ORIGINAL RESEARCH PAPER

Histopathology

ORBITAL MUCORMYCOSIS IN COVID-19 PATIENTS A SINGLE-CENTER EXPERIENCE FROM NORTH INDIA

KEY WORDS: Orbital Mucormycosis, COVID-19

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ABSTRACT

Background: Mucormycosis is a rapidly progressive & an extensively invasive disease. The notoriously common involvement of orbit by Mucor may endanger the vision, maiming the already perturbed COVID-19 patients. High mortality is found in COVID-19 cases with Orbital Mucormycosis as they are associated with high degree of cerebral involvement. **Aims & Objectives**: 1) To correlate histomorphology with clinical profile. 2) To identify histopathological features pointing towards severity and to know extent of involvement. **Materials & Methods:** This retrospective observational study was carried out on the debridement specimens and biopsies suspected of mycotic infection which were received between May 2021 and July 2021 in the Histopathology section of Pathology Department, SMS Medical College, Jaipur and cases of Orbital Mucormycosis were selected. Tissues were received in 10% formalin. They were processed and stained with Hematoxylin & Eosin (H&E) and other stains. Detailed clinical history was taken after obtaining an informed consent from the patients or their attendants. **Results:** Histopathological features like fungal load, degree of tissue necrosis, acute inflammation, perineural invasion, angioinvasion, presence of giant cell reaction and granuloma were identified and scored. Severity Score was calculated based on Histopathological Findings. Grade I – 10 Patients (33.3%), Grade II - 8 Patients (26.7%), Grade III - 12 Patients (40%). 33.3 % mortality was observed in GRADE III patients i.e., who had the highest severity according to the Histopathological findings. **Conclusion:** Biopsy from clinically affected sites and demonstration of hyphae by light microscopy are crucial to confirm the diagnosis and to determine the severity and extent of disease thus to define and re-evaluate the treatment response. The histopathological data gives definite evidence of high mortality in patients with COVID-19 associated Orbital Mucormycosis.

INTRODUCTION:

COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). It produces pneumonia-like symptoms and has recently shown association with fungal infections like Aspergillosis, Candidiasis, Mucormycosis and Cryptococcosis.[1]

Mucormycosis is an acute, invasive fungal infection that in a robust immune system rarely causes disease.(2,3) It has an incidence rate of 0.005 to 1.7 per million people worldwide. The exact incidence of Mucormycosis in India is not known due to lack of population-based studies. The estimated prevalence is 0.14 per thousand people, which is about 70 times higher in India than in global data. [4]

Indian Mucormycosis has certain unique features. It's association with uncontrolled Diabetes Mellitus is the predominant characteristic. [5] In susceptible conditions like uncontrolled Diabetes Mellitus, steroid therapy, transplant cases, cancer patients and other immunocompromised states, the disease usually starts in the Nose and Para-Nasal Sinuses after inhalation of fungal spores (Order Mucorales, most commonly by the species of Rhizopus Oryzae) from where it can spread into orbit and cranium.[6] Immunodeficient states and injudicious use of steroids may be responsible for the abnormal spike in the incidence of otherwise rare Sino-orbital Mucormycosis.[7]

Orbital Mucormycosis is the presentation of pathological symptoms in the orbit such as pain in eye, periorbital swelling, ptosis, blurring of vision, diplopia and external ophthalmoplegia. If left untreated, it is a vision-threatening and potentially fatal infection with high mortality rate amongst the patients with COVID-19.[8] It has been shown that delayed initiation of therapy by just 6 days increases mortality by two-fold.[9]

The objective of this study was to correlate histopathological features with clinical, radiological, and microbiological findings of COVID-19 patients with orbital Mucormycosis & to identify Histo-morphological features pointing towards severity and extent of disease so as to aid in early diagnosis and prompt intervention. This can help in appropriate management & better outcome of patients with COVID-19 associated Mucormycosis.

MATERIALS AND METHOD:

This was a retrospective observational study. During the unusual outbreak of Mucormycosis between May 2021 and July 2021, 750 surgical debridement specimens & biopsies of COVID-19 patients suspected of mycotic infection were received in the Histopathology section of Pathology Department, SMS Medical College, Jaipur.

Inclusion Criteria: All COVID-19 patients positive with RT-PCR or HRCT and having orbital involvement were included in the study.

Exclusion Criteria: Cases of non- COVID-19 mucormycosis, fungal infections other than mucor and patients lost to follow-

up were excluded from the study.

Out of 750 mycotic infections, 30 cases of Orbital Mucormycosis were obtained and included in the study. Demographic data including age and sex of patients, detailed history of clinical signs and symptoms, pre-existing co-morbidities, treatment history for COVID-19 infection, interval between COVID-19 infection and development of Mucormycosis symptoms, COVID-19 vaccination, anti-fungal treatment taken were obtained from all the patients. The laboratory, radiological and microbiological investigations were collected.

Procedure: All biopsy specimens were received in 10% formalin. Tissues were processed and stained with Hematoxylin & Eosin (H&E) stain along with special stains like Periodic Acid Schiff (PAS) and Gomori Methenamine Silver (GMS) (wherever required). Histopathological diagnosis of Mucormycosis was made on the basis of presence of broad aseptate hyphae with right angled branching on light microscopy. In our study we looked at the following variables as indicators and contributors of severity:

- Fungal load was quantified by seeing broad, aseptate hyphae with right angled branching in every high-power field.
- Tissue necrosis was described as non-viable tissue revealing fungal hyphae and was semi- quantified as percentage of tissue showing necrosis under low power.
- Neutrophilic cell infiltrate was measured in areas seen with fungal hyphae in 40X and graded depending on intensity of infiltrate.
- Granulomatous inflammation was observed as giant cell reaction or well-formed granulomas exhibiting fungal hyphae.
- The presence of fungal hyphae in the vessel wall and around the nerve was considered as definite evidence of angio-invasion and peri-neural invasion respectively.

Findings were used to assess the extent and severity of the disease and hence to correlate clinical and histopathological findings with disease prognosis. Patient's follow-up data was noted.

Detailed Informed consent was attained from the patients or their attendants before the beginning of study. Ethics clearance was duly obtained from Institutional Ethics Committee.

RESULTS:

Our study involved 19 males and 11 females with age ranging from 18 to 75 years among which 17 patients (56.67%) were above 50 years of age. 18 (60.0%) patients had type 2 Diabetes and 6 (20.0%) had a history of hypertension. Only 3 patients (10.0%) had received a dose of COVID-19 vaccine. The mean interval between COVID-19 and mucormycosis infection was 13.65 + 4.33 days.

Of the 30 Orbital Mucormycosis cases, right side of face was affected in 17 patients (56.67%), while left was involved in 13 (43.33%). The common presenting complaints were headache in 20 (66.67%), pain in eye and face in 17 (56.67%), periorbital swelling in 15 (50.0%), numbness in 13 (43.34%), facial swelling in 12 (40.0%) and eyeball movement restriction/loss in 7 patients (23.34%). Vision loss in 5 (16.67%), blurring of vision in 3 (10.0%), nasal irritation in 2 (6.67%) and tingling sensations in 1 patient (3.33%) were also present. Laboratory investigations revealed elevated ESR, CRP & D-dimer in all 30 patients. MRI and/or CT-scan of paranasal sinuses demonstrated involvement of maxillary sinus in 8 patients (26.67%), ethmoid sinus in 6 (20.0%) and frontal sinus in 3 patients (10.0%) accompanied by spread to orbit and orbital cellulitis in 12 (40.0%). KOH mount and Fungal culture showed mucormycosis in 17 (56.7%) patients while in 13 cases (43.3%) were found negative.

On light microscopy, 28 cases (93.3%) showed ischemic necrosis, hemorrhage, and congestion along with varying degrees of inflammatory infiltrates in all cases. Polymorphonuclear infiltrate was seen in 16 cases (53.3%) and graded as mild, moderate, and severe. Mixed inflammation and chronic inflammation were seen in 10 (43.3%) and 4 cases (13.3%) respectively. Histopathology confirmed Angio-invasion in 7 patients (23.4%) and peri-neural invasion in 8 (26.7%). In 5 cases (16.6%), granuloma formation was seen while giant cell reaction was present in only 1 patient (3.4%). Mycotic thrombi were seen in 4 (13.3%) cases. Retinal vessel involvement was seen in 3 (10.0%) patients. Periorbital fat and tissue were frequently invaded by the fungus i.e., in 22 (73.3%) and 5 patients (16.6%) respectively, followed by muscle in 4 (13.3%) and bone 2 in patients (6.66%).

Out of 30, treatment outcome was found to be successful in 26 patients whereas four patients expired while receiving treatment.

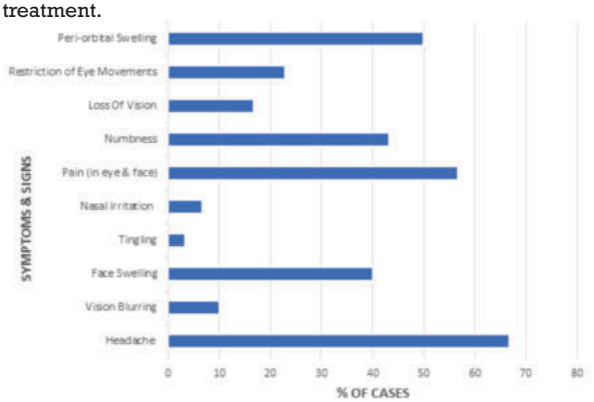

Chart 1- Clinical findings in orbital mucormycosis patients.

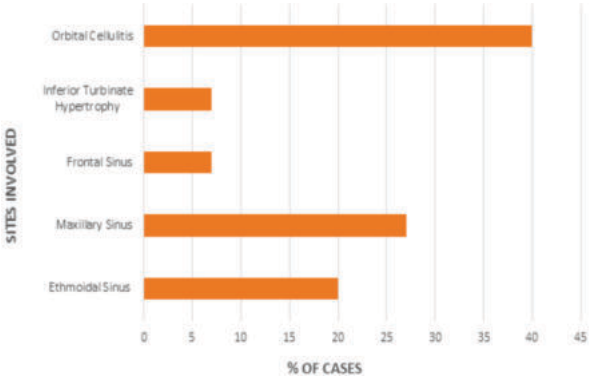

Chart 2- Radiological findings in orbital mucormycosis patients.

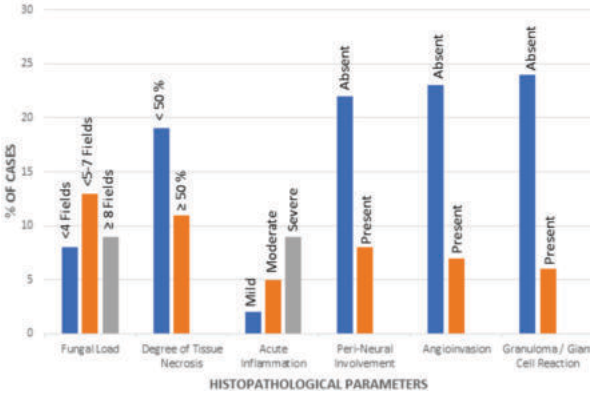

Chart 3- Histopathological parameters in orbital mucormycosis patients.

Table 1- Histopathology score and grades of severity in post COVID-19 orbital mucormycosis patients (N=30).

	Histo-Pathological Parameter	Score	No. Of Cases	% Of Cases
1	**Fungal Load**			
	< 4 Fields (Mild)	1	8	26.7
	5-7 Fields (Moderate)	2	13	43.3
	≥ 8 Fields (Severe)	3	9	30.0
2	**Degree of Tissue Necrosis**			
	< 50%	1	19	63.4
	≥ 50%	2	11	36.6
3	**Acute Inflammation**			
	Mild	1	2	12.5
	Moderate	2	5	31.2
	Severe	3	9	56.2
4	**Peri-Neural Invasion**			
	Absent	0	22	73.3
	Present	1	8	26.7
5	**Angio-Invasion**			
	Absent	0	23	76.6
	Present	1	7	23.4
6	**Granuloma / Giant-Cell Reaction**			
	Absent	0	24	80
	Present	1	6	20
SEVERITY SCORING				
Grade I		Score 3-5	10	33.3
Grade II		Score 6-8	8	26.7
Grade III		Score 9-11	12	40

Table 2- Data of cases showing severity grade III (score 9-11)

S. NO.	AGE/ SEX	SITE	CO- MORBIDITIES	GLOBE INVOLVEMENT	ANGIO- INVASION	RETINAL VESSELS INVOLVEMENT	PERINEURAL INVASION	OPTIC NERVE INVOLVEMENT	MYCOTIC THROMBI	FUNGAL LOAD	INFLAMM- ATION	GRANULOMA/ GIANT CELL REACTION	TISSUE NECROSIS	PARANASAL SINUSES INVOLVED	OUTCOME
1	42/F	left eye	DM 5 yrs	present	Present	present	Absent	present	Present	severe	severe	present	>50%	maillary, ethmoid & frontal	failure
2	72/F	right eye	DM + HTN 4 yrs	present	Present	absent	Present	absent	Absent	severe	moderate	present	<50%	maillary	successful
3	58/M	left eye	DM 12 yrs	absent	Absent	absent	Present	present	Absent	severe	severe	absent	>50%	ethmoid	failure
4	45/F	left eye	newly DM	present	Present	absent	Present	absent	Absent	Moderate	moderate	present	>50%	maillary	successful
5	48/M	right eye	newly DM	absent	Absent	present	Present	present	Present	Moderate	severe	present	>50%	maillary	successful
6	45/M	right eye	DM + HTN 4 yrs	absent	Absent	absent	Present	present	Present	severe	moderate	present	>50%	maillary, ethmoid, frontal & sphenoid	successful
7	56/M	right eye	newly DM	absent	Present	absent	Present	present	Absent	severe	severe	absent	>50%	maillary	failure
8	50/M	left eye	DM 8 yrs	absent	Present	absent	Absent	absent	Absent	severe	severe	absent	>50%	maillary, ethmoid & frontal	successful
9	62/M	left eye	HTN 15 yrs	absent	Absent	absent	Absent	absent	Absent	severe	severe	present	>50%	maillary	successful
10	55/M	left eye	nil	present	Present	present	Present	absent	Absent	Moderate	severe	absent	>50%	maillary & ethmoid	failure
11	25/M	left eye	nil	absent	Absent	absent	Present	present	Absent	severe	severe	absent	>50%	ethmoid	successful
12	58/F	left eye	DM 5 yrs	absent	Present	absent	Absent	present	Present	severe	severe	absent	>50%	maillary	successful

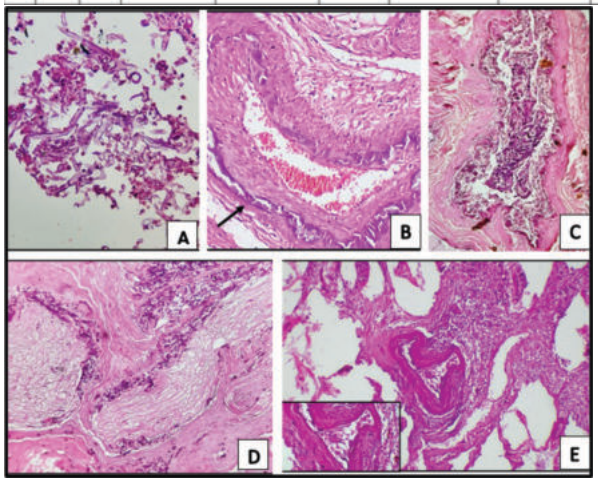

Figure 1- (A) H&E, 40X- Broad, aseptate, right angle branched hyphae of Mucor. (B) H&E, 10X- Angioinvasion by fungus (arrow). (C) H&E, 10X- Mycotic thrombi. (D) H&E, 10X- Peri-neural invasion by fungal hyphae. (E) H&E- Retinal vessel showing infiltration by mucor (inset).

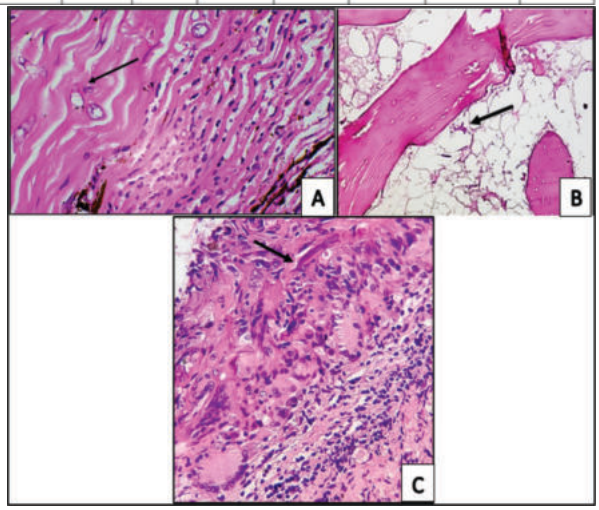

Figure 2. (A) H&E,40X- Uveal tissue invaded by fungal hyphae (arrow). (B) H&E, 10X- Fungal hyphae infiltrating fatty marrow. (C) H&E, 40X- Granuloma exhibiting fungal hyphae (arrow).

DISCUSSION:

Orbital Mucormycosis is a vision threatening and potentially fatal disease specially affecting patients with already existing co-morbidities such as diabetes, hypertension, AIDS, systemic corticosteroid use, neutropenia, hematologic malignancies, stem cell transplant and other immunocompromised states. [10] The incidence of mucormycosis varies from 0.005 to 1.7 per million population with case fatality rate of 46%. [11,12] A tremendous increase in the incidence of rhino-orbito-cerebral mucormycosis and its mortality had been observed in India during an unusual outbreak in 2021. Judicious diagnosis and management thus become vital in deciding the future outcome of these patients.[13] Radiology, microbiology and biopsy are the key diagnostic parameters for mucormycosis.

In our study, we found that out of all clinically and radiologically suspected cases, microbiology was helpful in detecting fungus in about 50% cases, while biopsy provided > 90% accuracy in detecting fungal infection. This was supported by Roy P. et al, they found strong association of histopathology with culture showing accuracy of 68.75% and advocated use of histopathology with culture and KOH mount for diagnosis.[14]

Radiology showed involvement of orbit as muscles thickening, orbital cellulitis, periorbital oedema, and affection of sinuses with varying frequency. Multiple sinuses were involved in most patients. However, maxillary sinus was frequently affected in our study. This is supported by Sree L et al [15], Jain K et al [7]. Maxillary sinus is commonly involved due to early implantation. No cerebral extension was seen in our study population.

There was a slight male preponderance with majority of patients suffering from a co-morbidity and received corticosteroid treatment during COVID-19 infection. Chouhan M et al. also showed similar results.[16]

The presence of tissue necrosis, hemorrhage, congestion, inflammatory infiltrate, granuloma formation and giant cell reaction are the key histopathology features which points in the direction of the presence of fungal organism as also demonstrated by Jain K et al [7], Goel A et al [17], Sree L et al [15] and others.

The ischemic necrosis was the most common histopathological feature seen in 93.3 % cases. Tissue necrosis due to invasion of blood vessels and subsequent thrombosis is the hallmark of invasive mucormycosis. Pale, basophilic necrotic areas are usually associated with high density of fungal hyphae and is important feature to assess the fungal load.

According to Cornely et al, the acute lesions show coagulative necrosis, hemorrhage, congestion, angio-invasion, neutrophilic infiltrate, and peri-neural invasion. [18] Chronic lesions show a pyogranulomatous inflammation with giant cell reaction or granuloma surrounding the fungal hyphae. Similar findings were seen in our study. Jose A et al. noted an increase in peripheral neutrophils with increased Neutrophil/ Lymphocyte ratio. These neutrophils are very effective and readily inactivate the fungus by generation of oxidative metabolites in immunocompetent host. [19]

Angioinvasion was seen in 23.4% cases. Blood vessels showed viable hyphae or necrotic foci in the wall associated with mycotic thrombi in 13.3% cases. Retinal vessel involvement was seen in 13.3% cases. These histopathological features were also noted in other studies (Sree L et al [15], Goel A et al [17], Jain K et al [7]), but with variable percentage. This could be due to difference in sample size, nature and amount of tissue submitted and inter-observer variation. Mucormycosis is an angio-invasive fungus that affects both artery and vein and is associated with hematogenous dissemination from original site of infection to other target organs.

In our study, optic nerve showed degenerative changes in 13.3% cases. Peri-neural invasion was seen in 26.7% patients mostly in cases of orbital exenteration and orbital debridement. This is in accordance with Goel A et al. [17] (27% cases), but contrasts with Sree L et al. [15] and Jain K et al. [7]. They found peri-neural invasion in 16% and 3.15% cases respectively. Frater et al. emphasized to rule out the presence of peri-neural invasion by fungal hyphae before excluding mucormycosis. Since loose facial sheath surrounds the nerve, it is a preferential site for fungal growth. [20]

In our study, 4 cases showed presence of mycotic thrombi out of which 2 also showed retinal vessel involvement by fungus, of which 1 patient died due to severity of disease. Similarly, globe involvement by fungus proved to be a deceptive finding and was associated with mortality in 50% of patients. Involvement of surrounding tissues like periorbital fat, bone and muscle give the extent of disease and are more relevant in the clinical setting. According to the severity grading, 12 patients in our study were categorized into GRADE III (Severe). 33.3% mortality was observed in these patients i.e., who had the highest severity according to the histopathological findings.

Therefore, it is deduced that studying histopathological features is important in early diagnosis of mucormycosis involving orbit and globe. It aids in prompt and appropriate management and helps to assess the prognosis of patients.

CONCLUSION:

COVID-19 associated Orbital Mucormycosis is a vision threatening infection with high mortality rate, generally affecting older persons with pre-existing co-morbidities. Majority of patients develop symptoms within a fortnight of COVID-19 infection. Prompt recognition of the clinical signs and symptoms and subsequent histopathological examination to distinguish Mucormycosis from other bacterial and fungal infections is vital for early treatment and favorable outcomes. A biopsy from clinically affected sites and demonstration of hyphae by histopathology are crucial for diagnosis and for determining the severity and extent of disease, thus to define and re-evaluate the treatment response. The histopathological data gives definite evidence of high mortality in patients with COVID-19 associated Orbital Mucormycosis.

Conflict Of Interest: There was no conflict of interest in the study undertaken.

Ethics Approval: Ethics Clearance was obtained from Institutional Ethics Committee – S.M.S. Medical College & Attached Hospitals, Jaipur.

Informed Consent: Written Informed Consent was obtained from all the patients or their attendants before the study.

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