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The Carpet Of Mucor In The Farm Of Covid- A Study Of COVID-19 Associated Rhinocerebral MucormycosisAt A Tertiary Care Centre

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ABSTRACT
Context: *Mucormycosis is being looked upon as one of the most dreadful complications during the pandemic of COVID-19. The co-existing immunosuppression, diabetes mellitus, and nosocomial infections are risk factors for the occurrence of mucormycosis in COVID-19 patients. The infection spreads rapidly and has a high mortality rate. The histopathologist has a key role in the early diagnosis of mucormycosis. The presence of characteristic hyphae, extensive angioinvasion with resultant vessel thrombosis, and tissue necrosis is the pathological hallmark of mucormycosis infections. The study was performed to study histomorphology of infection in COVID-19 associated rhinocerebral mucormycosis.*
Settings and Design: *This was a retrospective observational study conducted at the Department of Pathology, MGM Medical College and Hospital, Aurangabad, Maharashtra. The hospital attached to this college is a Tertiary Care Centre in India.*
Materials and Methods: *The study included 70 cases of rhinocerebral mucormycosis diagnosed during a period of four months from January 2021 to April 2021. The gross and microscopic findings include (a) characteristics of fungal hyphae, (b) type of inflammation and necrosis, (c) presence/ absence of sporangiophores, granulomatous reaction, angioinvasion, and perineural invasion, were noted, tabulated, and analyzed.*
Results: *The age range of patients was 32 to 86 years with a median of 55 years. There was male preponderance, and all patients were diagnosed with diabetes mellitus and had received steroids during treatment of COVID-19 infection. Characteristic fungal hyphae were seen in all cases with sporangiophores in six cases. Inflammation and necrosis were seen in all cases. The granulomatous reaction was seen in 42%, angioinvasion in 82%, and perineural invasion in 0.85% cases. Three cases revealed dual fungal morphology- One case revealed co-existing Candida and the other two with Aspergillus.*
Conclusion: *Rhinocerebral mucormycosis is one of the most dreadful complications in COVID-19 cases with an epidemic increase in incidence, risk factors being hyperglycemia, and immunosuppression. Histopathologist*

plays a significant role in early diagnosis. A high index of suspicion and early diagnosis would help the management of these cases.

Keywords: *COVID-19, Fungus, Mucormycosis, Rhinocerebral, Rhizopus*

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I. INTRODUCTION

Mucormycosis is being looked upon as one of the most dreadful complications during the current pandemic of COVID-19. The co-existing immunosuppression (either due to steroids or otherwise), pre-existing co-morbidities such as diabetes mellitus as well as the possibility of nosocomial infection are risk factors for the occurrence of mucormycosis in COVID-19 positive patients.

The histopathologist has a key role in the early diagnosis of this dreadful disease as diagnosing mucormycosis almost always requires histopathologic evidence of fungal invasion of the tissues. Imaging studies and culture may both be inconclusive/ insufficient and there is no reliable serological test as yet for diagnosing mucormycosis[1]. Recent studies have described increasing incidence of mucormycosis in Covid-19 patients, especially those with diabetes mellitus[2-6].

The study was performed to study histomorphology of infection in COVID-19 associated rhinocerebral mucormycosis (CARM). Literature of 36 published cases of CARM was also reviewed.

II. MATERIALS AND METHODS

This was a retrospective observational study conducted at the Department of Pathology at a Tertiary Care Centre in India. The study included 70 cases of rhinocerebral mucormycosis diagnosed over four months from January 2021 to April 2021.

Resected specimens of suspected cases were received in formalin in the Department of Pathology. Specimens were fixed overnight and grossed subsequently. Slides were stained with Haematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS) stains. The gross and microscopic findings include (a) characteristics of fungal hyphae, (b) type of inflammation and necrosis, (c) presence/ absence of sporangiophores, granulomatous reaction, angioinvasion, and perineural invasion, were noted, tabulated, and analyzed. The demographic, clinical details, and imaging findings of all cases were retrieved from medical case records.

III. RESULTS

A total of 70 cases of rhinocerebral mucormycosis were included in this study. The COVID-19 positive status of the patients was confirmed by RT-PCR test/ Antigen test in all cases. One of the patients was vaccinated (single dose 10 days before admission). The age range of patients was 32 to 86 years with a median of 55 years; 44 patients were above the age of 50 years. There was a male preponderance with a male: female ratio being 3.4:1.

All the patients were already diagnosed with diabetes mellitus before being admitted for mucormycosis. All patients had received steroids during treatment of COVID-19 infection. Two patients had co-existing ischemic heart disease. The presenting clinical features were maxillary sinusitis, eyelid oedema, periorbital facial pain, visual impairment/loss, proptosis [Figure 1], ophthalmoplegia, and focal neurological deficits. Sinusitis was the commonest presentation; in all the patients, at least one of the sinuses was affected. In addition, nasal involvement was present in 39 cases, orbital involvement in 11 cases, and cerebral involvement in three cases. Resected specimens included partial maxillectomy (11), nasal crust (39), orbital tissue (6), and orbital exenteration (2).

In all the cases, gross examination of infected tissue revealed congested, oedematous, violaceous, or black and necrotic tissue [Figure 2: A, B]. The histopathology section revealed characteristic broad, aseptate, thin-walled hyphae with non-parallel sides. Fungal hyphae had focal bulbous dilatations and occasional irregular branching. The hyphae appeared basophilic in H&E-stained sections and magenta-colored in PAS-stained sections [Figure 3: A-D]. Sporangiospores/ zygospores were seen scattered in many cases; well-formed sporangiophores were identified in six cases [Figure 3: E-F].

Acute (suppurative) or chronic (lymphoplasmacytic infiltrate/ granulomas) or mixed inflammation was present. Large areas of coagulative necrosis were seen in all cases. Necrosis of the vessel wall with mycotic thrombi was seen in 58 cases (82%). The foreign-body granulomatous reaction was present in 30 cases (42%). Perineural invasion was seen in 6 cases (0.85%). The overlying mucosa was oedematous inflamed and ulcerated with squamous metaplasia in some cases. These histomorphological features are shown in figure 4: A-D.

Three cases revealed dual fungal morphology- One case revealed co-existing Candida and the other two Aspergillus [Figure 5: A-C]. Candida species exhibit budding yeast cells with pseudohyphae, highlighted by

PAS stain. Aspergillus hyphae were narrow and septate with parallel walls and dichotomous acute-angled branching, highlighted by PAS stain. KOH mount revealed fungal hyphae in all cases. A culture report was available and correlated in 38 cases. Colonies of mucor appear "cotton furry" on fungal culture [Figure 6].

IV. DISCUSSION

In the present study, COVID-19 associated rhinocerebral mucormycosis was more common in males above the age of 50 years. All the patients were diabetic and had received corticosteroids. The commonest presentation was maxillary sinusitis. Orbital exenteration was done in two cases. Characteristic fungal hyphae of mucormycosis with inflammation and coagulative necrosis were seen in all cases with angioinvasion 82% and granulomatous reaction in 42% cases.

COVID-19 associated rhinocerebral mucormycosis cases have been published in various indexed-journals[2-6]. The median age of presentation considering all published CARM cases was 55 years; similar to that in the present study. There was male preponderance among reported cases as well, with a male: female ratio of 5.5:1. As documented by Moorthyet al[6], the male preponderance could be a reflection of the higher prevalence of COVID-19 in males in India[7].

During the pandemic, COVID-19-associated sepsis has emerged as a significant problem in India[8]. Elderly diabetic patients in India are significantly associated with severe disease[7]. In addition, COVID-19 is associated with a significant incidence of secondary infections, both bacterial and fungal probably due to immune dysregulation[2]. In a recent review, by Rawson et al, 8% of patients had secondary bacterial or fungal infections during hospital admission[9]. A complex interplay of factors, including pre-existing diabetes mellitus, previous respiratory pathology, use of immunosuppressive therapy, the risk of hospital-acquired infections, and systemic immune alterations of COVID-19 infection itself may lead to secondary fungal infections [2,6]. India has a high prevalence rate of diabetes mellitus, which is a known risk factor for infection by Mucor[10]. Nosocomial mucormycosis has been associated with iatrogenic immunosuppression and a variety of procedures or devices used in hospitals, including antifungal prophylaxis, bandages or medication patches, intravenous catheters, and even tongue depressors[8]. The occurrence of infection by Candida and Aspergillus in COVID-19 patients has been described.[6,11-13]

Mucormycosis is caused by zygomycetes of order Mucorales. Species belonging to the family Mucoraceae are isolated more frequently from patients with mucormycosis, Rhizopusoryzae (Rhizopusarrhizus) being the most common cause of infection[1]. Based on clinical presentation and the involvement anatomic site, mucormycosis is divided into six clinical categories: (i) rhinocerebral, (ii) pulmonary, (iii) cutaneous, (iv) gastrointestinal, (v) disseminated, and (vi) miscellaneous[1]. Mucormycosis is life-threatening and has a high mortality rate. Of the various categories, rhinocerebral mucormycosis is the commonest form[1-6,14]. In the present study maxillary sinus was the commonest site of infection. In a study by Murthy et al[6], all the patients had maxillary sinusitis.

Phagocytes of immunocompetent hosts kill Mucorales by the generation of oxidative metabolites and the cationic peptides defensins, and hence patients who are immunosuppressed/ neutropenic/ have dysfunctional phagocytes are at increased risk of developing mucormycosis. In patients with hyperglycemia and low pH(as in diabetic ketoacidosis), phagocytes are dysfunctional and have impaired chemotaxis and defective intracellular killing, predisposing these patients to Mucormycosis.[15] Patients with diabetic ketoacidosis have elevated levels of free iron in their serum; this unbound iron is an essential element for cell growth and development of Mucorales. Patients receiving deferoxamine during dialysis are also susceptible to Mucor infection.[16]

Rhizopus secretes rhizoferrin which supplies iron through receptor-mediated energy-dependent process[15]. Also, Rhizopus possess two homologs of hemeoxygenase which enable the pathogen to obtain iron from host hemoglobin. This mechanism explains the angioinvasive nature of the pathogen. R. oryzae spores can adhere to sub-endothelial matrix proteins including laminin and type IV collagen in vitro; the spores adhere to subendothelial matrix proteins significantly better than the hyphae[1]. Extensive angioinvasion results in vessel thrombosis and subsequent coagulative necrosis of host tissues. The spectrum of thrombotic microangiopathies owing to COVID-19 pathogenesis contributes to the aggressiveness of the disease[6]. In the current study, angioinvasion was identified in 58 out of 70 cases (82%). In a study by Sravani et al, angioinvasion was present in 25 out of 30 cases (83%)[14].

Nerve microenvironment and neurotropic factors secreted in a gradient along nerves may play a pivotal role in the pathogenesis of perineural invasion of the fungus. Perineural invasion indicates the advanced extent of invasion. In the present study, the perineural spread was identified in 6 cases (0.85%). In the study by Sravani et al, peripheral nerves were identified in 19 biopsies only and perineural spread was identified in 15 biopsies out of 30 (50%)[14].

A high index of clinical suspicion is required for the diagnosis of rhinocerebral mucormycosis. Imaging techniques may be suggestive of mucormycosis but are rarely diagnostic. The initial imaging study is frequently negative or has only subtle findings; the most common finding on computerized tomography (CT) scan of the

head or sinuses is a subtle thickening of sinus mucosa or extraocular muscles. The finding of bony erosion of the sinuses is strongly suggestive of the diagnosis in the appropriate clinical context[1].

Culturing organisms from a potentially infected site is rarely sufficient to establish the diagnosis of mucormycosis because the causative agent is ubiquitous, it may colonize normal persons, it is a relatively frequent laboratory contaminant and it may also be lysed during tissue grinding (while processing tissue specimens for culture). A sterile culture, therefore, does not rule out an infection. Also, a fungal culture is time-consuming and may delay appropriate therapy[1,6]. There are no reliable serologic, PCR-based, or skin tests for mucormycosis.

Therefore, the gold standard for the diagnosis of mucormycosis is histopathology of infected tissues. Biopsy thus remains the mainstay of diagnosis and the benefits of the procedure outweigh the risk, even at a site that is difficult to access[8].

Debridement and resection along with intravenous liposomal Amphotericin B is the treatment of choice for mucormycosis[4]. The gross examination of resected specimen reveals infected tissue which is congested, oedematous, violaceous, or black and necrotic. Thrombosed blood vessels may be identified. On histopathology, it is important to identify fungal hyphae and to distinguish mucormycosis from other fungi such as Aspergillus as the management differs. The hyphae of Mucorales are broader, infrequently septate, have non-parallel sides, and exhibit irregular non-dichotomous branching. In contrast, Aspergilli have narrower and septate hyphae with parallel walls and dichotomous acute-angled branching and. Aspergilli are better highlighted by special stains (PAS) as compared to mucor (which is better visualized by H&E)[17].

V. CONCLUSION

There are numerous complications in COVID-19 cases, mucormycosis being one of the most dreadful ones. There is an epidemic increase in the incidence of rhinocerebral mucormycosis in COVID-19 associated cases, risk factors being hyperglycemia and immunosuppression. Corticosteroids as always, are acting as a double-edged sword.

The histopathologist plays a significant role in early diagnosis by studying the histomorphology and special stains. A high index of suspicion and early diagnosis would help optimal management of these cases and reduce morbidity and mortality.

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Figure 1: Eyelid edema and chemosis with features of cellulitis

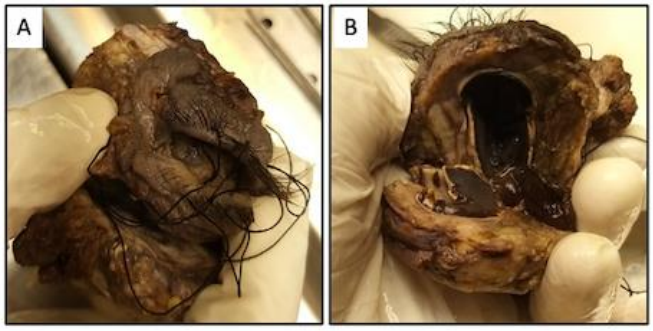

Figure 2: A- Specimen of exenteration of eyeball, **B-** Cut section of the same reveals large areas of necrosis.

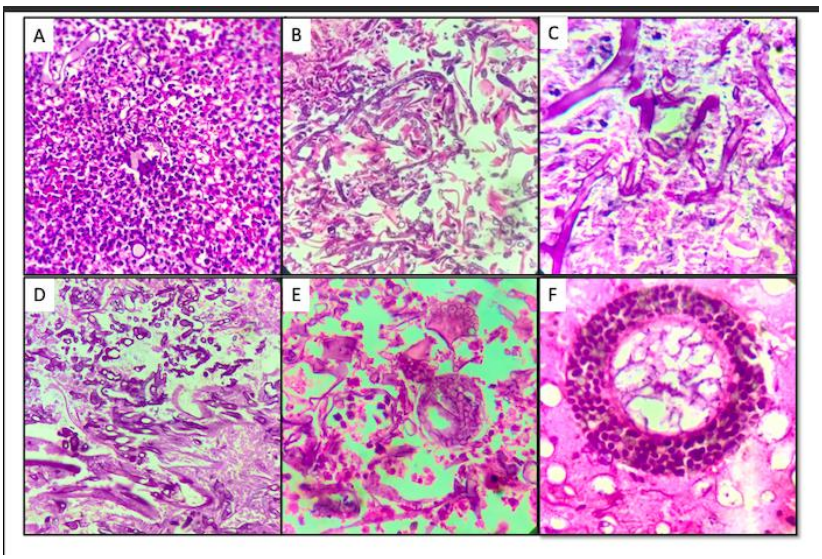

Figure 3: Histomorphology of mucormycosis: **3A**-H&E (10x): Fungal hyphae within suppurative inflammation, **3B**- H&E (10x): Ribbon-like broad thin-walled hyphae. **3C**- H&E (40x): Broad aseptate thin-walled fungal hyphae with non-parallel sides and irregular, non-dichotomous branching. **3D**- PAS stain (40x): Fungal hyphae highlighted by special stain. **3E**- H&E (40x): Fungal sporangiophore filled with brownish sporangiospores. **3F**- H&E (100x): Fungal sporangiophore transected transversely

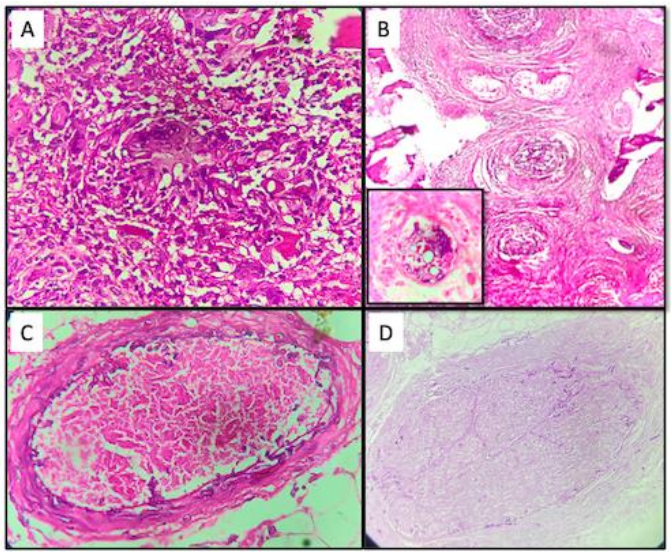

Figure 4: 4A- H&E (10x): Foreign-body granulomatous reaction to fungal hyphae. **4B-** H&E (4x): Vascular thrombosis with necrotic bone; inset reveals mycotic thrombus. **4C-** H&E (40x): Mural invasion by fungal hyphae. **4D-** H&E (40x): Perineural invasion by fungal hyphae

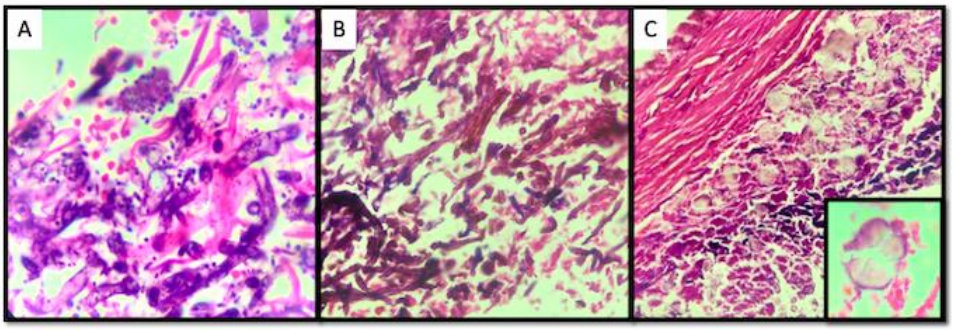

Figure 5: 5A: H&E (40x objective): Fungal hyphae of mucormycosis co-existing with budding yeast of Candida, **5B:** H&E (40x): Mixed infection- Fungal hyphae of aspergillus and mucor, **5C:** H&E (40x): Calcium oxalate crystals (fan-shaped- better seen in inset) surrounded by Aspergillus hyphae

Figure 6: Fungal culture plate showing whitish 'cotton furry' colonies of Mucor