Original Research Paper

Volume - 12 | Issue - 10 | October - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

HISTOMORPHOLOGICAL FEATURES OF MUCORMYCOSIS DURING COVID-19 PANDEMIC.

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ABSTRACT Introduction: Due to Corona Virus disease -19, India saw a surge of mucormycosis cases, associated with high death rate. India, during the month of May to July 2021 saw a surge of mucormycosis from all states, with close to 50,000 cases just in a span of 3 months. Objective: To examine the histopathological appearances of rhino-orbital/rhino-maxillary/sino-nasal mucormycosis in the backdrop of the ongoing COVID 19 pandemic. Material and methods: The study involved analysis of 194 nasal biopsy and enucleation specimens of suspected rhino-maxillary /rhino-orbital mucormycosis received from post-COVID-19 patients.KOH preparation and fungal culture was done . A preliminary review of the slides showing hyphal forms of fungal organisms with un-doubtful tissue / mucosal invasion was included. All samples were examined under Hematoxylin and Eosin stains. Data thus obtained were analyzed statistically. Results: The maximum number of patients with mucormycosis were in the age group of 41-50 years and 153 (78.9%) of them were males. Chronic type of inflammation was noted in 152 (78.4 %),coagulative type of necrosis was seen in 187(96.4%)cases. The dominant fungus were mucorales in 187 (96.4%), aspergillous along with mucorales in 2 (1.03%) and combination of mucorales and aspergillous identified in 5 (2.6%) cases. Conclusion: The present research was formulated, noticing an abrupt rise of mucormycosis cases during the second wave of COVID-19 .It is a life threatening disease .Therefore early screening and definitive diagnosis between mucor and aspergillous were much needed for proper management of patients.

KEYWORDS : Mucorales, Mucormycosis , COVID-19, SARS-CoV-2, Mycoses , aspergillosis.

INTRODUCTION

India is affected with resurging waves of Corona Virus disease -19 (COVID 19), ever since the World Health Organization (WHO) declared it as a pandemic in March 2020 [1], [2]. The infection continues relentlessly to become an ongoing public health problem affecting millions of people [1].

The COVID 19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), chiefly infects but not limited to the lungs, causing pneumonia, cardiovascular and even neurological disorders. About 7% of the individuals may develop co-infection with other microorganisms such as fungi or bacteria, further complicating the disease [3]

Fungi of the order mucorales belonging to the phyomycetes class are implicated in a potentially invasive and often fatal opportunistic infection called the Mucormycosis [4].Mucormycosis is described as potentially lethal infection amongst immunocompromised host particularly in those with diabetes, leukemia and lymphoma. Due to COVID-19, India saw a surge of mucormycosis, which was associated with high death rate. It was further complicated with reduced availability of antifungal medications.

India, during the month of May to July 2021 saw unexpectedly high reports of mucormycosis from all states, with a close to 50,000 cases just in a span of 3 months [5], [6]. The highest incidence of mucormycosis came from West of India followed by South India [6].

Untreated rhinosinus mucormycosis can develop to cavernous sinus thrombosis and cerebral invasion and 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. Histoptahological examination plays a key role in diagnosing the mucormycosis [7]. Therefore, the current research was initiated with the objective to examine the histopathological images of rhino-orbital / rhino-maxillary/sino-nasal mucormycosis in the backdrop of ongoing COVID 19 pandemic.

MATERIAL AND METHODS

This was an cross sectional observational study conducted in the Department of Pathology of a tertiary care teaching hospital from May – August 2021. Institutional Ethics committee approval was obtained, prior to the start of the research.

The study involved analysis of all biopsy samples of suspected rhino-maxillary /rhino-orbital mucormycosis received from post COVID -19 patients. Clinical details were retrieved from hospital information system. The demographic details like, patients age, sex, and COVID 19 status were obtained. A preliminary review of the slides showing hyphal forms of fungal organisms with un-doubtful tissue / mucosal invasion were included. Samples received from non-COVID patients were excluded from the study.

Basic microbiological methods such as KOH smear and fungal culture was used for the detection of MC in the received clinical specimen and morphology was studied under the microscope.

Tissues samples were examined macroscopically, processed as per the standard protocols and routine Hematoxylin & Eosin (H&E) staining was done to inspect the samples. Special stains for fungus namely Periodic Acidic Schiff (PAS) was utilized to confirm and/or to differentiate the fungal organisms and to highlight the cell wall of the fungus.

Mucorales genera were identified based on the characteristic histological findings like non-pigmented, wide (5–20 µm), thin walled, ribbon-like hyphae with pauci septations or aseptate, and right angle branching. On H&E staining, under 40 x magnification,hyphae will be empty looking as has been described in the literature [8], [9]. Aspergillus species was demonstrated by nonpigmented (hyaline), narrow, septated hyphae with acute-angle branching.

Two pathologists independently analyzed the histopathological slides and reached consensus. Parameters noted were site of biopsy, type of inflammation as acute, chronic, mixed suppurative and granulomatous. Inflammation was graded as 1- mild, 2-moderate and 3 - dense. Further, the presence of necrosis were studied.

The data thus obtained was analyzed using R software version 4.1.2 and Microsoft Excel 2016 for Windows. Categorical variables were represented in the form of frequency table and Continuous variables as Mean ± SD/ Median (Min, Max) form.

RESULTS

Between the study periods of 4 months (May - August 2021) total 237 histopathological samples were received in the department of Pathology .Out of which there were 194 nasal biopsies and enucleation specimens received from post covid patients. The samples received were from patients with a wide age range, ranging from 21 to 80 years and the mean age range was 41-50 years. There was a predominance of males (78.9%) affected with mucormycosis. The age and sex distribution is represented on Figs. 1 & 2.

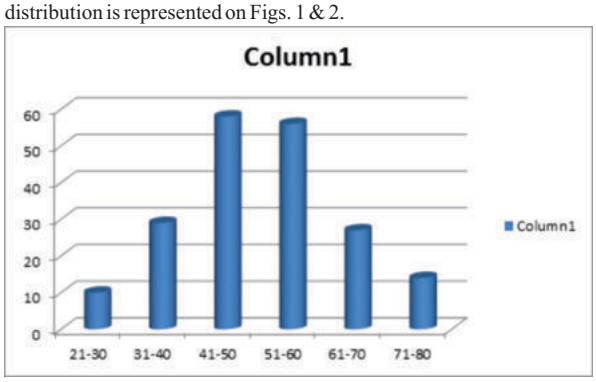

Fig. 1 Distribution Of Subjects According To Age.

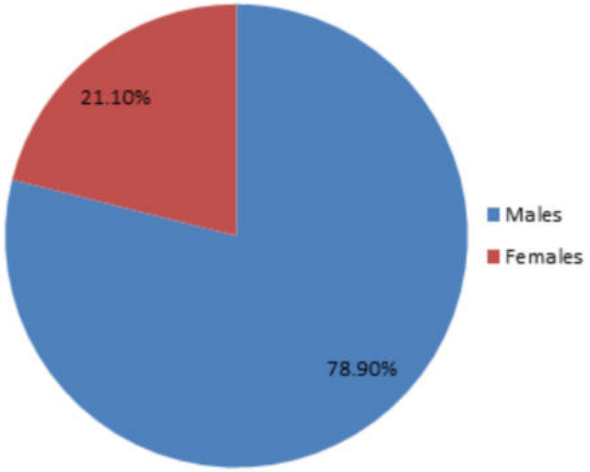

Fig. 2 Distribution Of Subjects According To Gender.

All patients were confirmed COVID 19 positive Macroscopic examination revealed the tissue samples to be predominantly gray-white to black in color.

Table 1. Summarizes The Type And Grading Of Inflammation And Necrosis Along With Type Of Organism

Table 1Distribution Of Type And Grading Of Inflammation Along With Type Of Organism.

Variables	Sub category	Number of subjects (%)
Granuloma	Absent	190 (98%)
	Present	04 (2%)
Type of Inflammation	Acute	07(3.6%)
	Chronic	152 (78.4%)
	Mixed	35 (18%)
Necrosis	Absent	16 (26.67%)
	Present A)Karyorrhetic B)Coagulative	43 (71.67%) 07(3.6%) 187(96.4%)
Grading of inflammation	Mild	0
	Moderate	24 (12.4%)
	Severe	170 (87.6%)
Mixed/ Only mucormycosis	Aspergillus	05 (2.6%)
	Mucormycosis	187 (96.4%)
	Asper+Mucor	02 (1.03%)

The predominant type of inflammatory response observed was chronic (78.4%), followed by mixed suppurative (18%) and the least was acute type (3.6%) which was seen only in aspergillus cases. Inflammatory infiltrate was mainly comprised of neutrophils, eosinophils, lymphocytes, macrophages and plasma cells. Patchy reactive lymphoid aggregates and multinucleated giant cell response were noted in a few cases.

The dominant fungus identified in the specimens were mucorales in 187 samples (96.4%), aspergillous in 5 samples(2.6%) and aspergillosis along with mucorales was identified in 02 samples (1.03%) .

Microscopic sections revealed wide (5–20 μm), thin walled, ribbon – like hyphae with pauci septations and right angle branching morphologically consistent with mucorales on H&E staining. Sections from two samples showed acute angled branching consistent with aspergillous.

Amount of fungal hyphal elements was identified more in the necrotic tissues. Inflammatory exudates predominantly of lymphocytes ,macrophages, areas of haemorrhage and thrombosis were exhibited in the surrounding tissue structures.

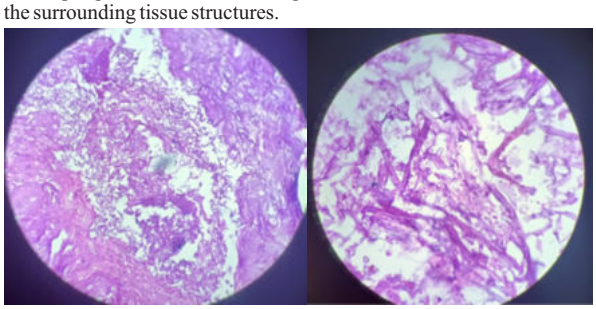

Fig.3
Histological findings of mucorales genera. (A)Microscopy sections of nasal mucosa with dense fungal growth by broad aseptate hyphae of mucor and areas of necrosis (haematoxylin-eosin stain, X10).

(B) (haematoxylin-eosin stain, X40) of the same

Our study group showed pathognomonic fungal morphology with the evidence of tissue necrosis which was mostly of coagulative type in mucor cases while karyorrhectic type was seen in aspergillus cases. Tissue inflammatory responses encountered were predominantly suppurative inflammation, mainly of neutrophils and granulomas formation. Fungal ball formation and sporule formation were less noticed. No Splendore-Hoeppli phenomenon was observed. All these case findings were described as histopathological characteristics to diagnose mucormycosis .

DISCUSSION

India was one of the worst hit countries with COVID and by the end of July 2021 had reported about 31 million cases [10]. It is now clear that COVID 19 weakens the host's immune system, making way for opportunistic secondary infections. Patients having recovered from COVID may develop fungal infections after a few weeks or months. COVID associated mucormycosis (CAM) may form about 0.3% of all co-infections [10].

Many states in India saw a sudden up surge of mucormycosis cases during the COVID second wave and it was declared an epidemic in the midst of an ongoing pandemic. The most common type noted clinically was that of Rhino-orbital-cerebral mucormycosis (CAROCM) [10], [11].

The spores of mucormycosis may gain entry into the human body via inhalation, ingestion or inoculation into an open wound, subsequently germinating inside the host into angioinvasive hyphae [2], [10].

The symptoms that most patients with CAROCM experience include facial pain and swelling, loss of vision, periorbital edema, etc. When the spores gain entry via inhalation, they enter the paranasal sinuses which later spread into the orbit and cerebrum. The fungi cause vascular invasion, thrombosis and necrosis [2], [10], [12], [13].

Various hypothesis proposed for the pathogenesis of CAROCM include the COVID associated lymphopenia, increase in pro-inflammatory markers, pulmonary damage, and hyperferretinemia, all of which favor fungal growth [2], [10], [14]

Frater et al. have described histopathological findings in

mucormycosis, where entirely neutrophilic response was seen in 50% of the cases, 25% had pyogranulomatous response, 5% showed only granulomatous response and 20% did not exhibit any inflammation. Whereas in the present study 78.4% showed chronic inflammation and neutrophilic response was seen only in 3.6% specifically in aspergillous cases.

Arora et al. studied the histopathological features of COVID associated rhino cerebral mucormycosis and the median age of the subjects was 57 years. Majority of the patients were males. Out of the 37-biopsy samples, soft tissue invasion was noted in 59%, necrosis with no cellular response was noted in 43%, acute suppuration in 5% of cases (n=2), granulomatous inflammation in 11% of the cases [15]. The results of the present study closely match the above study with age, sex and inflammatory reaction.

In a research by Jain et al. of COVID associated rhino cerebral mucormycosis, they also observed giant cell reaction in 33.3% of cases while necrosis was evident in more than 50% of the cases [11]. The results of the present study is similar to the above study.

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 [11].

A multicentric study by Patel A et al. compared mucormycosis with and without COVID association and identified 287 cases did not find any mixed fungi [16]. Similar results are also described by Ramadorai et al. [17]. Pakdel et al. in their study found Rhinomaxillary-orbital type to be most common (47%) followed by sino-orbital type (33%) and sino-nasal type was least common type (7%) [18]. In the present research, sino-nasal type was the most common site of involvement (96%) followed by rhino-orbital involvement(4%).

Certain immune-related factors in COVID-19 patients may allow subsequent opportunistic fungal infections such as mucormycosis to develop. COVID-19-related immunological dysregulation causes a reduction in T lymphocytes, CD4+T, and CD8+T cells, potentially altering innate immunity. Interleukin (IL) 4, IL-10, IL-17, and Interferon-gamma (IFN-g) are cytokines produced by CD4+and CD8+cells in response to fungal hyphae. The 'cytokine storm' may be exacerbated by the delayed IFN-γ response, extended hyperinflammatory state, and reduced CD4 and CD8 cell counts, which may enhance the severity of COVID-19 infection [19].

This study was a histopathology based research and positive results were confirmed on culture.

Limitations

A few limitations that were noted in the present research include the absence of recording and correlating clinical signs and symptoms of mucormycosis, and correlation with radiographic findings. Additionally, hospital or intensive care unit admission, receipt of oxygen, systemic disorders and other co-morbidities that the patients had, additional medications and drug history, vaccination status of the patients, evaluation of management and outcome/death were not included. Further research should be directed towards inclusion of these additional parameters to obtain a corroborative result.

CONCLUSION

The present research was formulated, noticing an abrupt rise of mucormycosis cases during the second wave of COVID-19 .It is a life threatening disease .Therefore early screening and definitive diagnosis between mucor and aspergillous by the histopathologist by various criterias setup were much needed for proper management of patients as treatment modalities of mucormycosis and aspergillous are different.

Conflict of Interest

Authors have declared that no financial / conflict of interest to disclose.

Acknowledgement

We express our gratitude to departments of Otorhinolaryngology, Medicine and Ophthalmology for their effort during the pandemic and associating us in patient management.

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