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Histomorphological study of mucormycosis in COVID-19 patients

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Abstract---Background: Mucormycosis is a rapidly progressive angioinvasive fungal infection which has a high rate of mortality and

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morbidity. Mucormycosis most commonly invades the nose and paranasal sinuses presenting as rhinosinusitis. We noted an upsurge in the number of patients affected with Mucormycosis during Covid-19 infection era. Aim: Our study aims to illustrate the histopathology of Mucormycosis in post-Covid-19 patients and also to study the fungal morphology by utilizing special stains. Materials and Methods: We prospectively analysed the histopathological findings of fungal elements in 260 different histopathological samples of mucormycosis in post-covid-19 patients. The clinical and histopathological findings were noted and tabulated. Statistical Analysis Used: The data collected was tabulated and coded in Microsoft excel. All the data of demographic and clinical characteristics were analysed by descriptive statistics and percentage. Tables and graphs were added wherever applicable. Results: Out of 260 cases analysed, most of the patients presented with periorbital swelling followed by headache and nasal block. Histopathologically, most of the cases showed chronic inflammatory reaction with wide areas of necrosis and granuloma formation. The fungal elements have been appreciated and sensitivity of different stains to appreciate the fungal structures have been compared. Conclusions: Histopathology is the gold standard in the diagnosis and confirmation of mucormycosis and this study in our institute has experienced the maximum number of mucormycosis cases. The morphology of the fungal elements has been studied in routine as well as special stains.

Keywords---COVID-19, mucormycosis, histopathology.

Introduction

Novel corona virus disease (COVID-19) caused by severe acute respiratory syndrome corona virus 2(SARS CoV-2) was first reported in China in December 2019 and caused a global public health issue and increased clinical burden of a wide range of illnesses[1]. Studies by Bedford J et al, Zhu N et al have shown that most patients of COVID-19 infection developed mild symptoms, however few have developed severe pneumonia, acute respiratory distress syndrome (ARDS) or even death.[2, 3]

Coronavirus disease (COVID-19) infection is associated with significant incidence of secondary infections, both bacterial and fungal probably due to immune dysregulation and are mainly seen in co-morbid, severely ill or immunocompromised patients. Additionally, studies by GeSong et al and Sahil Mehta et al, suggest that the widespread usage of steroids/ broad-spectrum antibiotics/ monoclonal antibodies as part of the armamentarium against COVID-19 may lead to the development/ exacerbation of preexisting fungal diseases.[4,5]

India has documented an alarming number of cases of mucormycosis, which is often a deadly fungal infection among covid-19 patients with high morbidity and mortality. Mucormycetes belong to the order Mucorales and sub phylum Mucoromycotina.[6] Most cases of mucormycosis results from inhalation of fungal

sporangiospores that have been released in the air or from direct inoculation of organisms into disrupted skin or gastrointestinal tract or nasal mucosa as stated in Farmakiotis D et al.[7]

According to Spellberg B et al., histopathology of tissue sections is dominated by inflammation which may be neutrophilic or granulomatous. Few cases may not show inflammation particularly in immunosuppressed patients[8]. Tissue infarcts and angioinvasion are more commonly seen in invasive diseases. Few cases show perineural invasion. Neutropenic patients display a more extensive angioinvasion when compared to non-neutropenic patients according to Frater JL et al[9]. There are few studies by by GeSong et al and Sahil Mehta et al, showing high probability of covid and post covid patients suffering from invasive mycosis[4,5]. This requires early detection by comprehensive diagnostic intervention to ensure effective treatment and subsequent reduction of mortality and morbidity. Microscopy (direct and histopathology) and culture of various clinical specimens are the cornerstones of diagnosing mucormycosis. Considering the slow growing nature of mucormycosis, the yield of culture results will be delayed and thus histopathology remains as a gold standard for early diagnosis which will help in effective management of patients. This study is undertaken to document histopathological findings of Mucormycosis in Covid-19 and post Covid-19 patients.

Objectives

- To study the histomorphological findings of Mucormycosis in post covid-19 patients.
- To delineate the fungal morphology by utilizing special stains.

Materials and Methods

Procedure

A prospective observational study has been done in Shri Atal Bihari Vajpayee Medical College and Research Institute for a period of 3 months from May 2021 to July 2021. The demographic details like age, sex, presenting complains, past history and co-morbidities and the surgical details were collected from the records.

The histopathological specimens like tissue scrapings, soft tissue bits, bony bits, hemimaxillectomy and toatal maxillectomy specimens and orbital exenteration have been received during the study period. The specimens like tissue scrapings, small and large tissue bits were preserved and fixed using 10% formalin. Maxillectomy/ any bony bits were decalcified using freshly prepared Nitric acid (5%). The calcified hard tissues/ bony bits were cut into small pieces (2-6mm) with a thin blade or bone saw and kept for fixation in adequate volume of 10% formalin (10:1 ratio). After fixation the tissue is thoroughly washed in running water to remove the excess formalin and then put in freshly prepared 5% Nitric acid for 12 to 24 hours.

Orbital exenteration specimens received were kept for fixation in greater volume of 10% formalin for at least 48 hrs. Once fixed, the exenteration specimen was oriented, the laterality was determined and the axial (anteroposterior), horizontal and vertical globe dimensions, the corneal diameter, length and diameter of the optic nerve were taken and then the grossing was done according to CAP Protocol for orbital exenteration specimens. Then the thorough sectioning of the specimen was done to include the areas affected by mucor and the optic nerve resected margin (inked and submitted prior to sectioning of the globe) to know whether there is involvement of optic nerve by mucormycosis.

All the representative bits were then processed in tissue processor and sectioned using rotary microtome. Four to five-micrometer-thick sections were taken and stained with Hematoxylin and Eosin (H&E) to study the different histomorphological features like tissue necrosis, angioinvasion, perineural invasion, fungal organisms and the other findings were documented, while PAS (Per Iodic Acid Schiff) stain, Reticulin stain, GMS (Grocott Methanamine Silver) stains were used to study detailed morphology of the highlighted fungal hyphae. Light Microscopic morphology of broad, aseptate ribbon-like fungal hyphae with right angled branching were considered diagnostic of mucormycosis. The histopathological findings of routine H and E and special stains along with clinical findings were analyzed.

Ethical clearance was sought from the institutional ethical committee (IEC NO: BLCMCRI/IEC/RP/052/21-22) and approval letter has been obtained. The written informed consent was obtained from all patients or relatives before the study for participation.

Inclusion criteria

All clinically suspected cases of mucormycosis in covid-19 patients who have undergone surgical debridement or any surgical procedures were included in our study.

Exclusion criteria

Mucormycosis cases of non-covid patients were excluded.

Statistical Analysis Used

The data collected was tabulated and coded in Microsoft excel. All data of the demographic and clinical characteristics were analysed by descriptive statistics and percentage. Tables and graph were added wherever applicable.

Results

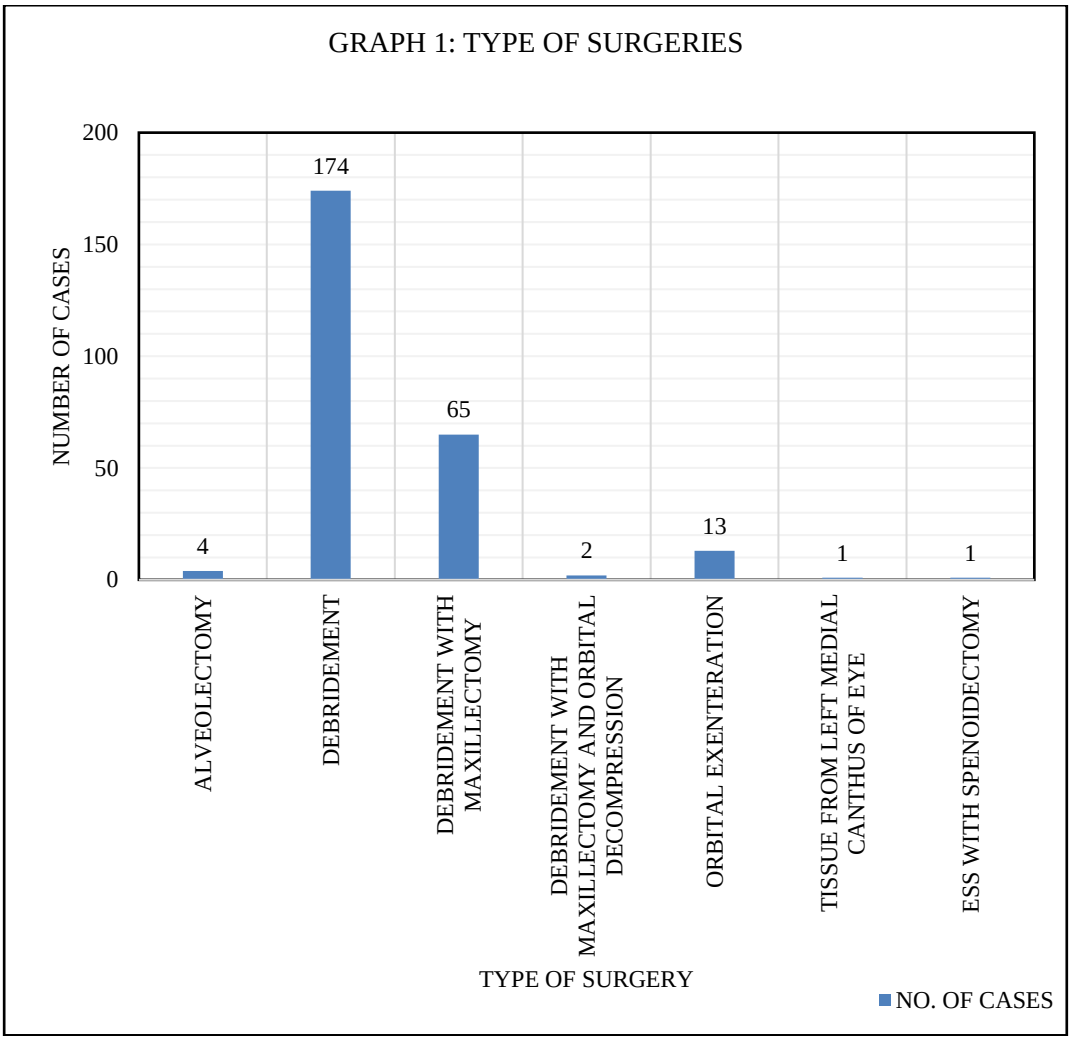
The present study involves the histopathological examination of 260 samples of Covid-19 positive patients with clinical suspicion of mucormycosis. Among them 217 (83%) were males and 43 (17%) were females with the male to female ratio of 5:1. The demographic details, laterality, clinical presentation and past history of all the patients are shown in table 1.

Most of the patients presented with periorbital swelling followed by headache, nasal block, fever and facial pain. Most of the patients (86.2%) had diabetes mellitus as the co-morbidity. The various surgical procedures have been done and the specimens were subjected for histopathological examination. The various surgeries done (graph 1) were Functional endoscopic sinus surgery (FESS) with debridement in 174 cases, FESS with debridement and maxillectomy in 65 cases, FESS with debridement, maxillectomy and orbital decompression in 2 cases, orbital exenteration in 13 cases, alveolectomy in 4 cases, sample of tissue from left medial canthus of eye in 1 case and endoscopic sinus surgery in 1 case.

Table 1
Demographic, clinical and investigation details of mucormycosis cases

	Number of cases	Percentage of cases
Age 1-20 20-40 40-60 60-80	 1 72 140 47	 0.4 27.7 53.8 18.1
Sex Male Female	 205 43	 78.8 16.5
Laterality Right Left Bilateral	 120 110 30	 46.2 42.3 11.5
Clinical Presentation Periorbital swelling Headache Nasal block Fever Facial pain Cough Ptosis Breathlessness Hemoptysis Seizures	 206 196 184 180 156 106 84 24 12 1	 79.2 75.4 70.8 69.2 60 40.8 32.3 9.2 4.6 0.4
Past History • Diabetes Mellitus • Tuberculosis • Chronic Kidney disease on Dialysis • Hematological	 224 1 1 1	 86.2 0.4 0.4

Malignancy • HIV	2	0.4 0.8



Among the 260 histopathological specimens studied, we observed a wide array of findings (figure 2 and 3) like chronic suppuration (n=244, 93.8%), minimal reaction (n=16, 6.2%), necrosis (n=179, 68.8%), granulomas (n=71, 27.3%), giant cell reaction (n=56, 21.5%), presence of fungal elements (n=177, 68.1%), angioinvasion (n=24, 9.2%) and perineural invasion (n=2, 0.8%). Most of the cases displayed chronic inflammatory reaction with neutrophils, lymphocytes and plasma cells with few of them showing giant cell response. The tissue exhibited variable degree of necrosis (n=179, 68.8%). Among them 106 cases (59.2%) showed necrosis in more than 50% of the tissue and 73 cases (41.8%) showed necrosis in less than 50% of the tissue. The fungal elements were noted in most of the cases (68.1%). Among them, broad, aseptate fungal hyphae with wide angled branching suggestive of mucormycosis were noted in the necrotic debris in 167

cases (94.4%) and mixed fungal infections with mucor and aspergillus were identified in other 10 cases (5.6%). We demonstrated the morphology of aspergillus as thin, septate hyphae with acute angled branching. Few cases (n=4) demonstrated the presence of fruiting bodies of Aspergillus species as well. Most of the fungal elements have been identified in routine haematoxylin and eosin stain (n=141, 54.2%), where as in 26 cases, fungal elements could not be identified on routine H and E staining, but have been picked up by special stains like periodic acid Schiff (PAS), Gomori Methenamine Silver (GMS) and Reticulin staining. The fungal hyphae were characteristically seen in the necrotic debris in most of the cases. The special stains were performed in all the samples received and the rate of detection of fungal elements were found to be more in special stains.

Few of the cases displayed angioinvasion (n=24, 9.2%) and perineural invasion (n=2, 0.8%) with fungal hyphae in the lumen of the blood vessel and in the nerve respectively. Among the 24 cases which showed angioinvasion, 19 cases of angioinvasion were detected in routine H and E stains and 4 cases of angioinvasion were detected in PAS stain and 1 case of angioinvasion detected in GMS stain. The perineural invasion was detected in 2 cases on routine H and E staining and in GMS staining.

Table 2
Number of cases showing specific histopathological features.

Sl. No	HISTOPATHOLOGICAL FEATURES	NUMBER OF CASES	PERCENTAGE (%)
1.	Chronic suppuration	244	93.8
2.	Minimal reaction	16	6.2
3.	Necrosis - Necrosis involving >50% of tissue - Necrosis involving <50% of tissue	179 - 106 - 73	68.8 - 59.2 - 40.8
4.	Granulomas	71	27.3
5.	Giant cell reaction	56	21.5
6.	Presence of fungal elements on H and E staining	141	54.2
7.	Presence of fungal elements on special stains	177	68.1
8.	Demonstration of Mucor	167	64.2
9.	Demonstration of mucor and aspergillus	10	3.8
10.	Angioinvasion	24	9.2
11.	Perineural invasion	2	0.8

Discussion

Mucormycosis is a serious but rare opportunistic fungal infection, mainly affecting elderly diabetic, immunocompromised individuals and spreads rapidly

causing infarction and necrosis in the host tissue. Corticosteroid therapy and a past history of chronic pulmonary disease were associated with a higher risk of invasive fungal disease according to Werthman-Ehrenreich A et al.[10]

The clinical presentations of mucormycosis are rhino-orbital-cerebral, pulmonary, cutaneous and disseminated. Diagnosis is made by clinical suspicion and histopathological examination. Weak host defenses are the major risk factors for invasive fungal diseases. Pulmonary mucormycosis is a relatively rare pulmonary fungal disease, which is difficult to diagnose early and lacks effective treatment according to Guarner J et al and Kim JH et al.[11, 12]

There is tremendous increase in the incidence of rhino-orbital-cerebral mucormycosis (ROCM) and mortality during Covid-19 infection, which was observed in several parts of India compared to other countries[13, 14]. The increased mucor cases in India could be due to many possible reasons such as high prevalence of uncontrolled diabetic status, poor regular health check-up, overdosage of steroids, underlying immunocompromised state, high loads of mucor spores in hospital and community due to humid climate and lack of personal hygiene according to studies by Prakash H et al, Jain K et al, Afroze SN et al and Kajs-Wyllie et al.[14,15,16, 17]

Mucormycosis is caused by the invasion of asexual spores of Mucorales which normally resides in the environment. These spores gain entry to the human body via routes of inhalation and settle in the oral and nasal mucosa. These spores will be restricted by the normal phagocytic and defense response in any immune-competent host. However, in an immunocompromised individual, germination will follow and there will be development of fungal hyphae which will be difficult to be eliminated by polymorphonuclear leukocytes.

We reported the clinical and histopathological characteristics of mucormycosis in covid and post-covid patients. Moderately severe pneumonia, steroid therapy, uncontrolled diabetes and malignancies are usually associated with mucormycosis. Mucorales being ubiquitous organisms, host environment becomes important factor for determining its pathogenesis. In our study, majority of the patients were known case of diabetes (86.2%) with few of them newly diagnosed (n=21, 9.3%). In a meta-analysis of 851 patients done by Jeong W et al., Diabetes mellitus acts as the major predisposing factor for the development of Mucormycosis[18]. The possible explanation for the increased incidence of Mucormycosis in diabetes mellitus and other immunocompromised states is because of the change in normal immunological response of the body to any infection. Hyperglycemia stimulates fungal proliferation by increasing the concentration of free iron and decrease in the anti-fungal inhibitory factors in the serum and also causes decrease in chemotaxis and phagocytic efficiency, thereby allowing them to survive in acid rich environment. There is evidence of increase risk of mucormycosis in patients with diabetic ketoacidosis as these organisms produce the enzyme ketoreductase which allows them to utilize the patient's ketone bodies. [16, 20] In addition, SARS Covid-19 virus destroys the beta cell integrity in pancreas and increase in risk of diabetes in Covid-19 patients[16, 19]. This explains the incidence of newly diagnosed diabetes mellitus (n=21, 9.3%) cases in our present study.

It is very important to distinguish mucormycosis from other bacterial and fungal infections mainly to aid in diagnosis and for early treatment and favorable outcomes. Histopathological examination is the gold standard in the diagnosis of suspected mucormycosis cases and we have examined 260 biopsy samples which were taken from the affected site.

Histopathologically, these lesions demonstrated chronic inflammatory response composed of lymphocytes, plasma cells, histiocytic inflammation, wide areas of necrosis, angioinvasion, perineural invasion, along with giant cell response and granulomas. Also noted are broad, aseptate, ribbon-like fungal hyphae branching at right angles. The histopathological differential diagnosis includes aspergillosis where the hyphae are septate and branches at acute angles. Few of the cases showed fruiting bodies. Also, we have observed necrosis as the predominant finding in 68.8% of cases. The presence of necrosis can be explained by the formation of luminal thrombi or luminal wall destruction by the angioinvasive nature of the hyphae which leads to infarction according to Muller JA et al[19].

We also performed special stains like PAS, GMS and Reticulin stain to highlight the hyphal structures. The special stains were helpful in identifying the degenerated and broken hyphal structures which were missed in routine H and E staining.

Conclusion

During Covid-19 pandemic, our institute experienced maximum number of mucormycosis cases, which helped us to study. Histopathology is the gold standard for identification of the fungal structures along with the fungal stains which helped us in difficult cases.

Declarations of interest: None

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Figure 1. Gross images- a) Specimen of Hemimandibulectomy. b) Specimen of orbital exenteration

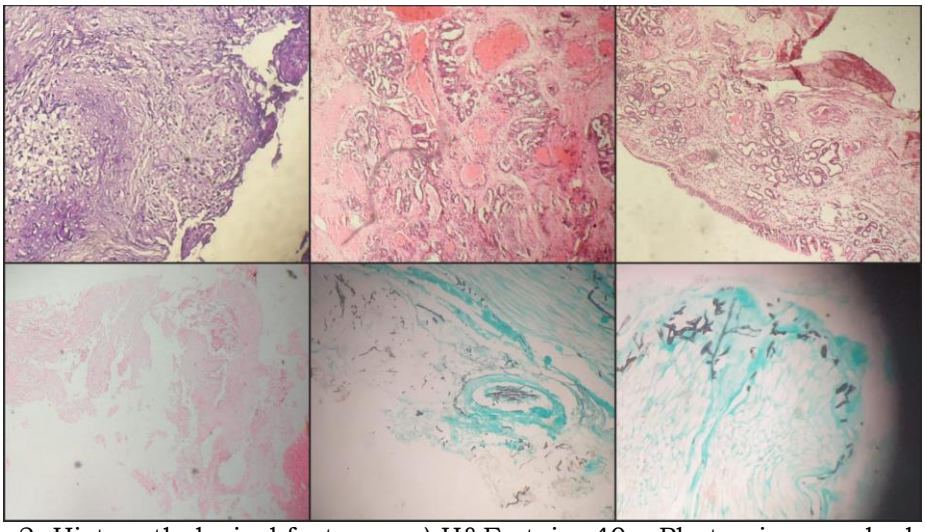

Figure 2. Histopathological features: a) H&E stain, 40x, Photomicrograph showing chronic inflammatory cell infiltrates. b) H&E stain, 40x, Photomicrograph of nasal mucosa showing seromucinous glands with mixed inflammatory cell infiltrates with congested blood vessels. c) H&E stain, 10x, Photomicrograph showing squamous metaplasia. d) H&E stain, 10x, Photomicrograph showing wide areas of necrosis. e) GMS stain, 40x, Photomicrograph showing angioinvasion of fungal structures. F) GMS stain, 40x, Photomicrograph showing perineural invasion of fungal structures

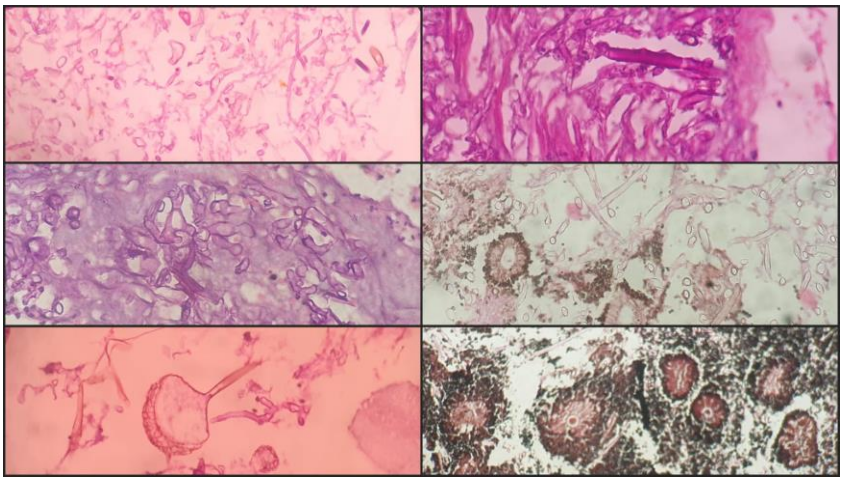

Figure 3. Microscopy of fungal structures: a) H&E stain, 40x, Photomicrograph showing broad, aseptate fungal hyphae consistent with mucor b) PAS stain, 40x, Photomicrograph showing broad, aseptate fungal hyphae consistent with mucor. c) PAS stain, 40x, Photomicrograph showing broad, aseptate fungal hyphae with few spore forms. d) GMS stain, 40x, Photomicrograph showing broad, aseptate fungal hyphae with spore forms and fruiting bodies. e) PAS stain, Photomicrograph showing 40x, fruiting bodies of mucor. f) Reticulin stain, 40x, Photomicrograph showing fruiting bodies of Mucor