Original Research Article

Clinicohistopathological spectrum of mucormycosis in covid–19 patients – Five months study in tertiary care hospital

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Abstract

Corona viruses are a group of viruses in which SARS – cov 1 strain causes severe acute respiratory syndrome. The new strain of coronavirus called SARS - cov-2 causes coronavirus disease 19(COVID-19).Corona virus disease 2019 may be associated with a variety of fungal and bacterial infections. Infection with mucormycosis in the form of Rhino-orbito-cerebral mucormycosis has reached epidemic proportion during this covid-19 pandemic with associated risk factors. This study was aimed to know the association of comorbidities and medications used ,with the development of mucormycosis which were confirmed by histopathological observation to improve early diagnosis thereby decreasing the morbidity, mortality and disability. Common age group affected , gender predilection are also studied. Mucormycosis may be manifested as different syndromes in humans especially in those with Diabetes mellitus and immunocompromised state. An observational and prospective study of five months from 15th Feb2021 to 15th July 2021, is done on patients with covid-19 associated with Rhino- orbito- cerebral mucormycosis with histological confirmation. All the patients admitted with confirmed covid-19 with suspected mucormycosis at Government General Hospital, Ongole, Andhra Pradesh are included in the present study along with clinical details obtained from clinicians. Clinico histological correlation was done and analysed in the present study: Overall 98 cases of mucormycosis in people with covid- 19 were reported in which 73(74.48%) were males and 25(25.51%) were females. Associated co-morbidities like Diabetes mellitus in 68 cases (69.38%), Diabetic ketoacidosis in 4.08%, steroid medication in 37 cases(37.75%) cases were seen. Other risk factors are also studied and analysed.Mucormycosis is mostly seen in nose and paranasal sinuses by 81 cseses(82.65%) followed by lungs 9 cases(9.18%) , oral cavity 5 cases (5.14%) and rhino- orbital cavity 3 csess (3.06%) .

Keywords: Covid-19, Mucormycosis, Rhino - orbital form.

Introduction

Mucormycosis or Zygomycosis is caused in humans by Rhizopus or Mucor or Rhizomucor. It is a rapidly growing aseptate or pauci septate broad hyphae(6-25µm), acutely branched ribbon like mould[2]. It causes angioinvasive disease resulting in thrombus formation, infarction and tissue necrosis. Infection with mucormycosis shows increased incidence in patients with uncontrolled Diabetes mellitus[3] and in immunosuppressed individuals due to lymphoma, leukemia, post transplantation or with drug usage. It also occurs as a hospital acquired infection such as ventilator associated pneumonia. Type 2 diabetes mellitus as a well known risk factor shows high prevalence rate in India[4]. The main cause for mucorale spores to germinate in COVID-19 patients is favourable environment like low oxygen(hypoxia),high glucose (diabetes mellitus, steroid induced hyperglycemia, diabetic ketoacidosis, high Fe+ levels, decreased phagocytosis due to neutropenia.

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These factors impair host defence mechanisms which may increase serum iron available to the pathogens growth. Rapid growth of the fungus occursdue to cell wall chemistry and genetic alteration. Invasive mucormycosis is commonly seen with long term use of corticosteroids particularly in debilitated patients. Combination of steroid therapy and diabetes mellitus will augment immunosuppression as well as hyperglycemia which in turn increases the risk of mucormycosis in a susceptible individual[5]. Spores and hyphae adhere to the receptors of endothelial cells by specific proteins causing angioinvasion and dissemination, mediated by lytic enzymes, proteases. The pathological hallmark is angioinvasion resulting in haemorrhages, infarction and secondary bacterial infection[6].Histopathological examination is highly effective in alerting clinicians to the presence of fungi and to guide the clinician in the choice of antifungal therapy, thereby reducing the morbidity, mortality and disability.

Methods

The present study is done in department of Pathology,Government Medical College, Ongole, Andhra Pradesh for five months period i.e from 15th February 2021 to 15th July2021 during second wave COVID-19 pandemic. Total nearly 8100 RTPCR confirmed covid-19 cases were admitted in Government General Hospital, Ongole. Out of 8100 cases 189 both treated and active covid-19 suspected cases for mucormycosis presenting with symptoms like nasaldischarge, nasal obstruction, swelling with or without pain in the cheek, reduced or loss of vision, proptosis, difficulty in swallowing and hemoptysis are taken for study. These cases after complete physical examination in relation to clinical history, radiological examination were subjected for surgical debridement, exenteration of painful blind eye and transbronchial lung biopsy wherever necessary . All these samples were sent in 10% formalin

to Pathology department for histopathological examination. In our study we aimed 1. To know most common age group and gender,2. To know most common symptom and 3. To know the clinico- histopathological spectrum. In total 98 cases, most common presentingsymptom is nasal discharge/ nasal.

Results

A total 189 covid-19 cases for suspected mucormycosis were subjected to study with the age group range between 20 - 80 years. Out of 189 cases, 89 cases showed pure mucormycosis infection,as shown in Figure 1 & 2. 9 cases showed mixed mucormycosis and asperigillous infection and 2 cases showed pure asperigillous infection as shown in Figure.3. Insufficient sample was observed in 4cases. 85 samples were negative for fungal infection. Mixed infection cases are also included in pure mucormycosis cases, so total (89+9) 98 cases of mucormycosis were observed. Maximum number of cases fall between 41 to 60 yearsof age as shown in Table1.

Table : 1 Age Distribution

Age in years	No of cases
< 10	01
11 – 20	03
21 – 30	09
31 – 40	15
41 – 50	32
51 – 60	28
61 - 70	08
71 - 80	02
Total	98

Males are predominantly effected constituting male: female ratio 3:1 as shownin Table 2.

Table: 2 Sex Distribution

Sex	No of cases
Male	73
Female	25

Obstruction in 81 cases followed by lung pneumonia in 9 cases presenting with fever and breathlessness, swelling & pain in the eyeball in 3 cases and oral ulcers predominantly in hard palate in 5 cases. Out of six exenterated eyeballs, 03(3.06%) samples showed mucormycosis on histopathological examination.Most common underlying risk factor observed is Diabetes mellitus. Out of 98 cases 68(69.38%) cases showed hyperglycemia as risk factor .As a part of treatment corticosteroids were used in 37 (37.75%) cases, along with Remidesivir and Tosulizumab.

Out of 98 cases ,diabetis mellitus is the most common risk factor seen in 68(69.38%) cases (among these 3 cases of rhino orbital regionwith history of diabetes mellitus showed positivity for mucormycosis), followed by excess steroid usage in 37 (37.75%)cases., 9 (9.1%)cases were suffered from covid19 induced / ventilator associated pneumonia , 02(2.04%) cases had h/o Non Hodgkinslymphoma , 01(1.02%) case had h/o treatedAcute myeloid leukemia and 01(1.02%) case was HIV positive. Oral ulcers were observed in 5(5.1%) cases. .as seen in Table 3.

Table.3. Risk Factors

S.No	Risk Factors	No Of Cases
1	Diabetis mellitus / diabetic ketoacidosis	68(69.38%)
	a. Uncontrolled Diabetis	43(43.87%)
	b. Corticosteroid induced	25(25.51%)
2	Excess Steroid Usage	37(37.75%)
	a . Corticosteroid	25(25.51%)
	Induced	12(12.24%)
	Dm	
	b .Immunisupression	
	Due To	
	Steroids	
3	Pneumonia	09 (9.18%)
4	Oral Ulcers	05(5.14%)
5	NHL	02(2.04%)
6	AML	01(1.02%)
7	HIV	01(1.02%)
	TOTAL	98(100%)

Discussion

The most common fungal infection in the present study is mucormycosis also known as black fungus ,isan aggressive rapidly progressive angioinvasive opportunistic infection caused by bread mould fungi including Mucor, Rhizopus, Lichtheimia, Canninghamellabelonging to class Phycomycetes , first described by Paultauf in 1885[7] which waslater coined as mucormycosis by Baker. These fungi are commonly seen in soil, ondead & decaying

matter, rarely infecting immunocompetent. Giant cell angioinvasion, thrombus formation, infarction and necrosis of the underlying tissue is pathologic hallmark of mucormucosis. Mucormycosis can be differentiated from other fungal infections by having broad hyphae (6-25µm) and acute branching with more lower angle, absent or pauci septae. Gold standard for clinical diagnosis of mucormycosis described by Smith et al in 1950 was black necroticturbinates, blood tinged nasal discharge, facial pain, facial puffiness in the form of periorbital and perinasal swelling, proptosed eyeball & ophthalmoplegia, cranial nerve palsies[8]. Although mucormycosis is rare in healthy individuals several predisposing factors like Diabetes mellitus with or without ketoacidosis, Corticosteroid therapy, Acquired immunodeficiency syndrome, Hematological malignancies like lymphomas, leukemias, hospital acquired pneumonias, prolonged neutropenia, Hemochromatosis/increased Fe+ content, open wounds will lead to development of Mucormycosis[9].Most commonly mucormycosis involves nose and para nasal sinuses, orbit, lung, GIT, central nervous system, skin, jaw bones, joints, heart, kidney and mediastinum. Most common clinical manifestations are fever, unilateral facial pain, headache, nasal congestion, epistaxis, hemoptysis, visual disturbance and lethargy.Rhino-orbital-cerebral mucormycosis(ROCM) is a rare opportunistic infection seen mostly with immunosuppression with underlying conditions like diabetes mellitus, malignancies and steroid therapy[10].

In the present study 89 cases were positive for mucormycosis, 9 cases were positive for both mucormycosis and aspergillosis and 2 cases showed only asperigillosis. Recognition of fungus by histopathologival examination is more reliable than culture(Medicalmicrobiology 18th edition).

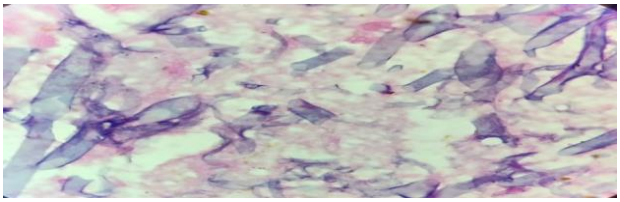

Fig.1. Shows broad aseptate fungal hyphae (mucor) in40X, H&E,

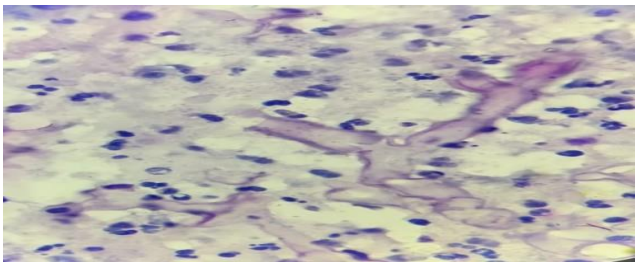

Fig.2. Shows broad aseptate fungal hyphae (mucor) in 40X ,H&E,

In the present study nose and paranasal sinuses are involved in 81 cases which are compared with the study of Yohai et al,[11] followed by lungs, oral cavity and orbital cavity as seen in Table.4.Rhino orbito cerebral mucormycosis meansspectrum of involvement ranging from sinonasal and sino orbital to orbital and sinonasal to orbital to CNS involvement

Table.4 Most common sites.

Site	No of cases/ Percentages
Nose and paranasal sinuses	81(82.65%)
Lungs	09(09.18%)
Oral cavity	05(05.14%)
Rhino-orbital cavity	03(03.06%)
Total	98(100%)

Suspected cases were put for surgical debridement , for exenteration of eye ballsand samples were sent for histopathological examination In our study of the samples from nose and paranasal sinuses 68 cases were confirmed histopathologically as mucormycosis withassociated underlying factors like dibetes mellitus with or without ketoacidosis (69.38%) which can be compared with study of K.I. Peterson et al[12].In our study out of 98 patients, 37 (37.75%) cases were having h/o corticosteroid therapy as a part of treatment for covid-19 infection. Nearly half of the patients showed newly onset(steroid induced) hyperglycemia and remaining showed uncontrolled diabetes mellitus with precipitation of diabetic ketoacidosis which might be the cause for development of mucormycosis[5].In our study 9 (9.1%) cases of pneumonia got affected with mucormycosis. Samples were collected by transbronchial lung biopsy. Out of 9 cases 1 necropsed lung specimen of treated post covid patient with h/o suspected hematological abnormality was observed and kept for histopathological examination which was confirmed[13]. In remaining , eight cases showed mixed mucormycosis and asperigillous infection on histopathological examination and this study was in comparison with the study of A. K. Johnson etal[14].

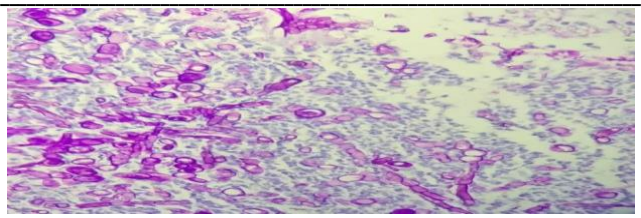

Fig.3.shows thin septate fungal hyphae(aspergillus) in 40X , PAS.

In our study six exenterated eyeballs with a h/o painful blind eye were subjected for histopathological examination. Out of six samples 3(3.06%) samples showed positive for mucormycosis infection. Out of three cases two cases are having h/o uncontrolled hyperglycemia and newly onset diabetes mellitus each[15]. Rhino orbito cerebral Mucormycosis remains infrequent association with patient whose only risk factor is positive retroviral infection[16] andit is associated with high mortality[17]. In our study only one case was positive for mucormycosis on HPE with a h/o HIV in covid 19 patient presenting as scalp skin involvement.Invasive fungal infections are important cause of morbidity and mortality in immunocompromised patients with hematological malignancies like lymphomas and leukemias. While the candidiasis and asperigillosis are accounting for majority of above said cases, mucormycosis have emerged as increasingly relavent and lethal cause of invasive fungal infection[18]. This finding was observed even more frequently in covid pandemic. In our study one covid patient with a h/o acute myeloid leukemia under remission showed mucormycosis withincidence of 1.02% which was compared with the study of Anna Skiada et al[19]. In our study fulminant pneumonia caused by mucorales was observed in two immune compromised patients with a h/o Non hodgkins lymphoma constituting 2.04%[20].In our study of 98 cases , out of five cases showing oral cavity involvement in form ofhard palate ulcers two cases are showing mixed mucormycosis and asperigillousinfection and three cases are showing onlymucormycosis infection with h/o poorly controlled diabetes mellitus and/or immune compromised [21].Till now reported cases of mucormycosis may not be the accurate prevalence rate or it may be an under estimation of real burden in society as it is hard to make accurate microbiological or histopathological diagnosis in the present raising covid-19 pandemicity[22].

Conclusion

Successful treatment depends upon early diagnosis of infection to allow prompt therapy with control of underlying predisposing factors, to prevent advancement of disease as well as morbidity and mortality.

By prompt control of diabetes mellitus, by curtailing unnecessary use of excess corticosteroids in covid-19 pandemic opportunistic fungal infections can be reduced.Fungal culture is necessary to identify the genus, but the recovery of mucorales isdifficult in culture. No serological test is available,radiological methods are not specific, and fresh sample is required for PCRexamination.

Even though biopsy is an invasive procedure a confirmed diagnosis of mucormycosis can be done by the presence of characteristic hyphae in the involved tissue. Histopathological examination seems to bethe alternative in the diagnosis of invasive fungal infections.

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