings, history of comorbidities, COVID-19 infection & treatment related details were noted from the histopathology requisition form and the patient's cases sheets and recorded in predesigned proforma.

Adequate representative tissue samples were received in 10% neutral-buffered formalin for histopathological examination. The tissue specimens were examined for gross characteristics.

Macroscopically visible necrotic areas or blackish areas were selected for processing and block preparation. Five-micrometer-thick sections were stained with routine hematoxylin and eosin (H&E) stain for histopathology evaluation and special stains like Gomori Methenamine Silver (GMS) and Periodic Acid Schiff (PAS) were used to highlight the fungal morphology. The slides were examined independently and blindly by three different pathologists.

Histopathological findings were recorded including various morphological patterns of fungal organism as well as other tissue reaction like presence of acute/chronic inflammation, presence of tissue necrosis, granulomatous/giant cell reaction, tissue invasiveness of fungal organism, presence/absence of vascular and perineural invasion and presence of other significant associated findings if any were noted.

Mucorales genera were identified based on the characteristic histological findings like of broad aseptate non-pigmented, wide (5–20 μm), ribbon-like fungal hyphae with right angle branching on H&E staining, under 40 x magnification, Aspergillus species was demonstrated by nonpigmented (hyaline), narrow, septate hyphae with acute-angle branching with/ without presence of conidial forms. The pathological diagnosis of the lesion was established on the basis of the characteristic fungal hyphae in the background of necrotic and inflamed tissue.

All the findings were entered in excel sheets; descriptive statistical analysis was done. Appropriate charts and graphs were prepared in terms of frequency.

RESULTS

The suspected cases of rhino-orbital - cerebral Mucormycosis in patients with known covid history (tested positive by RTPCR) admitted to our institute were included in this study. During the study periods of 2 months (may - June 2021) biopsy specimens from 236 patients of clinically suspected post COVID fungal rhinosinusitis were received at histopathology section, pathology dept.

Out of 236 specimens received, 211 (89%) showed presence of tissue invasive fungal organism. The samples received were from patients with a wide age range, ranging from 20 years to 85 years and the mean age was 50.9 years. A male predominance 145 (69%) was observed with a male to female ratio of 2.2:1 (Chart 1,2).

SEX DISTRIBUTION

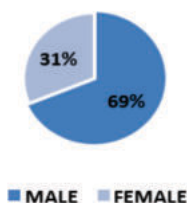


Chart 1: Sex Distribution

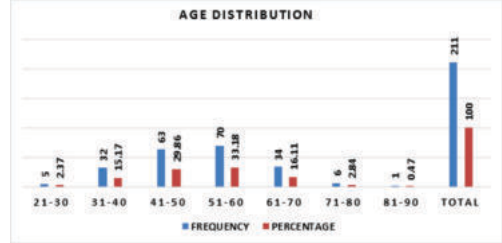


Chart 2: Patient Distribution According To Age

Required clinical details for data analysis was possible to retrieve in 110 cases. Based on which following results were drawn. All the study cases were confirmed COVID 19 positive and were clinically diagnosed with fungal rhinosinusitis after a mean of 22.67 days post covid diagnosis. 46% patients had moderate to severe Covid requiring O2 support and 53% had mild infection of Covid 19 and recovered with oral medication. 72.72 % of patients had received systemic corticosteroid therapy. 76.36 % patients were diabetic, and 20% patients were hypertensive. (Table 1)

All patients presented with one or other local or constitutional symptoms or signs. The most common complaint at the time of presentation was facial swelling (45%), followed by headache (44%), pain in teeth and gums (12%), proptosis (10%), and eye pain with periorbital swelling (8%). (Table 1)

Table:1 Clinical Details Available In 110 Suspected Rhino Orbital Fungal Infection In Post Covid Patients

CLINICAL DETAILS	FREQUENCY (n = 110)	PERCENTAGE (%)
DIABETES MELLITUS STATUS		
DIABETIC	84	76.36%
NON-DIABETIC	26	23.63%
HYPERTENSION STATUS		
HYPERTENSION	23	20.90%
NO HYPERTENSION	87	79.09%
STEROID		
YES	80	72.72%
NO	30	27.27%
O2 STSTUS		
GIVEN	51	46.36%
NOT GIVEN	59	53.63%
DAYS FROM COVID		
<20 DAYS	62	56.36%
>20 DAYS	48	43.63%
SYMPTOMS		
FACIAL SWELLING	51	45.45%
HEADACHED	49	44.55%
PAIN IN GUMS AND TEETH	14	12.73%
PROPTOSIS	11	10.00%
PERIORBITAL CELLULITIS	10	8.18%

Out of 211 cases of tissue invasive fungal infection, the most common location was the nasal cavity and paranasal sinuses 178 (84.36%) followed by the orbital 32 (15.17 %). Intracranial extension was noted in 01 (0.47%), Among the paranasal sinuses, the maxillary sinus was most commonly involved 137 (65%) followed by ethmoid sinus.

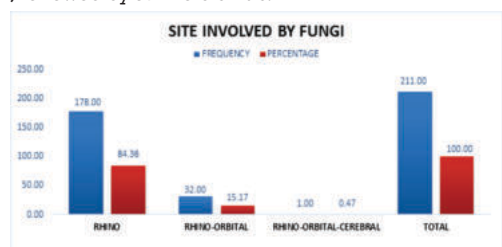


Chart: 3 Site Of Involvement

Macroscopic examination revealed the tissue samples to be predominantly greyish white to black in colour and mainly soft to friable in consistency. Under the light microscope in H&E staining, out of 236 specimens received, 211 cases showed fungal hyphae morphologically characteristic of Mucormycosis, aspergillus or both with prominent inflammation and tissue necrosis (Image 1-4). Extensive areas of necrotic tissues were more often associated with abundant fungal load. Most of the tissue samples showed suppurative and mixed type of inflammatory response mainly comprised of neutrophils, eosinophils, lymphocytes, macrophages and plasma cells. Multinucleated giant cell response/granulomatous inflammation were noted in a few cases 46 (21 %) (Image 6,7). Areas of haemorrhage and thrombosed vessels, necrotic bony fragments were also exhibited in the tissue examined. Some crystalloid material was also seen in submucosal areas in few cases.

The dominant fungus identified in the specimens were mucorales in 152 samples (72.04%), mixed aspergillus, along with mucorales was identified in 50 samples (23.70%) whereas aspergillus alone was noted in 09 cases (4.27 %). (Chart 4). The histological findings of mucorales genera and aspergillus H&E (Image 1-4) and special stains (PAS &GMS) (Image 9,10) are represented on aspergillus fungal forms also showed conidial forms/ Many black pigmented fruiting bodies along with fungal colonies. (Image 3)

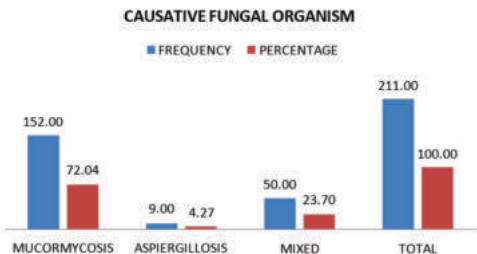


Chart: 4 Morphological types of fungus identified in the specimen

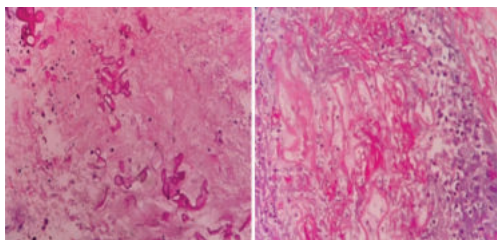


Image 1,2: Broad nonseptate fungal hyphae morphologically consistent with Mucormycosis

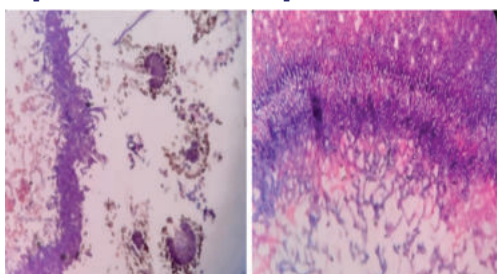


Image 3,4: Thin branching septate hyphae morphologically consistent with Aspergillus

Angio invasion was identified in 82 (38.56%) of tissue samples (Image 8). It was evident by the presence of fragmented fungal elements within vascular lumen, invading the blood vessel wall associated with formation of thrombi in some vessels and surrounding tissue necrosis (Image 5). Perineural / neural invasion was identified in 4 (1.9%) of samples with the invasion of the nerve bundles by fragmented fungal hyphae.

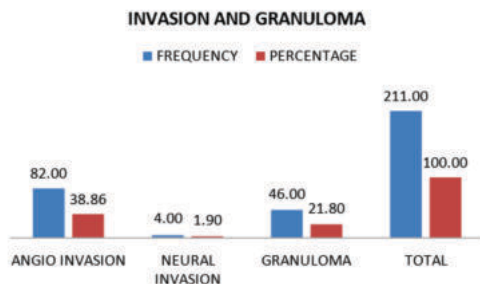


Chart: 5 Frequency of Angio – Perineural Invasion and Granulomatous response

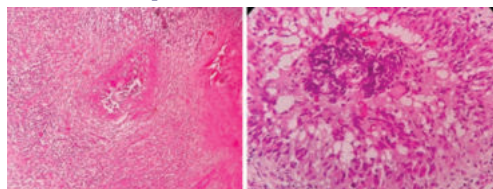


Image 5: Tissue Necrosis

Image 6: Granulomatous Inflammation

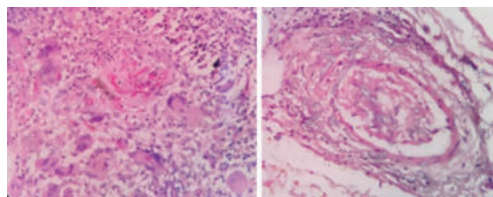


Image 7: Foreign Body Giant Cell Reaction

Image 8: Angioinvasion By Fungi

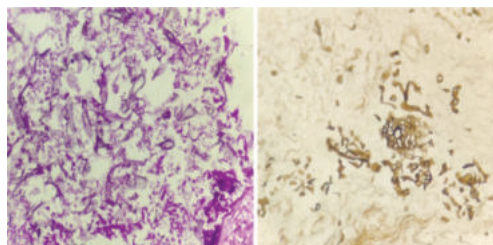


Image 9: Special Stain PAS

Image 10: Special Stain GMS

DISCUSSION

Mucormycosis is an aggressive opportunistic fungal infection also known as “phycomycosis or zygomycosis”. Rhizopus is one of the commonest fungi causing this disease. (7)

A significant increase in number of cases of covid linked Mucormycosis was reported in India during 2nd wave with sudden increase during the period between April and July 2021. The reason behind a sudden surge in these cases may be explained by the presence of a high load of Mucor spores in the hospital and community due to the tropical and humid climate, the high prevalence of uncontrolled or latent diabetes cases, poor monitoring of the dose and duration of steroids used to treat COVID-19 cases, and poor maintenance of frequently used oxygen mask. (3) in the present study 84 (76.36 %) cases were found to be diabetic and systemic corticosteroid therapy was given in 80 (72.72 %) of patients. Study done by Laxmi et al and Awdhesh et al also showed strong association between diabetes mellitus and use of corticosteroids in the treatment of COVID-19 and development of Mucormycosis in their studies. The possible mechanisms for increased growth of mucor would be uncontrolled hyperglycemia and precipitation of diabetic keto acidosis due to corticosteroid intake which decrease the phagocytic activity of white blood cells. (8) Patients with diabetes and hyperglycemia often have an inflammatory state that could be potentiated by the activation of antiviral immunity to SARS-

CoV2, which might favor secondary infections⁽⁶⁾

In the present study, 152 (72.04%) cases of COVID associated rhino- orbital- cerebral Mucormycosis were noted, mixed infection with mucor and aspergillus was identified in 50 (23.70 %) of cases and only aspergillus was noted in 09 (4.27 %) of cases. Jain et al. also observed that out of 90 cases of COVID associated rhino cerebral Mucormycosis, mixed infection with aspergillus was noted in one patient and with candida in two cases. Pal et al. also found four cases of Mucormycosis along with aspergillosis, in 99 patients of COVID associated Mucormycosis. Nidhya et al found mucorales in 96.67%, samples out of which aspergillus, along with mucorales was identified in 20% samples whereas a combination of mucorales and candida was noted in 13.33% cases. Present research identified higher percentage of Mucor and aspergillus mixed infection, which could be due to the environmental and geographical difference in the distribution of the fungi.

In the present research the sino-nasal type was the most common site of involvement in 178 (84.36 %) followed by rhino-orbital involvement 32 (15.17%). Cerebral involvement by Mucormycosis was identified in 1 (0.4%) case. Awdhesh and Nidhya et al also found sino-nasal type to be the most common site of involvement (76%,88.9% respectively) followed by rhino-orbital involvement. Maxillary sinus was the commonest sinus involved followed by Ethmoid sinus in our study.

The direct histological examination of scraping or biopsies of involved tissue demonstrating broad, irregular, non-septate hyphae in the background of necrotic and inflamed tissue is diagnostic of mucorales. Histopathology not only distinguishes the presence of the fungus in the specimen but also identifies the blood vessel invasion. It can furthermore reveal the presence of coinfections with other fungal organisms like Aspergillus sp.in debrided tissue. Special stains such as Grocott methenamine- silver (GMS) and periodic acid-Schiff (PAS), help identifying fungal morphology by highlighting the fungal wall and also gives a better visualization of the surrounding tissue. High fungal density was specifically noted in areas of extensive tissue necrosis in present study; hence the necrotic areas must be sampled well. Infarction and haemorrhage are due to the angioinvasive nature of the hyphae that cause vessel wall destruction and luminal thrombosis. The varying pattern of inflammation observed in the present study could explain the virulence of the organisms, type of viral strain and the immune status of the patient. The prognosis is generally good for patients with enhanced immune mechanisms, which leads to the formation of granulomatous inflammation with low fungal load. In the current research,21.8 % of the cases showed granulomatous inflammation. In our study fungal hyphae invading blood vessel (Figure 4b) (angioinvasion) was seen in 82 cases (38.86%).

CONCLUSION

Mucormycosis can be a serious complication in cases of post COVID- 19 infection. A triad of uncontrolled diabetes mellitus, rampant use of steroids and possibilities of immunosuppression due to COVID-19 pathophysiology is mainly responsible for sudden surge of covid associated Mucormycosis. All efforts should be made to control glucose levels and to appropriately use the steroids.

A proper diagnosis to distinguish Mucormycosis from other bacterial and fungal infections is vital for early treatment and favorable outcomes. Histology not only helps to give early diagnosis by identifying fungal morphology but also defines the morphology of the tissue reaction, and the presence of tissue or blood vessel invasion which help predicting disease progress and severity and guide the clinician to optimize treatment accordingly.

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