

**CLINICOPATHOLOGICAL STUDY OF MUCORMYCOSIS IN RESECTED SPECIMENS
OF COVID-19 PATIENTS: AN INSTITUTIONAL STUDY****Dr. Manjula K.*¹, Dr. Sowjanya Lakshmi R.V.², Dr. Azeem Mohiyuddin S.M.², Dr. Kalyani R.²**¹Associate Professor of Pathology, Chikkaballapur Institute of Medical Sciences, Chikkaballapura - 562101.²Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar 563101.***Corresponding Author: Dr. Manjula K.**

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INTRODUCTION

Coronavirus disease 2019 (Covid-19) is an infection caused by severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2).^[1] Covid19 has been associated with a wide range of opportunistic bacterial and fungal infections. Aspergillus and Candida have been reported as the main fungal pathogens for co-infection in people with COVID-19.^[2] Recently, several cases of mucormycosis in people with COVID-19 have been increasingly reported worldwide, in particular from India.^[3,4]

Mucormycosis is an opportunistic infection caused by fungi belonging to the order Mucorales. It is a highly invasive fungal infection affecting predominantly the immunocompromised patients. Rhizopus spp. is the most common organism responsible for 70% of all cases of mucormycosis.^[3, 5, 6] These saprophytic fungi are widely in nature, and infection is acquired by exposure to their sporangiospores. Mucormycosis infections are characterized by extensive angioinvasion that results in vessel thrombosis and subsequent tissue necrosis.^[7, 8]

Worldwide the prevalence of mucormycosis varies from 0.005 to 1.7 per million populations. In India its prevalence is 80 times higher compared to developed countries.^[2] Diabetes mellitus (DM) is the main risk factor associated with Mucormycosis. Next common risk factor is long term use of corticosteroids, Even a short course of corticosteroid therapy predisposes to mucormycosis, especially in DM. Hematological malignancies, organ transplants, chronic liver and kidney diseases, immunodeficiency disorders are the other risk factors which predispose the individual for acquiring Mucormycosis.^[2,5,8,9,10] There are five clinical forms of mucormycosis. Rhinocerebral, pulmonary, disseminated, gastrointestinal and cutaneous type of mucormycosis.^[8]

Over the past few months our institution has seen a sudden rise in mucormycosis, maximum number of cases were seen in the month of April 2021 and May 2021, later incidence gradually reduced. Operating theaters were being occupied by patients undergoing radical surgical procedures for Mucormycosis. Here, in this observational study, we are sharing our institutional experience of

Mucormycosis in resected specimens of Covid-19 patients.

METHODOLOGY

This is an observational study done in the department of pathology. Only those cases with clinical features suggestive/suspicious of mucormycosis and exhibiting characteristic broad septa ribbon-like hyphae with wide-angle branching diagnostic of mucormycosis at histopathology were taken for the study. Those patients with clinical features suspected of mucormycosis but could not be confirmed at histopathology were excluded from the study. Patient's clinical presentation details, laboratory investigation details, radiological findings and other available details of investigation were collected. Also details regarding co-morbidities, clinical management, and operative details were collected. Gross details of the resected specimens were noted.

Routine processing of the tissue was done in Histopathology. Tissue sections were stained with Hematoxylin and Eosin (H&E) Special stains (PAS and GMS) were used to confirm the mucormycosis. These slides were evaluated for the presence or absence of organism, and the following histopathological features were studied in details and findings were tabulated. Fungal load (high/low) Angioinvasion, perineural invasion, intravascular thrombosis, coagulation necrosis, tissue infarction, granulomas and nature of inflammatory cells (mild to moderate/ dense inflammatory cells)

RESULTS

A total of 85 cases of mucormycosis were studied in the present study. Male to female ratio was 2.86:1. The most

common age group affected was 40- 49 years, median age was 44.5 years, accounting to 29.41% (table 01). The most common clinical presentation was sinonasal obstruction/ growth, followed by facial pain (table 02). The most common associated risk factor was immunosuppressive therapy (78%) followed by Diabetes mellitus.

The tissue sent for histopathological examination was predominantly greywhite and friable. The characteristic hyphae of mucormycosis were broad, thin walled,

nonparallel and predominantly aseptate with right-angle branching, some were folded, twisted and they are better visualized with H and E stains than with special stains (Figure 01). The most common histopathological finding associated with mucormycosis was acute suppurative inflammation and coagulative necrosis (Table 03 and Figure 02). Cases having acute suppurative inflammatory reaction showed low fungal growth, where as high fungal load were associated with sparse to mild inflammatory cell reaction in the tissue sections studied. Granulomatous tissue response was seen in 21 cases.

Table1: Shows age distribution of mucormycosis cases.

Age (years)	Number of cases	Percentage
20- 29	04	4.7
30- 39	12	14.11
40-49	25	29.41
50-59	14	16.47
60-69	16	18.82
70 and above	14	16.47
Total	85	100

Table2: Shows various clinical signs and symptoms of mucormycosis cases.

Clinical feature/ signs	Number of cases	Percentage (%)
Sinonasal obstruction/growth /pain/discharge	38	44.7
Facial pain / head ache	17	20.0
Swelling around the eyeball	08	9.4
Teeth pain	05	5.88
Reduced vision	8	9.41
Ptosis/proptosis	6	7.05
Difficulty in breathing	3	3.5
h/o loose stools	2	2.1
Total	85	100

Table3: Shows various histopathological changes seen in mucormycosis cases.

Histopathological changes	Number of cases	Percentage of cases
Acute suppurative inflammation	64	75.29
Low fungal load	64	75.29
High Fungal load	21	24.70
Coagulation necrosis	77	90.01
Degree of Inflammatory cell exudates – mild to moderate	21	21.70
Degree of Inflammatory cell exudates – marked	64	75.29
Fibrinoid necrosis of the vessels	35	41.17
Granulomatous tissue response	30	3.29
Congestion	40	47.05
Intravascular thrombosis	38	44.70
Angioinvasion	40	47.05
Neural/ perineural invasion	10	11.76
Combined infection	08	9.41

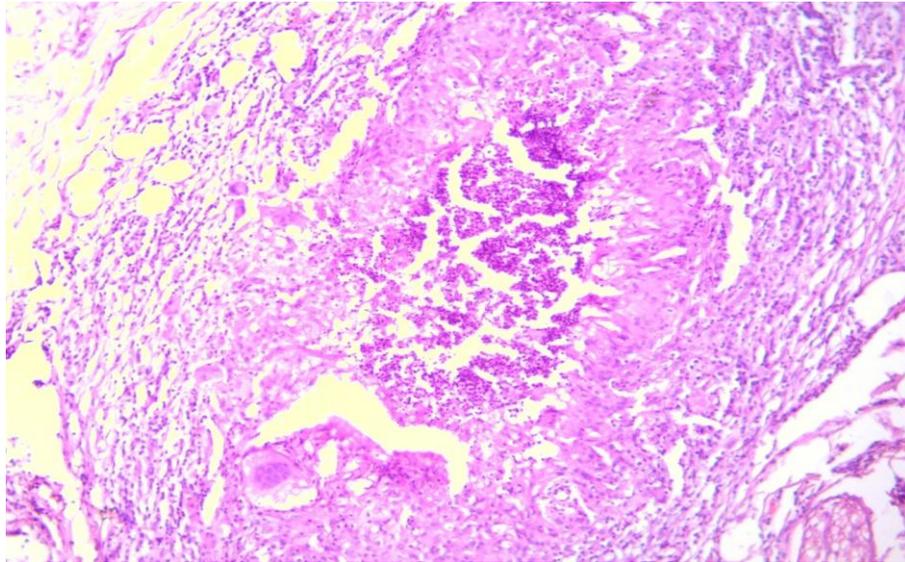


Figure:1 Acute suppurative inflammation with granulomatous tissue response H&E, 200X.

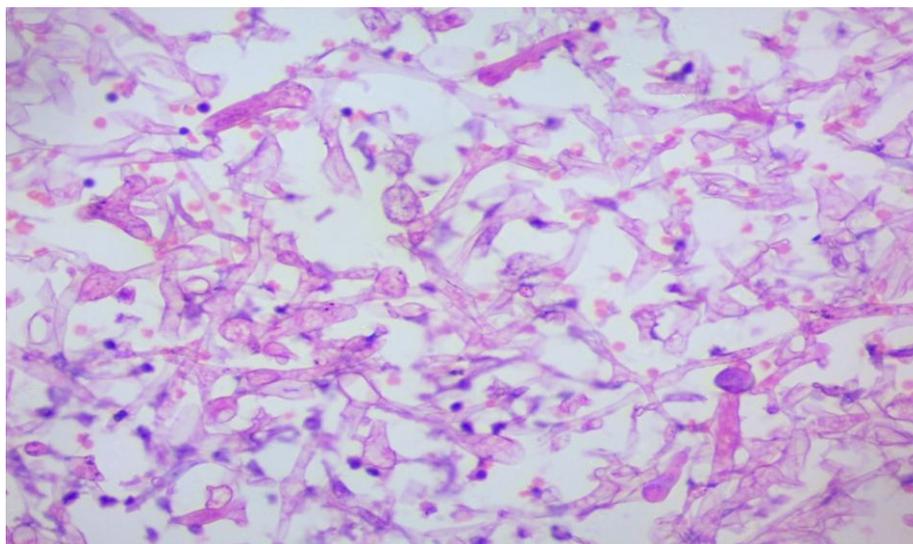


Figure:2 Characteristic hyphae of mucormycosis H&E,400X.

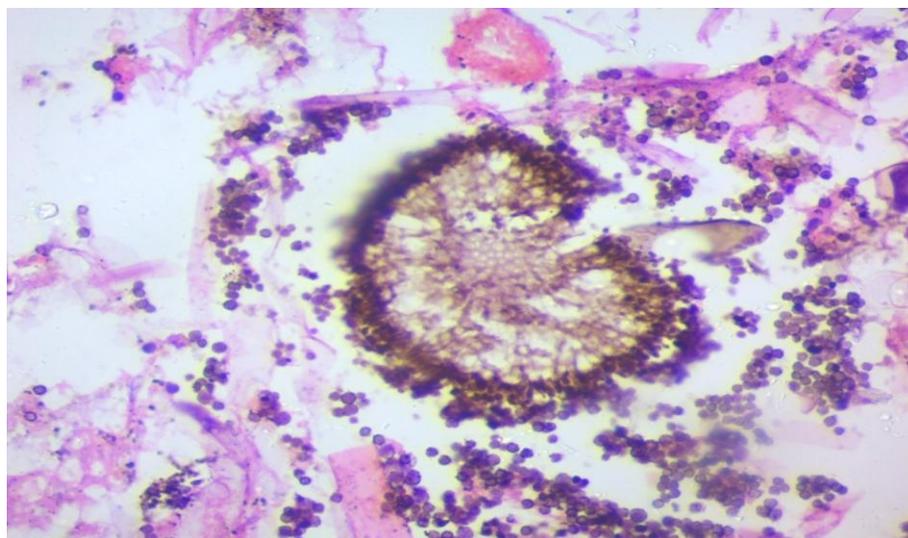


Figure: 3 Mucormycosis and Aspergillosis H&E, 400X.

DISCUSSION

In second wave of Covid 19, Mucormycosis was the most deadly co infection responsible for the mortality and morbidly associated with COVID 19 infections. Here we are sharing our institutional experience with mucormycosis tertiary care hospital.

In our study mucormycosis was predominantly seen in males, male to female ratio was 2.86:1. This is similar to study done by Singh AK *et al.*^[2] The most common age group affected was 40- 49 years, median age was 44.5 years. In other similar studies, Median age varies from 40.43 to 56.3 years.^[3,10,11,12] Hyperglycemia was the most common risk factor associated with mucormycosis in patients with Covid 19 accounting to 78%. In a study done by Singh AK *et al.*^[2], hyperglycemia was seen in 83% of cases, whereas in a study done by Ramaswami A *et al.*^[4] hyperglycemia was observed in 67.3%. In our study, 77% of patients were on immunosuppressive therapy, which is similar to study done by Singh AK *et al.*^[2] Commonest clinical type in our study was Rhinocerebral mucormycosis accounting to 96%, similar to study done by Singh AK, Bala *et al.*^[3] Ramaswami A *et al.*^[4] and other.^[13,14] Review of literature has shown that, in Covid 19 infected patients, nasal mucociliary clearance which is a primary innate defense mechanism will be profoundly delayed throughout the course of infection and may persist for a long time.^[15]

In our study acute suppurative inflammation was the most common histopathological feature observed in tissue sections accounting to 75.29%. Complete blood count in these patients showed neutrophilia with an increased neutrophil to lymphocyte ratio. This is similar to study done by Li G *et al.*^[6] Neutrophils play a major role in killing the fungus by generating the oxidative metabolites.^[5,16] Fungal load was low in these cases.

Coagulative necrosis was seen 90% of cases. It varied from grade one to grade four where there will be no viable tissue was found. In a study done by Goelet *et al.*^[17], necrosis varied from 2 to 90%. Granulomatous tissue response with giant cells was seen in 30% of cases; Castillo *et al.*^[18] in their study have found that multinucleate giant cell granulomas may be correlated with the disease with better prognosis however we could not correlate with disease prognosis.

In our study Angioinvasion with vacuities was seen in 47.05% of cases (Figure 3). In a study done by Ben Ami *et al.*^[19] angioinvasion was present in all the cases where as in a study done by Goel *et al.*^[17], angioinvasion was seen in 51% of cases. Angioinvasion is a distinctive pathognomonic feature of mucormycosis which ultimately leads to vessel thrombosis and subsequent tissue necrosis.^[8]

Combined infections (Mucormycosis and Aspergillosis) were seen in eight cases (Figure 4), Jain *et al.* in their review article have reported that, 11 cases with mixed

infections. Significant increase in invasive fungal infection was evident in patients suffering from COVID-19 which could be due to immunosuppression and other pre-existing co morbidities.^[20] and also Baddley JW *et al.*^[21] in their study, found that incidence of invasive fungal infections varies, often related to heterogeneity in patient populations, surveillance protocols, and definitions used for classification of fungal infections.

CONCLUSION

Mucormycosis is a highly invasive fungal which cannot be diagnosed by rapid tests. In many patients affected with mucormycosis, the outcome is poor. Early diagnosis and reversing the predisposing factors are most important. Controlling the infection requires increased awareness, better tests to diagnose at the earliest, controlling the diabetes and usage of corticosteroids wisely.

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