MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.12.227.231

The Role of Histopathology with Special Emphasis on Frozen Section Biopsy in Detection of COVID Associated Mucormycosis

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Key Words
COVID-19 associated mucormycosis, frozen section biopsies, histomorphology, comorbid conditions like diabetes mellitus

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Received: 20 August 2024
Accepted: 10 November 2024
Published: 23 November 2024

Citation: Vijaya Lakshmi Seetha, Tholem Sripriya, Sanjay Kishve and GVRN Krishna Kanth, 2024. The Role of Histopathology with Special Emphasis on Frozen Section Biopsy in Detection of COVID Associated Mucormycosis. Res. J. Med. Sci., 18: 227-231, doi: 10.36478/makrjms. 2024.12.227.231

ABSTRACT
Mucor mycosis is a well described fulminant fungal infection that presents acutely. COVID-19 associated mucor mycosis (CAM) is a life threatening infection which is on rise during second wave especially in immuno compromised individuals like uncontrolled Diabetes Mellitus, Hematological malignancies etc. Early diagnosis on frozen section biopsies decreases turn around time compared to permanent sections and helps in early initiation of therapy thus decreasing the mortality and morbidity due to rapid extension of Mucor mycosis within no time of infection. Objective is to study the role of histopathology in mucor mycosis and to evaluate the statistical diagnostic accuracy of frozen section in post COVID-19 patients. Present study was carried out in Histopathology section, Department of pathology, ESIC, Sanathnagar, Hyderabad. It's an retrospective cross sectional study with sample size of 60 tissue biopsies including frozen sections from ENT Department over a period of 1 year from January 2021 to December 2021. Among 20 frozen sections, 12 were diagnosed as mucor mycosis that were confirmed on permanent HandE sections and special stains like GMS, PAS. 08 frozen sections were negative for mucor, out of which 01 came positive on permanent HandE sections. COVID-19 associated mucor mycosis were seen in 26 cases(76%) among 35 total mucor positive cases. 100% positivity is seen among diabetic patients. Although permanent sections still remain the gold standard, Frozen section biopsies is a specific and sensitive method to make a quick initial diagnosis of mucor mycosis that allows immediate surgical intervention and early initiation of antifungal therapy with Amphotericin B.

INTRODUCTION

COVID-19 associated mucormycosis (CAM) is a rapidly progressive opportunistic and life threatening infection that have increased in India especially in the second wave of COVID-19 pandemic[1]. The incidence of mucor mycosis in general population in pre-covid era is very low 0.005-1.7/million population, but there is a significant rise in the incidence of occurrence with COVID-19 pandemic[2],typically affecting immuno compromised individuals like uncontrolled Diabetes Mellitus, hematological malignancies and who underwent organ transplantation. The clinical approach to the diagnosis of CAM has low sensitivity and specificity due to its close proximity to the signs and symptoms of complicated sinusitis like nasal block, crusting, discharge, facial pain, swelling, edema, headache, fever with neurological signs if intra cranial extension is present[3,4]. The hallmark of mucor mycosis is tissue necrosis resulting from angioinvasion and thrombosis[5]. Early diagnosis and intervention of CAM are key to improve patient outcome. To avoid delay inherent in tissue processing, evaluation of biopsies, intraoperative consultation has shown promising results in facilitating early surgical intervention and treatment[6]. However, a definitive diagnosis is based on demonstration of fungal hyphae for mucor mycetes in biopsies of affected tissue in HandE sections and special stains like PAS, GMS stains.

MATERIALS AND METHODS

This study was done at ESIC Medical College and Hospital, Sanathnagar, referral territory care teaching hospital, Hyderabad over a period of 12 months from January 2021 to December 2021. Retrospectively all sinonasal biopsies sent from ENT department for frozen section and histopathology with clinical suspicion for CAM were retrieved. After the list of records were identified, the data collected were included as age, sex, comorbidies, frozen and histopathology reports. A total of 60 cases of post COVID-19 patients were included in the study in which various tissue samples like FESS, Maxillectomy and ethmoidectomy done.

Histopathology Methods: Biopsy samples sent for frozen section were freezed at temperature -24°C with Leica bio system cryostat. Out of 60 samples received, 20 sent for frozen section biopsy. Remaining samples were preserved in 10% neutral buffered formalin. Biopsy material after paraffin embedding, were sectioned at 5 µm tissue sections, stained with routine Haematoxylon and Eosin dye. Specials stains like PAS,GMS, ZN stain were performed on suspicious cases of mucor mycosis on HandE to confirm the presence of fungal hyphae on permanent sections. The morphological criteria for mucor mycosis included were large, broad, aseptate or very few irregular septa and wide angled branching hyphae. Samples were analyzed for following parameters like fungal load, granulation tissue, inflammatory infiltrates, necrosis, angioinvasion and bony invasion. Histopathology findings were reviewed by two senior faculty independently and blindly. If discrepancy between two pathologists notice, the case was discussed with another faculty to gain consenses.

RESULTS AND DISCUSSIONS

A total of 60 cases of sinonasal biopsies from ENT department with clinical suspicion of mucor mycosis were studied. Out of which 32(53.3%) were males, 28(46.6%) were females.35 cases were mucor positive. Among 60 cases, 34 were COVID positive of which 26(76%) were mucor positive and 26 were COVID negative of which 9(34%)were mucor positive. Patients with diabetes were 16 out of 60 with all of them: 16 (100%) positive for mucor mycosis, 44 were non diabetic with 19 (43%) of them showing mucor positive. (Table 01).

Table 01: Relation Between Mucor Positive with COVID and DM Status

Clinical status	No of Mucor Positive cases	No of Mucor Negative cases
Diabetes Mellitus	16 (100%)	0
Non Diabetes Mellitus	19 (43%)	25 (56%)
COVID Positive	26 (76%)	8 (23%)
COVID Negative	9 (34%)	17 (65%)

Out of 60 cases, 16 were Diabetic and post COVID showing mucor positive (100%)., 44 were Non Diabetic among which 19 were post COVID showing mucor positive(43%). Total of 20 cases were sent for Frozen, 12 cases were positive for mucor and 08 cases were negative for mucor, of which 01 was positive on permanent sections.(Table 02).

Table 02: Frozen and Histopathological Correlation

	HPE Positive	HPE Negative
Frozen Positive cases	12 (true positive)	0 (false positive)
Frozen Negative cases	01 (false negative)	07 (true negative)

Tissue samples were stained with HandE and special stains like Periodic Acid Schiff(PAS), Gomori Methanamine Silver (GMS) were performed to demonstrate fungal hyphae. Histomorphological features seen are characteristic fungal hyphae that are broad, aseptate and ribbon like with wide angled branching confirmed with special stains. Fungal load was mild in 14 (40%) cases, moderate in 10(28%), severe in 11 cases(31%). Necroinflammation is seen in 28 cases(80%).Granulation tissue with inflammatory cell infiltrates noted in 24(68%), giant cell reaction in 8(22%), Angioinvasion in 08(22%), bony invasion in 2 (5%) cases. 4 cases showing co-infection with other fungal organisms like Aspergillus, Actinomycetes, Rhizopus. Fig 1-7 and Table 03).

Table 03. List of Histomorphological Features with Percentage of Cases Exhibiting the Features

S.No	Histomorphologic feature	No.of cases
1	Fungal load- Mild	14 cases(40%)
	Moderate	10 cases (28%)
	Severe	11 cases (31 %)
2	Necroinflammation	28 cases (80%)
3	Granulation tissue with Inflammatory cell infiltrates	24 cases (68%)
4	Giant cell reaction	8 cases (22%)
5	Angioinvasion	8 cases (22%)
6	Bony invasion	2 cases (5%)

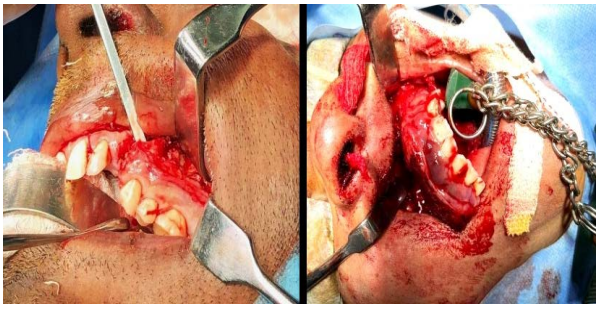

Fig. 1: Gross Intraoperative Images of Necrotic Maxillary Bone

Statistical Analysis: Use fullness of Frozen section biopsies prior to surgery has been proved with the sensitivity of 92.3%, Specificity of 100% and Accuracy of 95% with the cases we got. Also we evaluated the risk of infection with mucor mycosis among COVID patients, we have calculated relative risk with confidence level of 95%, Relative Risk is greater than 1, that is 2.2: confidence interval lower boundary- 1.262 and upper boundary-3.8 indicating increased risk of infection among exposed group of patients with COVID. Also we have calculated the relative of infection with mucor mycosis among Diabetics and Non diabetics which is also greater than 1, to be exact is 1.5 with confidence interval of lower boundary-1.313 and upper boundary-1.873 indicating the increased risk of infection among Diabetic patients

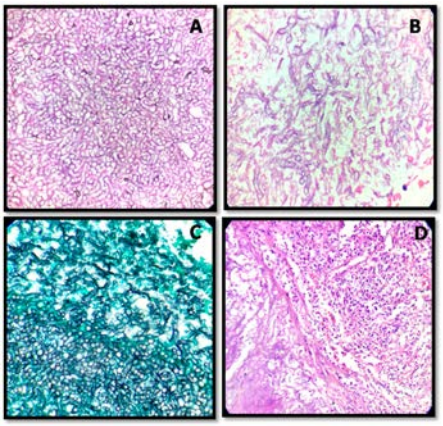

Fig. 2: A) HandE Fungal (Broad Aseptate) Elements
B) Coinfection Mucor with Aspergillus
C) GMS Stain Highlighting Fungus, 5) Necro Inflammation

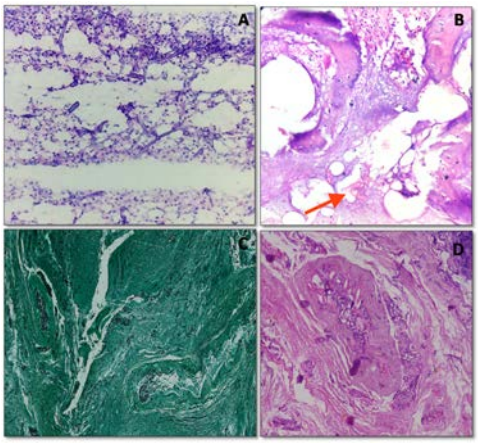

Fig. 3: A) Frozen Section Image Showing Fungal Hyphae
B) Necrotic Bony Fragments with Fungal invasion
C) Low Power View of Vascular Invasion, Giemsa Stain
D) High Power View of Vascular Invasion, PAS Stain

Coronavirus disease COVID-19, caused by SARS-CoV-2, is the leading cause of emergency global pandemic[7,8]. during which usage of corticosteroids as a first line drug of choice for the treatment has increased, thus increasing number of patients affected with mucor mycosis post COVID, typically among immuno compromised patients[2-4]. The survival rate of patients infected with mucor mycosis has increased to 85% since last 30 years with appropriate treatment[9] without which there is significant progress of disease with intracranial extension leading to death. Early diagnosis, early initiation therapy and surgical debridement of infective tissue have increased survival rates[10-12]. Studies on frozen section biopsy analysis in diagnosing the invasive fungal sinusitis are limited. In 2007, Gadiali *et al.* showed frozen section biopsy as a useful tool in early diagnosis of invasive fungal sinusitis among 20 patients, with 84% sensitivity and 100 % specificity[13]. A study was done on a group of 7 patients to evaluate use of the frozen sections in the diagnosis of mucor mycosis in which 6 out of 7 were positive, suggesting its use in early diagnosis[14]. A recent study in 2016 done on 31 tissue frozen sections by Claire *et al.* showed similar results of its usage as early diagnostic tool[15]. In a study done by Papagiannopoulos[16], 2017 which was a retrospective study showed sensitivity of 72.7%, specificity of 100%, PPV-100% and NPV-64.7%. Recent study on Accuracy of intraoperative frozen sections in detection of Acute

Invasive Fungal Rhinosinusitis by Rahaf[17] showed sensitivity of 88.5% , specificity of 100%, PPV-100% and NPV- 90.6%. Our study showed similar results as above mentioned studies on the frozen sections with sensitivity of 92.3%, Specificity of 100% and Accuracy of 95%, thus establishing the significance of frozen section biopsies prior to surgery as early diagnostic tool of mucor mycosis in post COVID patients. Our study showed similar histomorphological features with the study done by I. Sree Laksmi[18] like grossly, necrotic bony tissue (fig. 3a,b), histopathological features of mucor mycosis showing non-septate, broad, ribbon like fungal hyphae (fig. 3c), confirmed on special stains like GomoriMethanamine Silver stain (fig. 3e), areas of necroinflammation(fig. 3f), necrotic bony invasion with fungal hyphae (fig. 3g). Fungal load was quantified by number of fungal hyphae present in ×400 microscopic fields: mild-≤3 fields, moderate-4-7 fields, severe-≥8 fields. In a study done by Kavitha Jain *et al.* among 95 cases, fungal load was mild in 10 cases(10.5%), moderate in 65 cases(68%) and severe in 20 cases(21%)[19]. Strong association between diabetes mellitus and development of mucor mycosis was concluded by the studies done by John[20], Kumar[21], Sharma[1] Similar results were observed in our study with 100% occurrence of mucor in Diabetic patients. Intraoperative Frozen section studies support their use in surgical planning and debridement with respect to intraoperative rapid pathological interpretation thus allowing minimally invasive approach. Also ability to limit the surgical debridement to pathologically confirmed cases with frozen helps in decreasing patient morbidity[15]. The limitations of the study include the small sample size, with only 20 frozen section biopsies, single hospital center experience, and short-term follow-up. Further extensive research studies with a larger group of patients are needed for standardization.

CONCLUSION

Clinical suspicion, early diagnosis of Mucor mycosis in post COVID-19 patients are essential for better treatment outcome. Rapid initiation of anti-fungal therapy, immediate surgical intervention could affect the prognosis and survival rates of the patients that is possible with the frozen section biopsies. Even marginal status for the surgical debridement can be assessed by the frozen sections with proper guidelines that prevents extensive removal of the normal tissue and complete removal of the infected tissue.

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