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Original Research Article

Association of serum iron studies in COVID associated mucormycosis with stage of the disease

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ABSTRACT

Background: Mucormycosis is a rare and lethal invasive fungal infection caused by opportunist and ubiquitous fungi. India has reported surge in cases of post COVID 19 mucormycosis due to the increasing frequency of risk factors like corticosteroid therapy, uncontrolled diabetes, DKA, neutropenia and iron overload and hence warrants a detailed study to identify potential contributing factors. Aim of this study was to study the clinical profile of patients with CAM and to correlate serum iron studies with severity and extent of disease in CAM patients.
Methods: This prospective crosssectional study was conducted on 75 patients with CAM admitted to Bowring Hospital, Bangalore. Detailed history with clinical evaluation and appropriate investigations done and data was analysed.
Results: The mean age of the subjects was 48.19 with 52 males and 23 females. Among 75 patients 90.7% were unvaccinated against COVID, 62.7% had oxygen and steroid use, with diabetes mellitus as most common comorbidity. Rhino orbital-cerebral mucormycosis was the most common clinical presentation. Patients with stage 4 CAM had elevated levels of inflammatory markers LDH (292), D-dimer (457), CRP (74.64), mean serum iron (50.37) and TIBC (255.37). Case fatality rate was 12%.
Conclusions: The results of this study revealed significant correlation between the clinical severity of CAM and higher mortality, with increased serum iron levels and inflammatory markers. Therefore patients with elevated levels of available serum iron are uniquely susceptible to mucormycosis infection, suggesting dysregulated iron metabolism in its pathogenesis.

Keywords: Mucormycosis, SARS-CoV-2, Serum iron profile

INTRODUCTION

Mucormycosis (Zygomycosis) is a rare and lethal invasive fungal infection, often acute and extremely severe caused by opportunist and ubiquitous fungi belonging to the class Phygomycetes, subclass Zygomycetes, order Mucorales, family Mucoraceae; usually by the following species: *Absidia corymbifera*, *Apophysomyceselegans, Cunninghamella bertholletiae*, *Mucor rouxii*, *Rhizomucor pussillus*, *Rhizopus arrhizus*, and by species of the genus *Saksenaea spp*. The species mentioned above suppose the third cause of invasive fungal infection after *Aspergillus* and *Candida* species in

humans.[1] This fungus usually resides as a commensal of the nasal mucosa and in conditions of immunosuppression like diabetes, ketoacidosis, solid organ transplant, severe burns, etc. can germinate in the nasal cavity and paranasal sinuses to invade the palate, orbits and brain, often leading to death.[2] It is acquired by the establishment or implantation of the fungal spores in the oral, nasal and conjunctival mucosa (rhino-orbito-cerebral), by inhalation (pulmonary), or by the ingestion of contaminated food (digestive) as they quickly colonize nutrients rich in simple carbohydrates being glucose its main energy source.[3]

Coinfection in patients with coronavirus disease 2019 (COVID-19) has been reported on multiple series, being bacterial in origin the most frequent; and fungal infection being reported only in severe cases.[4-6] India has reported surge in cases of post COVID 19 mucormycosis over the past few months due to the increasing frequency of risk factors like corticosteroid therapy, uncontrolled diabetes, diabetic ketoacidosis, neutropenia and iron overload. Increased level of available serum iron is most important risk factor to infection by mucorales. Iron is essential for growth and virulence of fungi of the class Zygomycetes.[7,8] Present study is to correlate the serum iron studies in COVID associated mucormycosis with stage of the disease.

METHODS

This study was an Institution based observational study conducted on patients with COVID associated mucormycosis between July 2021 and November 2021 in Bowring and Lady Curzon Hospital, Bangalore. Approval and clearance were obtained from the institutional ethics committee.

The study included patients diagnosed with COVID associated mucormycosis either clinically, radiologically or microbiologically of age more than 18 years. Exclusion criteria included patients not willing to give informed consent, patients with hemoglobin <10gm/dl and with history of blood transfusion in the past 3 months.

Case record was used to record the duration of disease, history of treatment and complications. Patients included underwent the following laboratory investigations which included complete blood count, liver function test, renal function test, serum electrolytes, serology, quantitative CRP, Serum LDH, D-dimer, serum iron studies and serum ferritin.

Patients were followed up for outcome measures which included course in hospital, requirement of ICU admission, requirement of oxygen therapy and severity of disease was followed up until discharge or death.

A power analysis was established by G*power, version 3.0.1(Franz Fauluniversitat, Kiel, Germany). A sample size of 72 would yield 90% power to detect significant differences, with assuming the effect size of 0.5 and significance level at 0.05.

Descriptive statistics of the explanatory and outcome variables were calculated by mean, standard deviation, median and IQR (based on data distribution) for quantitative variables, frequency and proportions for qualitative variables. Inferential statistics like Chi-square test was applied for qualitative variables. The level of significance is set at 5%.

RESULTS

A total of 75 cases of COVID-19 associated mucormycosis were included according to the proforma detailed in the methodology and the data obtained thereby are presented and analysed below.

Table 1: Distribution of the subjects based on age.

Age	Frequency	Percent
21 to 35 years	14	18.7
36 to 50 years	32	42.7
51 to 65 years	23	30.7
> 65 years	6	8.0
Total	75	100.0

Of the 75 patients in the study, majority were in the age group 36- 50 years (42.7%) with mean age being 48.19 years (Table 1). 23 (30.7%) patients were females and 52 (69.3%) were males. The female:male (F:M) ratio was 0.44:1 (Table 2).

Table 2: Distribution of the subjects based on gender.

Gender	Frequency	Percent
Females	23	30.7
Males	52	69.3
Total	75	100.0

Table 3: Distribution of the subjects based on comorbidities.

Co-morbidities	Frequency	Percent
DM	45	60.0
DM, BA, HTN	1	1.3
DM, hepatitis B	1	1.3
DM, HTN	13	17.3
DM, IHD	1	1.3
Hypothyroidism	1	1.3
NIL	13	17.3
Total	75	100.0

The most common comorbidity was diabetes mellitus alone 45 (60%), followed by DM with hypertension 13

(17.3%), DM with ischemic heart disease 1 (1.3%), DM with bronchial asthma 1 (1.3%), DM with hepatitis B 1 (1.3%), hypothyroidism 1 (1.3%). 13 patients (17.3%) did not have any comorbidities (Table 3).

Out of 75 patients in the study, 68 (90.7%) were not vaccinated for COVID 19 and 7 (9.3%) had received 1 dose of COVID 19 vaccine (Table 4).

Table 4: Distribution of the subjects based on vaccination status.

Vaccination status	Frequency	Percent
No	68	90.7
Yes 1 dose	7	9.3
Total	75	100.0

Table 5: Distribution of the subjects based on oxygen and steroid uses.

Oxygen and steroid use	Frequency	Percent
No	28	37.3
Yes	47	62.7
Total	75	100.0

Out of 75 patients in this study, 47 (62.7%) were O2 dependent and treated with steroids and 28 (37.3%) were non O2 dependent (Table 5).

The most common presentation was Rhinoorbital cerebral mucormycosis 29 patients (38.7%) followed by rhinoorbital mucormycosis 28 (37.3%) and sinonasal mucormycosis 14 (18.7%), sinonasal sinusitits 2 (2.7%), and sinonasal mucormycosis with aspergillosis 1 (1.3%) (Figure 1).

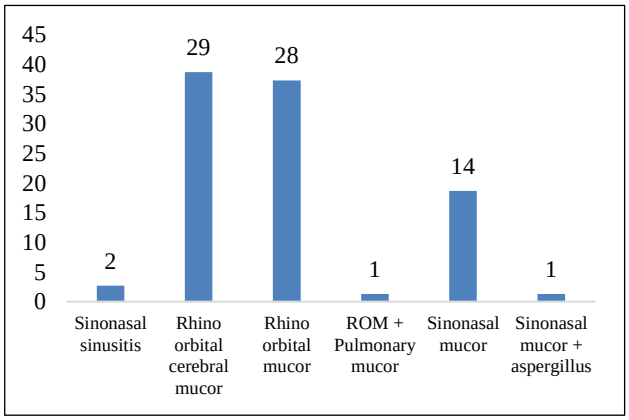

Figure 1: Distribution of the subjects based on diagnosis.

Based on the staging the most common presentation in Rhino orbital cerebral mucormycosis was stage 4A-12 patients (16%) and stage 4B-11 patients (14.7%), in rhino orbital mucormycosis was stage 3A-13 patients (17.3%) and stage 3B-11 patients (14.7%) (Table 6).

With increase in the stage of mucormycosis the inflammatory markers increase, and mean serum iron and TIBC also increase (Table 7 and 8).

Among the total 75 patients with Covid associated mucor 9 patients died with a case fatality of 12% and remaining 66 patients (88 %) were discharged (Table 9).

Table 6: Distribution of the subjects based on stages of mucor.

Stages of mucor	Frequency	Percent
2A	1	1.3
2B	7	9.3
2C	7	9.3
2D	2	2.7
3A	13	17.3
3B	11	14.7
3C	4	5.3
4A	12	16.0
4B	11	14.7
4C	5	6.7
4D	2	2.7
Total	75	100.0

Table 7: Cross-tabulation of stages of mucor with inflammatory markers.

Inflammatory markers	Stages of mucor	Minimum	Maximum	Mean	S.D	Chi-square value	p value
LDH	Stage 2	133.00	657.00	292.24	146.94	4.89	0.29
	Stage 3	123.00	578.00	299.19	120.52		
	Stage 4	140.00	643.00	298.00	148.17		
CRP	Stage 2	8.20	84.04	38.06	20.30	3.3	0.26

Continued.

Inflammatory markers	Stages of mucor	Minimum	Maximum	Mean	S.D	Chi-square value	p value
	Stage 3	8.16	84.00	44.75	24.57		
	Stage 4	6.41	210.00	74.64	47.57		
D Dimer	Stage 2	119.00	826.00	457.29	199.03	2.49	0.28
	Stage 3	224.00	1038.00	449.93	186.51		
	Stage 4	111.00	938.00	494.37	249.18		

Table 8: Cross-tabulation of stages of mucor with iron levels.

Iron levels	Stages of mucor	N	Minimum	Maximum	Mean	S.D	Chi-square value	p value
Serum iron	Stage 2	17	19.00	108.00	38.59	21.26	4.35	0.11
	Stage 3	28	15.00	68.00	34.69	12.86		
	Stage 4	30	11.00	129.00	50.37	29.62		
TIBC	Stage 2	17	159.00	335.00	232.76	65.17	3.67	0.45
	Stage 3	28	110.00	324.00	233.27	56.52		
	Stage 4	30	105.00	742.00	255.37	119.00		
T saturation	Stage 2	17	10.00	36.00	20.65	7.55	5.6	0.23
	Stage 3	28	7.00	35.00	15.73	6.53		
	Stage 4	30	4.00	36.00	15.60	8.67		
Transferrin	Stage 2	17	105.00	242.30	155.92	45.20	4.51	0.1
	Stage 3	28	105.00	267.70	176.24	42.35		
	Stage 4	30	97.00	214.00	147.85	32.86		
Ferritin	Stage 2	17	201.00	1209.00	599.79	280.68	3.07	0.21
	Stage 3	28	76.51	1684.40	483.65	402.26		
	Stage 4	30	79.00	2000.00	516.60	460.57		

Table 9: Distribution of the subjects based on outcome.

Outcome	Frequency	Percent
Death	9	12.0
Discharged	66	88.0
Total	75	100.0

DISCUSSION

Mucormycosis is an angioinvasive infection commonly seen in immunocompromised patients with risk factors being uncontrolled diabetes mellitus, use of steroids, neutropenia, extremes of age, iron overload, malignancy, AIDS etc with recent surge in cases of mucormycosis post COVID 19 infection.[6] Present study is done to focus on clinical profile of patients with COVID associated mucormycosis and to correlate the serum iron studies with the stage of the disease.

In the present study, we observed that 32 patients belonged to 36-50 years of age. 23 patients (30.7%) of the study population were females and 52 patients (69.3%) were males. The female:male (F:M) ratio is 0.44:1.

In this study, 61 patients (81.3%) had diabetes mellitus as the most common comorbidity, followed by hypertension in 13 patients (17.3%), ischemic heart disease in 1 patient (1.3%), bronchial asthma in 1 patient (1.3%), hepatitis b in 1 patient (1.3%), hypothyroidism in 1 patient (1.3%). 13 patients (17.3%) of the cases had no comorbidities in our study. In a study done by Mohindra et al, 16 out of 27 patients had diabetes mellitus as the predisposing factor. Several mechanisms like hyperglycemia and acidosis are known to impair phagocyte movement and killing by both oxidative and nonoxidative mechanisms and acidic pH causes dissociation of free iron from sequestered proteins.[2]

Total 47 patients (62.7%) were oxygen dependent and were treated with corticosteroids, and 33 patients (44%) were given remdesivir. In a study done by Jesil et al demonstrated that stage of covid associated mucormycosis had a significant correlation with use of oxygen therapy, corticosteroids and severity of COVID 19. Corticosteroids have a tendency to cause lymphopenia and defective T cell activity along with inducing hyperglycemia and this coupled with Covid 19 induced systemic pro inflammatory state with hyperferritinemia, systemic immune alteration (reduced T lymphocyte activity) leads to increased risk of invasive mucormycosis.[9]

In this study it was observed that the most common presentation was rhinoorbital cerebral mucormycosis, followed by rhinoorbital mucormycosis and sinonasal mucormycosis.

In this study it was observed that patients with stage 4 Covid associated mucor had elevated levels of inflammatory markers CRP, LDH and d dimer. A study done by Nitya et al showed that elevated inflammatory markers as with the severity of Covid 19 infection helped to facilitate fungal thriving and angioinvasion and vascular thrombosis.[10]

In this study it was observed that the mean serum iron (50.37), TIBC (255.37) and serum ferritin (516.60) were significantly higher in stage 4 Covid associated mucor as compared to stages 2 and 3 which are less invasive.

A study done by Tabassum et al demonstrates that iron is an essential micronutrient required for fungal growth, metabolic processes and proliferation. Fungi assimilate iron by various mechanisms like release of high affinity iron permeases, siderophores, heme oxygenase which helps obtain iron from the host hemoglobin. Factors like hyperglycemia, steroid use, acidosis, severity of Covid 19 infection as seen by systemic inflammation all cause increased serum iron levels facilitating fungal growth.[11]

A study done by Ibrahim et al showed that iron depletion or chelation by deferiprone can act as a novel therapy for refractory mucormycosis infections.[1]

In a study done by Simple et al showed that hyperferritinemia not just acts as marker of systemic inflammation but also indicates the increased free iron (causes apoptosis of hepatocytes releasing free iron and ferritin to cell exterior) allowing growth and invasion by the fungi and also causes upregulation of glucose regulated protein (GRP78) involved to evade endothelial cells by the fungi.[12]

It is observed that among 75 patients in the study a significant 15 patients (20%) had documented episodes of DKA and remaining 60 patients (80%) had no documented episode of DKA. In a study done by Ibrahim et al it was demonstrated that in patients with DKA due to acidic pH of the blood there is proton mediated dissociation of iron from transferrin leading to increased serum iron availability and facilitation of growth of Mucorales.[1]

It is observed in our study that the case fatality rate of Covid associated mucor is a significant 12% (9 patients) and remaining 88% (66 patients) were discharged.

A review of CAM cases by Martin et al showed a mortality of 49% particularly in patients with cerebral or pulmonary involvement indicating that covid associated mucormycosis is associated with high mortality and morbidity.[13]

This study has also some limitations and includes the relatively small sample size that limited our power to draw formal conclusions for large population.

CONCLUSION

The results of this study revealed significant correlation between the clinical severity of CAM and higher mortality, increased serum iron levels and inflammatory markers in this population of patients. Therefore, patients with elevated levels of available serum iron are uniquely susceptible to mucormycosis infection, suggesting dysregulated iron metabolism in its pathogenesis.

Further studies need to be done to study the effectiveness of iron chelators like deferiprone for refractory mucormycosis infections.

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