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Original Research Article

Histopathological Study of Mucormycosis at a Tertiary Care Hospital

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Abstract:

Study Background / Introduction: Mucor is the most common genus causing human mucormycetes in most cases followed by rhizopus & lichtheimia. It is associated with significant lymphopenia, long-term neutropenia, malnutrition, intravenous drug use and non-judicious use of corticosteroids. Mucormycosis commonly affects the nasal and paranasal sinuses, upper respiratory tract,lungs, angio-invasion and brain. Usually air-borne spores contaminate food and lab specimens producing infections. To confirm the diagnosis tissue sections are stained with Hematoxylin & Eosin (H& E), PAS / GMS stain. Patients present with clinical features of Periorbital edema, blurring of vision, nasal/paranasal congestion, crusting, dyspnea, cough, mild fever. There are reports of cases in literature with mucormycosis in poorly controlled diabetes mellitus while post covid invasive mucormycosis is the subject of interest due to its aggressive invasiveness & rapid mortality. Early clinical and lab diagnosis is the need of the hour with rational use of antifungal and broad spectrum antimicrobial treatment. Mortality accounts to 30-70% due to angioinvasion with complete cut off of blood supply, thrombosis, tissue infarction & extensive necrosis. Imaging findings on MRI with contrast gadolinium scan shows hyperdense foci within left ethmoid and sphenoid sinuses with orbital extension. Microscopic examination shows respiratory epithelial lining with stroma showing fungal elements in the form of broad aseptate hyphae with wide angle branching, fungal granulomas, foreignbody giant cells dense mixed inflammatory infiltrate with predominant eosinophils and neutrophils admixed areas of necrotic tissue. Gomori's silver methenamine stain highlights black pigmented Broad aseptate hyphae. Additional confirmatory method by Immunofluorescence with Robin blue/Calcofluor white stain for fungal elements also reveals Mucor species. Fungal cultures are diagnostic for fungal elements obtained after 48hrs on Sabouraud's/Potato dextrose agar. The present study aims to Evaluate histopathological diagnosis and microbiological evidence in clinically diagnosed mucormycosis in post covid patients.

Aim of the study: This study is conducted with an aim to Diagnosis of invasive Mucormycosis on Histopathological examination at a tertiary referral hospital. Rhino cerebral Mucormycosis is the current life-threatening infection commonly affecting immunocompromised patients especially post COVID-19 within 6 weeks having poor glycemic control, Intravenous drug users, ICU patients, post chemotherapy, iron overload, persistent neutropenia and follow-up cases of hematological malignancies. Histopathological features of mucormycosis showing severe fungal load, large areas of necrosis, presence of angioinvasion and perineural invasion were associated with poorer prognosis and decreased survival rate. Thus, histopathologists can help in assessing prognosis at the time of tissue diagnosis so that clinicians could optimize treatment accordingly.

Primary Objective: In the general population attending the tertiary care centre to determine the following characteristics of Mucormycosis a) The age wise distribution b) Histomorphological patterns associated aggressiveness.

Seconday Objective: a) Frequency of occurrence of infections b) Can analyse associated high risk factors c) To determine the significance of histopathological examination.

Justification of study: The frequency of mucormycosis will be analysed and assessed histomorphological features with special attention to angio invasion especially in high risk patients like post covid patients, immunosuppressive patients and Diabetic patients. To determine accurate histopathological diagnosis is essential for treatment and determining the prognosis. ICMR recommended multidisciplinary team approach for early diagnosis and management is highlighted. This present study emphsis Histopathological examination also one of the the gold standard tests like microbiological fungal culture for early diagnosis of mucormycosis.

Study area: The present study is a observational retrospective study to be conducted at Government Pudukottai Medical College and Hospital, Tamil Nadu over a period of 2 years from January 2021 to December 2022 in the Department of Pathology. **Study population:** The data pertaining to samples of tissues infected with mucormycosis received for histopathological evaluation to the Pathology laboratory of our tertiary care hospital. **Study design:** This study is hospital based Retrospective study. **Time duration:** 2 years. **Sample size:** All the

resected specimens and biopsies pertaining to our study received and reported in our Pathology Department Inclusion criteria: All the cases of mucormycosis reported in the Department of Pathology in a tertiary care centre in south India from January 2021 to December 2022 were included in the study. **Exclusion criteria:** Patients diagnosed with small biopsies and same was diagnosed in resected specimens means, the small biopsy datas may be excluded. **Data collection Methods:** Approval from institutional ethical committee was taken before undertaking the study. All the required Datas/Materials for this study will retreive from the Pathology departmental registers which will be analysed later. **Informed consent:** NA. **Adverse events monitoring:** Not applicable. **Follow up:** Not applicable. **Statistical analysis:** Data will be summarized using percentages, mean, and standard deviation. Categorical variables will be analysed using Chi-square test and fisher's exact test. Significance will be assessed at P value <0.05. All data will be entered in excel format and statistical analysis will be done through SPSS software version 26.0 charts and Graphs will be expressed using OriginmPro v9.1.

Introduction

Mucor is the most common genus causing human mucoromycetes in most cases followed by Rhizopus & Lichtheimia. It is associated with significant lymphopenia, long-term neutropenia, malnutrition, intravenous drug use and non-judicious use of corticosteroids [1]. Fungi of the order Mucorales belonging to the phycomycetes class are implicated in a potentially invasive and often fatal opportunistic infection called the Mucormycosis [2].

Mucorales hyphae are non-septate or pauci-septate with a variable width of 6-16microns. To confirm the diagnosis tissue sections are stained with Haematoxylin & Eosin (H & E), PAS and GMS stain. Patients present with clinical features of Periorbital oedema, blurring of vision, nasal/paranasal congestion, crusting, dyspnoea, cough, mild fever [3]. The mode of transmission is through inhalation of spores. Surgical debridement and amphotericin-B remains the mainstay of treatment.

The angio-invasive nature and rapidity of dissemination of these molds make it potentially life-threatening. Early diagnosis and treatment may prevent the rapid progression of the disease since the reported mortality rates from intra-orbital and intracranial complications are 50-80%. The fatality rate is as high as 90% in cases of intracranial involvement [4].

According to a report, mucormycosis may develop in 0.25% of COVID-19 patients, anywhere between 15 and 30 days, post infection [5]. This particular disease also received a lot of media attention as "Black fungus" owing to the colour of the necrotic tissue. The Department of surgical Pathology involved in this research, during the second wave of the pandemic, started to receive several emergency biopsies of mucormycosis [6]. Untreated rhino sinus 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. Histopathological 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.

Materials and Methods

A observational retrospective study was conducted at Government Pudukottai Medical College and Hospital, Tamil Nadu over a period of 2 years from January 2021 to December 2022 in the Department of Pathology. Thirty nine post COVID – 19 patients were included in the study, whose tissue biopsy samples included functional endoscopic sinus surgery. The study involved analysis of all biopsy samples of suspected rhino-maxillary /rhino-orbital mucormycosis received in post COVID -19 patients. Clinical details were retrieved from hospital information system. The demographic details like, patients age, sex, diabetic status, glycemic control and COVID 19 status were obtained. A preliminary review of the slides showing hyphal forms of fungal organisms with un-doubtful tissue / mucosal invasion was included. Samples received from non-COVID patients were excluded from the study.

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) and Gomori Methenamine silver (GMS) were 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. Candida species were diagnosed based on the morphological findings such as yeasts (3–5 µm) forms of smaller size admixed with pseudohyphae and/or hyphae forms [10].

Tissue samples received were studied for the following microscopic details.

1. Fungal morphology
2. Fungal load, graded as mild, moderate and severe
3. Presence and absence of tissue necrosis
4. Angioinvasion and perineural invasion
5. Presence/absence of granuloma

Invasive fungal sinusitis was diagnosed when hyphal forms were present in sinus mucosa or submucosa, blood vessel or bone.

Results

Between the studies periods of 2 years (January 2021 to December 2022) biopsy samples from thirty nine patients with post COVID infections were studied. The samples received were from patients with a wide age range, ranging from 20 years to more than 70 years and the mean age was 51.5 years. There was a predominance of 29 males (74.35%) affected with mucormycosis.

The age and sex distribution is represented on Figs. 1 & Table 1.

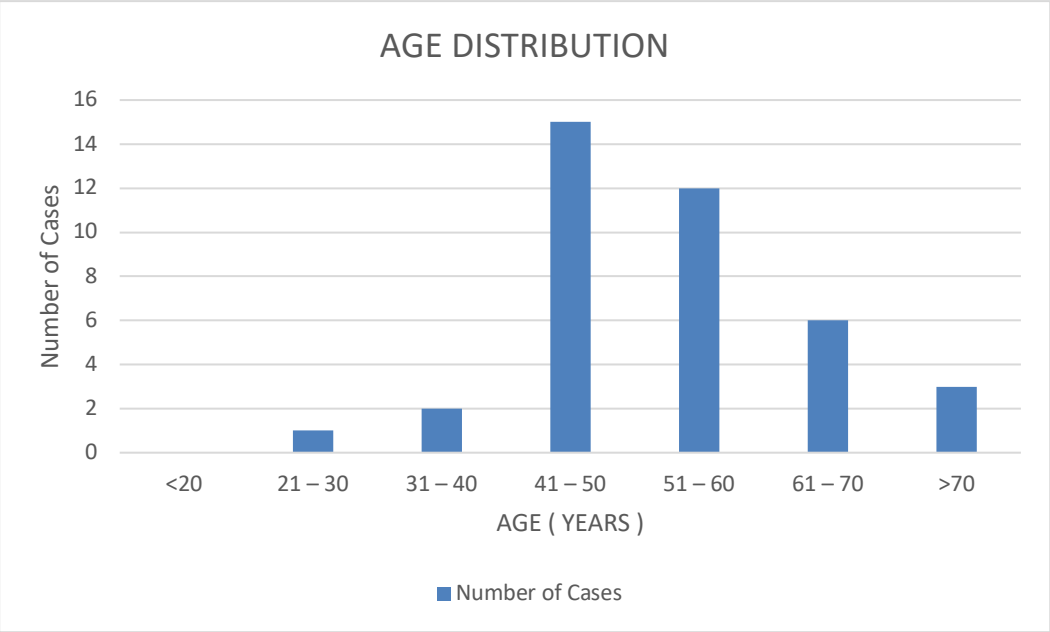

Figure 1:

Table 1: The Age and Sex distribution

Age (Years)	No. of Cases
<20	0
21 – 30	1
31 – 40	2
41 – 50	15
51 – 60	12
61 – 70	6
>70	3

Macroscopic examination revealed the tissue samples to be predominantly gray-white to black in color. Tissue necrosis was observed in 76.92% of cases. The dominant fungus identified in the specimens were mucorales in 37 samples (94.87%), aspergillous was identified in 2 samples (5.13%).

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 with fragmented, folded and thick-walled spherical structures were also observed. Special stains were done (PAS &GMS) in some of the cases with sparse fungal load to demonstrate the fragmented/ sparse fungal wall and also the vessel wall invasion by the fungus.

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

The histological findings of mucorales genera and special stains (PAS &GMS) are represented on Fig. 2. Sections with granulomatous inflammation consisting chiefly of multinucleate giant cells and surrounded by cuff of neutrophils, eosinophils, lymphocytes and plasma cells. These were noted in 33.33% of cases. No central necrosis was observed in the granulomas. The number of fungal organisms noted in these cases was less when compared to nongranulomatous inflammatory response type cases.

Angio invasion was identified in 53.84% of samples. It was evident by the presence of fragmented fungal elements invading the blood vessel wall or inside their lumen with thrombi formation. Adjacent tissue shows areas of ischemic necrosis. Special stains were done in difficult cases to highlight the elastic lamina of the vessel wall and also to identify the fragmented fungal structures. Tissue necrosis was identified by granular basophilic material with karyorrhectic debris. Tissue invasion was noted in 76.92% of the samples with extensive areas of mucosal tissue necrosis. Bony invasion seen in 12.82% of the samples and were evident by the presence of hyphal structures in the trabecular marrow spaces.

Perineural / neural invasion was identified in 10.25% of samples with the invasion of the nerve bundles by fragmented fungal hyphae observed in the periorbital soft tissues. Cases with combined mucor, candida and or aspergillus show similar tissue reactions with the predominance of neutrophilic respons. Many black pigmented fruiting bodies and oxalate crystalloids were noticed in combined mucor and aspergillus infection. We did not encounter Splendore-Hoeppli phenomenon, bony/vascular invasion by Aspergillus/ Candida species.

Table 1 summarizes the invasion pattern of the infection and Table 2 summarizes the type and grading of inflammation.

Our study group showed pathognomonic fungal morphology with the evidence of tissue necrosis, angio invasion and perineural invasion. 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 and were similar to non- COVID mucormycosis. Except the formation of granulomas were observed in immunocompetent individuals of non-COVID mucormycosis.

Table 2: Distribution of Invasive Pattern of the Infection

Variables	Sub Category	No. Of Patients (%)
Tissue invasion	Absent	9
	Present	30
Angioinvasion	Absent	18
	Present	21
Bony Invasion	Absent	34
	Present	5
Perineural invasion	Absent	35
	Present	4

Table 3: Distribution of Type and Grading Of Inflammation Along With Type of Organism

Variables	Sub Category	No Of Subjects
Granuloma	Absent	26
	Present	13
Type of Inflammation	Acute	7
	Chronic	6
	Mixed	26
Necrosis	Absent	9
	Present	30
Grading of inflammation	Mild	3
	Moderate	9
	Severe	27
Mixed / Only mucormyosis	Mixed (Aspergillus)	5
	Only Mucormycosis	34

Table 4: Site of Infections

Site of Infection	Number
Nasal Cavity	17
Maxillary Sinus	15
Rhino orbital	5
Rhino maxillary	2

Table 5: Microscopic Results of Histopathological Examination with H & E Stain

S.No.	Impression	No. Of. Cases
1.	Mucormycosis	15
2.	Angioinvasive Mucormycosis	22
3.	Mixed (Aspergillus + Mucormycosis)	2

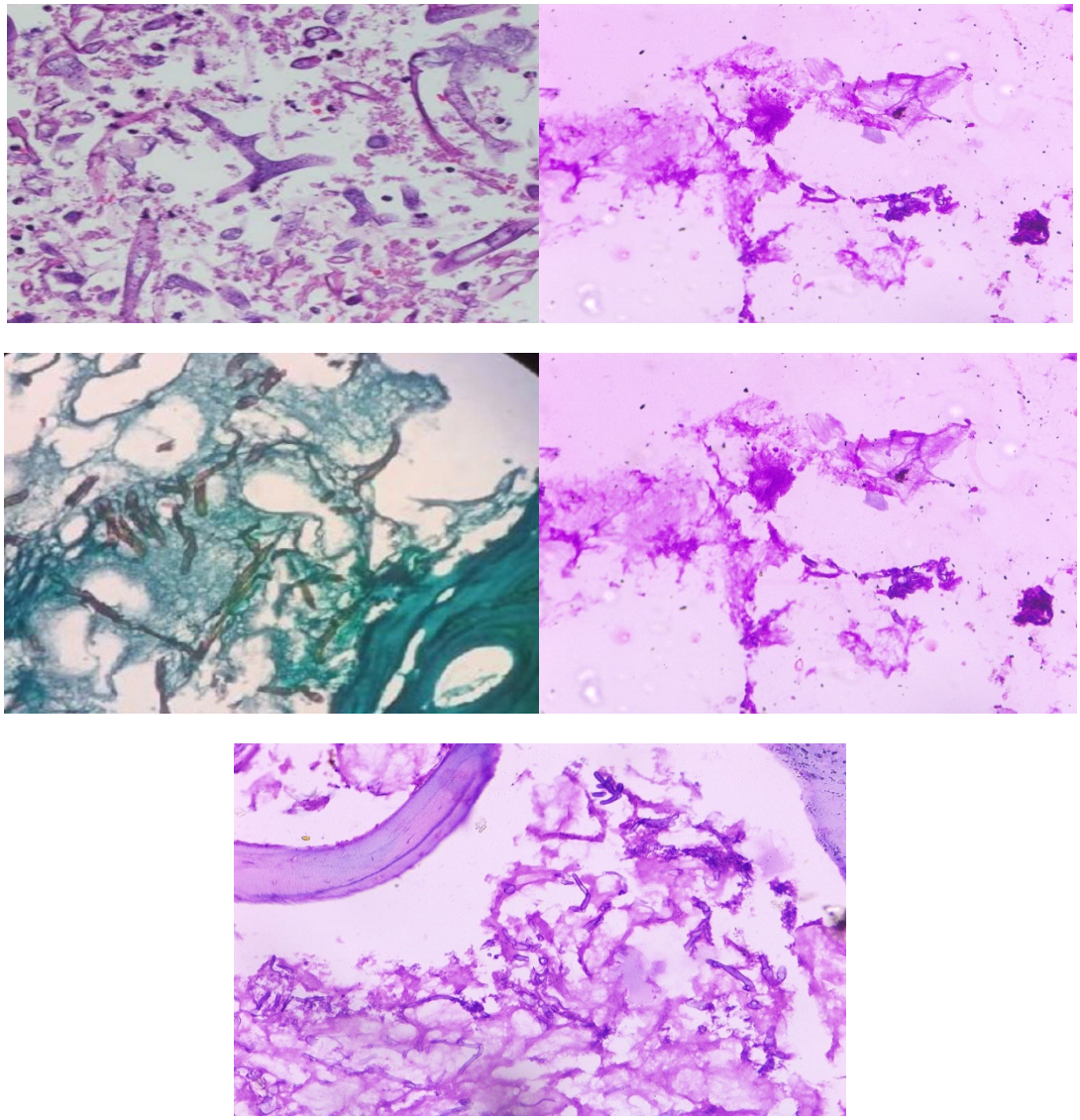

Figure 2:

Discussion

India was one of the worst-hit countries for COVID, with around 31 million cases recorded by the end of July 2021 [11]. It is now obvious that COVID-19 inhibits the host's immune system, allowing for opportunistic secondary infections. Patients who have healed from COVID may acquire fungal infections within a few weeks or months.

COVID-associated mucormycosis (CAM) may account for around 0.3% of all co-infections [11]. During the COVID second wave, several Indian states had a significant increase in mucormycosis

cases, which was designated an epidemic among a continuing pandemic.

The most prevalent kind clinically seen was rhino-orbital-cerebral mucormycosis (CAROCM) [11,12]. Mucormycosis comprises a group of infections caused by the fungi belonging to the order Mucorales and family Mucoraceae. Rhizopus oryzae is the most common cause of infection. They are seen in soil and decaying matter and are transmitted by airborne asexual spores [13].

Mucormycosis infiltrates blood vessels and bone trabeculae as wide, aseptate or slightly septate ribbon-like hyphae ranging from 5 µ to 20 µm. Septate hyphae with acute-angle branching ranging from 3 to 5 µm appear as Aspergillus sp. or other hyaline moulds. These organisms can be spotted on microscopy in sites of suppurative tissue necrosis [14]. 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 [11].

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 [11,15,16]. Several hypotheses for the pathophysiology of CAROCM include COVID-associated lymphopenia, an increase in proinflammatory markers, pulmonary injury, and hyperferretinemia, all of which promote fungal development [11,17].

Additionally, diabetic ketoacidosis, free radical-induced endothelial inflammation, and hepcidin activation have all been implicated in the pathophysiology of COVID mucormycosis [18]. Uncontrolled diabetes mellitus, the use of systemic corticosteroids, prophylactic broad-spectrum antibiotics that disrupt nasal commensals, allowing pathogenic fungi to invade, immune-suppressive medications and diseases, organ transplants, and haematological malignancies have all been identified as risk factors for CAROCM [11, 15]. Interestingly, inhaling steam, which was frequently recommended by many practitioners in India to treat nasal congestion in COVID, was discovered to damage the sensitive nasal mucosa, allowing mucorale invasion [19].

Chouhan et al. in their research found 41 patients who satisfied the diagnostic criteria of CAROCM and accordingly, had a male preponderance (n = 28), 36 patients had a history of steroid use, 40 patients were diabetics and majority of them had received oxygen while in the hospital [18].

The current study had a similar male preponderance (74.35%) Two systematic reviews conducted in the past on CAROCM have identified diabetes mellitus as the most common comorbidity followed by hypertension. The biopsy samples obtained from the patients in the present research were also diabetics.

Frater et al. have also 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. They further observed angioinvasion in 100% of cases and perinural inflammation in 90% of cases. They opined that since loose facial sheath surrounds the nerve, perineural invasion of the fungi could take place with ease [23].

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, steroids had been used in 45% of the cases and diabetes mellitus was the predisposing factor in 98% of the cases. 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 [24].

The results of the present study closely match the above study with age, sex and predisposing factor being similar. Soft tissue invasion was seen in a higher percentage with 76.9% of the specimens, abscess formation in the present study.

In a study of COVID-associated rhinocerebral mucormycosis by Jain et al., angioinvasion was observed in 100% of the cases (n = 95), soft tissue invasion in 58% of the cases, bone invasion in 6.3% of the cases, and nine cases showed the presence of peripheral nerves, three of which showed perineural invasion. They also found a large cell response in 33.3% of the cases, as well as necrosis in more than half of them [12]. In terms of invasions, the results of the current study are comparable to those of the previous study, with the exception that the proportion of engagement in the current study was significantly greater. This might be related to a larger proportion of patients with immunocompromised conditions.

Jain et al. also discovered that out of 90 instances of COVID-associated rhinocerebral mucormycosis, one had a mixed infection with aspergillus and two had candida [12]. Pal et al. discovered four incidences of mucormycosis combined with aspergillosis in 99 individuals with COVID-associated mucormycosis. [20]

In the current investigation, combined infection with aspergillus was found in 6.89% of patients. In the current study, two cases of mucormycosis with Aspergillus niger revealed aspergilloma, which consisted of an extracellular fungal ball surrounded by granulation tissue and an outer layer of fibrosis.

Patel A et al. conducted a multicentric research to examine mucormycosis with and without COVID relationship and found 287 patients, 65.2% of which were COVID-associated and 62.7% of which were diabetic. They discovered that the rhino-orbital type was the most prevalent, and there were no mixed fungus [28].

Ramadorai et al. [25] found similar results. In their investigation, Pakdel et al. discovered that the most prevalent form was rhinomaxillary-orbital (47%) followed by sino-orbital (33%), with sino-nasal being the least common kind (7%) [30]. In the current study, a larger proportion of patients had increased glycosylated haemoglobin (82%). The most prevalent form of involvement was sino-nasal, followed by rhino-orbital.

Cornely et al. have described the histopathological picture of mucormycosis and accordingly the acute lesions show hemorrhagic necrosis, angioinvasion, coagulative necrosis, neutrophilic infiltration and perineural invasion.

Chronic lesion on the other hand, shows a pyogranulomatous inflammation with giant cells along with a deeply eospinophilic material surrounding the pathogen, the Splendore- Hoeppli phenomenon [12]. The mucormycosis specimens in the current research showed similar classic features such as angioinvasion, granulomatous inflammation along with perineural invasion.

Mucormycosis cases in India during the second wave were estimated to be 70 times higher than the worldwide incidence and there was a sudden increase during the period between April and July 2021 [12].

The same trend was noticed in the present research as well. Interestingly and additionally, the present research identified a change in the histopathological appearance of mucormycosis during the month of May 2021 which was predominantly necrotic tissue with abundant fungal organisms. When the pandemic started to decline, during the month of June 2021, the histopathology showed more of granulomatous type of inflammation with fewer organisms.

The literature does not address the change in histological appearance of mucormycosis during the rise and fall of the second wave of COVID 19, which was seen in the current study. The variable pattern of inflammation seen in the current study may explain the virulence of the organisms, the kind of viral strain, and the patient's immunological condition.

Patients with improved immune systems, which results in the creation of granulomatous inflammation with minimal fungal burden, have a good prognosis. Mucormycosis infection with granulomatous inflammation is extremely uncommon, with only a few case reports available [26]. In the current study, 23% of the patients had granulomatous inflammation with fungus in the cytoplasm. Interestingly, the bulk of these occurrences were recorded in.

Conclusion

The current study was designed after noting a dramatic increase in mucormycosis patients during the second wave of COVID-19. This infection was primarily related with diabetes mellitus. During the pandemic's initial stages, the expected histological appearances of angioinvasion, bone and soft tissue invasion, and a larger percentage of perineural invasions were all present.

The unanticipated feature was the alteration of inflammatory pattern, which was more of granulomatous type with reduction in tissue and vascular invasion coinciding the decline of the COVID second wave.

This substantiates that as immunity evolves, the host response varies for secondary opportunistic infections. Histopathological examination and fungal culture are the gold standard tests for early diagnosis of mucormycosis.

References

1. Sen M, Lahane S, Lahane TP, Parekh R, Honavar S. Mucor in a viral land: A tale of two pathogens. Indian J Ophthalmol. 2021; 69(2):244– 52.
2. R. Meher, V. Wadhwa, V. Kumar, D. Shisha Phanbuh, R. Sharma, et al., COVID associated mucormycosis: A preliminary study from a dedicated COVID Hospital in Delhi, Am. J. Otolaryngol. 2022;43 (1):103220.
3. (Sarkar S, Gokhale T, Choudhary SS, Deb AK. COVID-19 and orbital mucormycosis. Indian J Ophthalmol. 2021; 69(4):1002–4.
4. (Deutsch PG, Whittaker J, Prasad S: Invasive and non-invasive fungal rhinosinusitis-a review and update of the evidence. Medicina (Kaunas). 2019; 55.
5. Thakar, D. Lal, “Black fungus”: a perspective on the coronavirus disease 2019 (COVID-19)-associated rhino-orbital mucormycosis epidemic in India, Int Forum Allergy Rhinol. 8 2021; 1278–1279.
6. (J.M. Aranjani, A. Manuel, H.I. Abdul Razack, S.T. Mathew, COVID-19-associated mucormycosis: evidence-based critical review of an emerging infection burden during the

pandemic's second wave in India, PLoS Negl. Trop. Dis. 2021;15 (11): e0009921.
7. D.P. Gupta, S. Gupta, C.K. Shah, S.R. Sreevidya, Clinical study of surge of mucormycosis in COVID-19 pandemic: a Tertiary Care Center Study [published online ahead of print, 2021 Aug 4], Indian J. Otolaryngol. Head. Neck Surg. 2021.
8. O.A. Cornely, A. Alastruey-Izquierdo, D. Arenz, S.C.A. Chen, E. Dannaoui, B. Hochhegger, et al., Mucormycosis ECMM MSG Global Guideline Writing Group, Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium, Lancet Infect. Dis. 2019;19 (12): e405–e421.
9. M. Karanth, P. Taniere, J. Barraclough, et al., A rare presentation of zygomycosis (mucormycosis) and review of the literature, J. Clin. Pathol. 2005;58: 879–881.
10. J. Guarner, M.E. Brandt, Histopathologic diagnosis of fungal infections in the 21st century, Clin. Microbiol Rev. 2011;24 (2): 247–280.
11. J.M. Aranjani, A. Manuel, H.I. Abdul Razack, S.T. Mathew, COVID-19-associated mucormycosis: evidence-based critical review of an emerging infection burden during the pandemic's second wave in India, PLoS Negl. Trop. Dis. 2021;15 (11): e0009921.
12. 12.K. Jain, A. Surana, T.S. Choudhary, S. Vaidya, S. Nandedkar, M. Purohit, Clinical and histology features as predictor of severity of mucormycosis in post-COVID-19 patients: an experience from a rural tertiary setting in Central India, SAGE Open Med. 2022;10: 20503121221074785.
13. O. Anyapu, S. Parvatini, P. Kalivarapu, R. Kuna, J. Suvvari, Surge and scare of mucormycosis in the backdrop of COVID-19 pandemic, J. Clin. Diagn. Res. 2022;16 (3): EC15–EC18.
14. S. Kandasamy, S. Muthuraju, A. Vasugi, M. Chandrasekar, R. Murugan, P. Inbasekaran, P. R, Clinicopathological study of mucormycosis in COVID-19 patients: experience from a tertiary care center in South India, Cureus. 2022; 14 (3): e23016.
15. M. Chouhan, B. Solanki, N. Shakrawal, Rhino-orbital-cerebral mucormycosis: fungal epidemic in a viral pandemic, J. Laryngol. Otol. 2021;135 (11): 981–986.
16. G. Reid, J.P. Lynch 3rd, M.C. Fishbein, N.M. Clark, Mucormycosis, Semin Respir. Crit. Care Med. 2020;41 (1): 99–114.
17. I.C.N. Rocha, M.M. Hasan, S. Goyal, T. Patel, S. Jain, A. Ghosh, et al., COVID-19 and mucormycosis syndemic: double health threat to a collapsing healthcare system in India, Trop. Med. Int. Health. 2021;26 (9): 1016–1018.
18. Jose, S. Singh, A. Roychoudhury, Y. Kholakiya, S. Arya, S. Roychoudhury, Current understanding in the pathophysiology of SARS-CoV-2-associated rhino-orbito-cerebral mucormycosis: a comprehensive review, J. Maxillofac. Oral. Surg. 2021;20 (3): 373–380.
19. S.K. Kumar, S. Chandran, A. Mohanty, M.K. Jha, Black fungus: possible causes of surge in cases in the second pandemic in India, J. Fam. Med. Prim. Care. 2021; 10 (11): 4322–4323.
20. R. Pal, B. Singh, S.K. Bhadada, M. Banerjee, R.S. Bhogal, N. Hage, et al., COVID-19-associated mucormycosis: an updated systematic review of literature, Mycoses. 2021; 64 (12): 1452–1459.
21. A.K. Singh, R. Singh, S.R. Joshi, A. Misra, and Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India, Diabetes Metab. Syndr. 2021;15 (4): 102146.
22. S. Sharma, M. Grover, S. Bhargava, S. Samdani, T. Kataria, Post coronavirus disease mucormycosis: a deadly addition to the pandemic spectrum, J. Laryngol. Otol. 2021; 135 (5): 442–447.
23. J.L. Frater, G.S. Hall, G.W. Procop, Histologic features of zygomycosis: emphasis on perineural invasion and fungal morphology, Arch. Pathol. Lab. Med. 2001;125 (3): 375–378.
24. R. Arora, R. Goel, S. Khanam, S. Kumar, S. Shah, S. Singh, et al., Rhino-orbito-cerebral-mucormycosis during the COVID-19 second wave in 2021 - a preliminary report from a single hospital, Clin. Ophthalmol. 2021;15: 3505–3514.
25. Ramadorai, P. Ravi, V. Narayanan, Rhinocerebral mucormycosis: a prospective analysis of an effective treatment protocol, Ann. Maxillofac. Surg. 2019; 9 (1): 192–196.
26. Z. Chi, C. Zhang, W. Wang, Granulomatous mucormycosis of the temporal bone extending into temporomandibular joint and infratemporal fossa: a case report, Ear Nose Throat J. 2021; 1455613211022079.