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Role of inflammatory markers in assessing the severity of Mucormycosis in covid 19 patients.

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Abstract:
Background*: In humans, free iron is unavailable to pathogens due to the binding of iron to transferrin and ferritin, which is a defence mechanism against Mucorales. The virus interacts with the hemoglobin molecule through ACE- 2 Receptor during infection, resulting in hemolysis and iron overload. In covid- 19 infection serum, ferritin is released into the circulation in more quantities. It loses its iron content after getting released into the circulation, thereby increasing the levels of iron in the serum and further leading to activation of reactive oxygen species. Patients with high serum ferritin levels are more prone to get a severe grade of mucormycosis.*
Methodology*: Data was collected from medical records of 60 cases of mucormycosis with RT-PCR confirmed COVID-19 positive in less than 2 months duration who were admitted in mucormycosis ward of SVRRGH Tirupati, chittor district, Andhra Pradesh from July 2021 to August 2021 as per pre-structured proforma, and data is analysed using logistic regression in spss software version 21.*
Results*: 60 patients with COVID-19 and Rhino-Orbital Mucormycosis were observed. The median age is 49.65 years, with males and females 68.33% and 21.67%, respectively. The mean ferritin level is 302.4993. The mean d- dimer is 1.1523, and the mean CRP is 4.9517. serum ferritin levels are well correlated with the stage of mucormycosis, showing the higher ferritin levels having severe mucormycosis involvement (p: <o.001% and r: 0.5). However, d- dimer levels and CRP levels did not significantly correlate with the severity of mucormycosis.*
Conclusion*: Serum ferritin can be used as an indicator for assessing the severity of mucormycosis;*
Key words*: COVID-19, Mucormycosis, Serum ferritin*

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I. Introduction:

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome (SARS-CoV-2). Opportunistic infections like bacterial and fungal infections have been associated with COVID-19. Among them, Aspergillus and Candida were found to be more common. Mucormycosis cases have been found to be increased in covid-19 patients [1].

Mucormycosis is an angio-invasive disease caused by the fungi that belong to the group Mucorales. Rhizopus Oryzae is responsible for 60 % of infections in humans. Rhizopus oryzae is a fast-growing organism at 3mm per hour at 36^0C that allows mucor to grow fast when optimal conditions are met. Immunocompromised patients, like those with haematological diseases and diabetes mellitus, are more prone to getting mucormycosis. The mode of contamination is through the inhalation of fungal spores. The prevalence of mucormycosis worldwide ranges from 0.005 to 1.7 per one million population. In India, it was about 80 times higher when compared to developed countries with a prevalence of 0.14 as India has more number of patients with diabetes mellitus [1] [2].

There are many factors in COVID-19 patients which combinedly added together, resulting in increased proliferation of Mucorales. Low oxygen(hypoxia), High glucose (diabetes and steroid-induced hyperglycemia), acidic medium (metabolic acidosis and diabetic ketoacidosis), high iron levels (increased serum ferritin), and decreased phagocytic activity of white blood cells (steroid-induced, SARS-CoV-2 mediated) and prolonged hospitalisation [1].

In humans, free iron is unavailable to pathogens as serum iron is unavailable to pathogens as it is bound to storage proteins like transferrin and serum ferritin, which constitutes the most important host defence mechanism against Mucorales. During SARS-CoV-2 infection, more serum ferritin is released into the circulation in response to the increased inflammatory response. Ferritin protects the cells from excessive free iron and its toxic effects by binding with it. Iron content that is a part of it will get released soon after releasing serum ferritin into the circulation. Iron is an important factor in the growth and metabolism of Mucorales, helping in proliferation. Free serum iron in the circulation will further signal the liver to increase the production of more ferritin [3][5].

Role of iron in the growth of Mucorales: iron is an important element in the growth of any organism. Iron has a role in the synthesis of DNA as it is a cofactor of ribonucleotide reductase and it regulates the cell cycle by activating cyclin dependant kinase complexes. iron binding proteins like transferrin and lactoferrin which are partly saturated with iron will help in maintain an extremely low levels of iron in the serum (10^{-18} M). 10^{-6} to 10^{-7} M of iron is required for the growth of pathogens like candida, aspergillus and the zygomycetes [6].

Based on anatomical locations, mucormycosis is divided into categories like rhino-cerebral, pulmonary, cutaneous, gastrointestinal, disseminated and miscellaneous. However, the most common presentation is rhino-orbital-cerebral mucormycosis [2] [4].

Staging of mucormycosis [7]:

Table 1

Stage of mucormycosis	Symptoms
1: involvement of nasal mucosa 1a: limited to the middle turbinate 1b: involvement of inferior turbinate or ostium of the nasolacrimal duct 1c: involvement of the nasal septum 1d: bilateral nasal mucosal involvement	Nasal stuffiness, nasal discharge, foul smell, epistaxis
2: involvement of paranasal sinuses 2a: one sinus 2b: two ipsilateral sinuses 2c: > two ipsilateral sinuses and/or palate or oral cavity 2d: bilateral paranasal sinus involvement or involvement if zygoma or mandible	Symptoms in stage 1 puls facial pain, facial oedema, dental pain, systemic symptoms (malaise and fever)
3: involvement of the orbit 3a: nasolacrimal duct, medial orbit, vision unaffected 3b: Diffuse orbital involvement (>1 quadrant or > 2 structures), vision unaffected 3c: central retinal artery or ophthalmic artery occlusion or superior ophthalmic vein thrombosis, involvement of the superior orbital fissure, inferior orbital fissure, orbital apex, loss of vision 3d: bilateral orbital involvement	Symptoms in stages 1 and 2 plus pain in the eye, proptosis, ptosis, diplopia, loss of vision, infraorbital and facial V1 V2 nerve anaesthesia
4: involvement of the CNS 4a: focal or partial cavernous sinus involvement and or involvement of cribriform plate 4b: diffuse cavernous sinus involvement and/or cavernous sinus thrombosis 4c: involvement beyond the cavernous sinus, involvement of the skull base, internal carotid artery occlusion, brain infarction 4d: multifocal or diffuse CNS disease	Symptoms in stage 1 to 3 plus bilateral proptosis, paralysis. Altered consciousness, focal seizures

Aim: The role of inflammatory markers in assessing the severity of mucormycosis in covid-19 patients.

Objectives:

1) To document the stage of mucormycosis radiologically by MRI brain.
2) to estimate the levels of serum ferritin, D-dimer and CRP in COVID-19 patients with mucormycosis.
3) to assess the relationship between inflammatory markers and severity of mucormycosis in covid-19 patients.

Materials and Methods:

Study design: A hospital-based retrospective study

Study period: study period is for two months (from July 2021 to August 2021)

Inclusion criteria:

1) patients with mucormycosis with a history of RT-PCR positive for SARS-CoV-2 less than 2 months duration.
2) Age more than 18 years

Exclusion criteria: severe anaemia

Methodology: Data was collected from medical records of 60 cases of mucormycosis with RT-PCR confirmed COVID-19 positive in less than 2 months duration who were admitted in mucormycosis ward of SVRRGH Tirupati, chittor district, Andhra Pradesh from July 2021 to August 2021 as per pre-structured proforma, and data is analysed using logistic regression in spss software version 21.

II. Results:

1. **AGE DISTRIBUTION**: Out of 60 patients, Most common age group affected was 41-50 years(35%) followed by 51-60 years(25%). Least common group affected was 21-30 years (5%). The mean age of the study population was 49.65 years.

S.NO	AGE GROUP	FREQUENCY (N)	PERCENT(%)
1	20-30	3	5
2	31-40	10	16.66
3	41-50	21	35
4	51-60	15	25
5	61-70	11	18.33
	Total	60	
	Mean±SD	49.65±11.65	

2. SEX DISTRIBUTION: Out of 60 patients, 22 patients were females (36.66%), and 38 patients are males (63.33%). Male predominance was observed in the present study with a male to female ratio of . In both males and females most commonly involved age group was 41-50 years followed by 51-60 years.

S.NO	Age group (years)	Male (N)	Female(N)	TOTAL
1	20-30	2	1	3
2	31-40	6	4	10
3	41-50	14	7	21
4	51-60	10	5	15
5	61-70	6	5	11
	TOTAL	38	22	60

3. STAGING OF MUCORMYCOSIS WITH AGE AND SEX DISTRIBUTION:

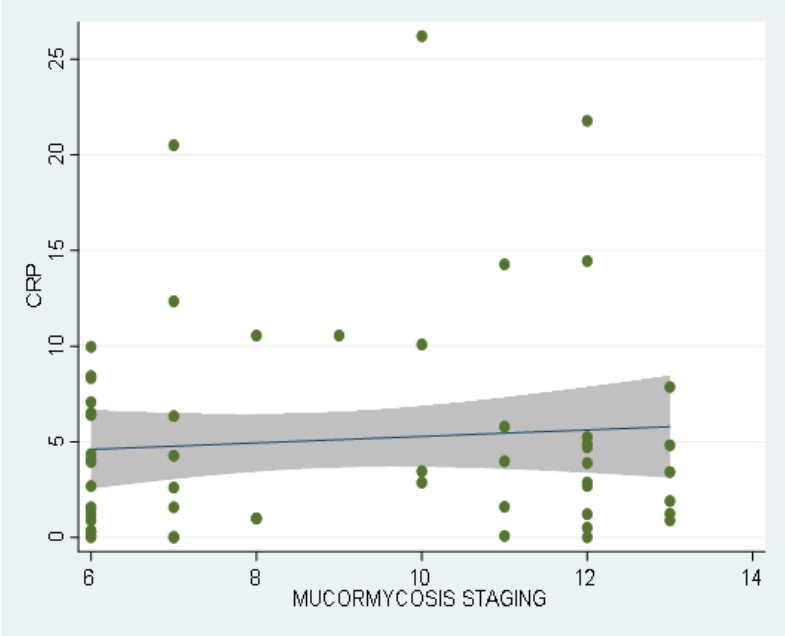
s.no	Stage of mucormycosis	Male (N)	Female (N)	Total (N=60) (PERCENTAGE %)
1	Stage1	0	0	0 (0%)
2	Stage2	15	18	33 (55%)
3	Stage3	17	4	21 (35%)
4	Stage4	6	0	6 (10%)

In the present study most of the patients suffered with stage 2 Mucormycosis ( ) followed by stage 3( ) . stage 1 mucormycosis was not seen in the present study. Among males stage 3 ( ) was coomon followed by stage 2 ( ). Among females, stage 2 was most common ( ) followed by stage 3 ( ). No one was suffered with stage 4 disease in the female group.

S.NO	STAGE	MALE	FEMALE
1	1	0	0
2	2	15	18
3	3	17	4
4	4	6	0
5	TOTAL	38	22

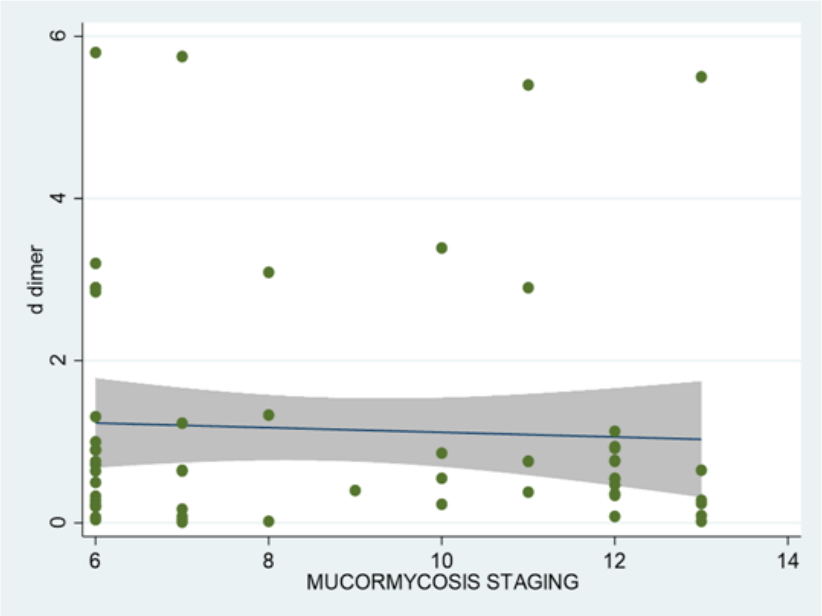
In males, 41-50 years age group was commonly affected with stage 2 followed by 61-70 years and in stage 3 51-60 years age group was most affected followed by 41-50 years. In females, 41-50 years age group was commonly affected followed by 51-60 years and 31-40 years. Only % of females in the age group of 61-70 years affected with stage 3 disease.

To apply the logistic regression, qualitative data in the given sample was converted to quantitative data (1a to 4d is represented as 1 to 16, respectively). The correlation coefficient for mucormycosis grading with CRP and D- dimer is 0.02 and -0.07, respectively, which means there is no correlation between CRP and D-Dimer with severity of mucormycosis. In this study, there is a moderate positive correlation between serum ferritin and severity of mucormycosis as the correlation coefficient(r) is 0.54 and p-value is 0.00, which is depicted in figure 5.



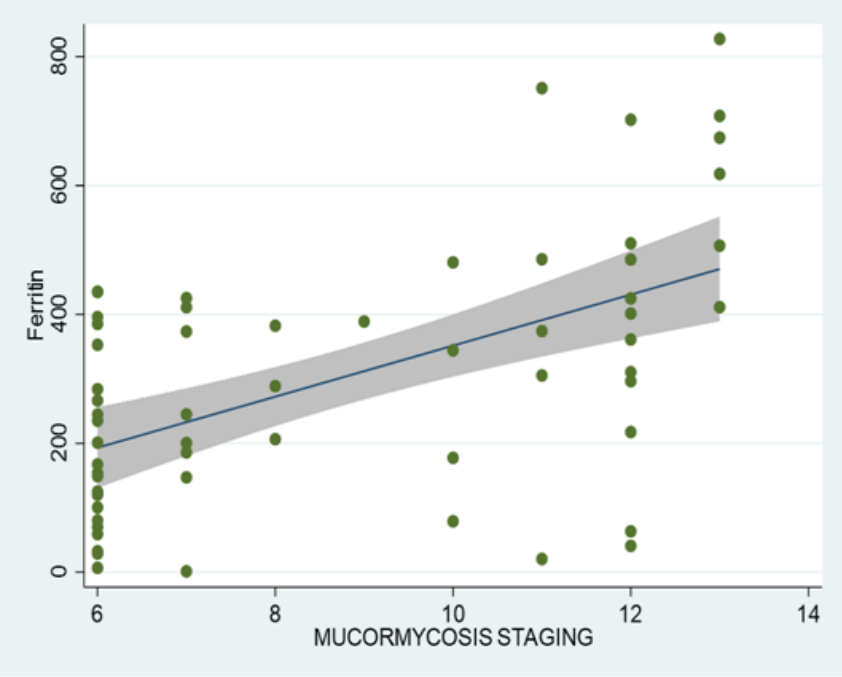
slope = **.0243139** (± .0375596), p-value (0.**517**)

correlation = **0.0840**, p-value (**0.5233**)



Slope = -.0726623 (±.1572671) p-value (0.644)

Correlation = -0.0532, p-value (0.6863)



Slope =0 .0064406 (±.001462), p-value (<0.000)

Correlation = 0.5433 , p-value (0.0000)

III. Discussion:

In our study, the correlation between the severity of mucormycosis and inflammatory markers was assessed, and the study showed a positive correlation between the severity of mucormycosis and serum ferritin levels as the correlation coefficient is 0.54.

In a study done by Bhanuprasad et al., serum ferritin was significantly higher in the patients of mucormycosis with COVID-19 patients with mean serum ferritin of 490.88 ± 521.9 and p-value of 0.041. CRP levels did not show any significance, with a p-value of 0.135. The severity of mucormycosis and serum ferritin levels were not assessed in this study [8].

In a study done by selarka et al., mean age was 55±12.8 years and male preponderance was observed with 74.5%. Mean serum ferritin of 357±280.3 was observed which was well correlated with mean serum ferritin level of males in our study which was 380.49±180.40. In this study ,ferritin levels are not correlated with the stage of mucormycosis [9].

In a review article written by Ibrahim et al., increased free iron in the serum was shown as one of the riskfactor for mucormycosis, as the organism grows poorly in the serum and the growth inhibition was reversed when exogenous iron was added. Furthermore, increased incidence of invasive mucormycosis with mortality of 80% was seen in the patients who were treated with deferoxamine, which acts as a siderophore by supplying unavailable iron to the fungus [10].

A study done by boelaert et al, which was done on guinea pigs by administration of different chelators, showed that animal survival was shortened with administration of desferoxamine for 4 days (p-value < 0.05) rhizopus growth was sevenfold higher in the animals which were given deferoxamine. This study showed that the severity of the disease is increased with increased availability of serum iron [11].

Alekseyev et al, reported a case of covid-19 with rhino-orbital-cerebral mucormycosis in which serum ferritin level was found to be very high (3044 ng/ml) [12].

Revannavar SM, et al reported a case of rhino-orbital-cerebral mucormycosis with non-ketotic diabetes with COVID-19. In this report, inflammatory markers like serum ferritin (180.2 mg/dl), CRP (68.35) and D-dimer (0.8 ug/ml) were found to increased more than the reference range [13].

IV. Conclusion:

In our study male preponderance was observed and serum ferritin level correlates well with the severity of mucormycosis as the correlation coefficient is 0.54 and p value is less than 0.00 and further studies needed.

Limitations: 1) It is a retrospective study.
2) Serum iron levels are not included in the present study.
3) Duration between covid-19 and mucormycosis is not assessed in this study.

References:

[1]. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: A systematic review of cases reported worldwide and in India. Diabetes Metab Syndr. 2021 Jul-Aug;15(4):102146. doi: 10.1016/j.dsx.2021.05.019. Epub 2021 May 21. PMID: 34192610; PMCID: PMC8137376.

[2]. Agnihotri AK, Vij M, Aruoma OI, Yagnik VD, Bahorun T, Villamil ME, et al. The Double Trouble: COVID-19 Associated Mucormycosis a Focused Review and Future Perspectives. Glob J Med Pharm Biomed Update 2021;16:4.

[3]. Jose A, Singh S, Roychoudhury A, Kholakiya Y, Arya S, Roychoudhury S. Current Understanding in the Pathophysiology of SARS-CoV-2-Associated Rhino-Orbito-Cerebral Mucormycosis: A Comprehensive Review. J Maxillofac Oral Surg. June 2021:1- [PMC free article] [PubMed].

[4]. Alan M. Sugar, Mucormycosis, Clinical Infectious Diseases, Volume 14, Issue Supplement_1, March 1992, Pages S126–S129, https://doi.org/10.1093/clinids/14.Supplement_1.S126.

[5]. Artis WM, Fountain JA, Delcher HK, Jones HE. A mechanism of susceptibility to mucormycosis in diabetic ketoacidosis: transferrin and iron availability. Diabetes. 1982;31:1109–1114. doi: 10.2337/diacare.31.12.1109.

[6]. Symeonidis AS. The role of iron and iron chelators in zygomycosis. Clin Microbiol Infect. 2009 Oct;15 Suppl 5:26-32. doi: 10.1111/j.1469-0691.2009.02976.x. PMID: 19754753.

[7]. Honavar, Santosh G Code Mucor, Indian Journal of Ophthalmology: June 2021 - Volume 69 - Issue 6 - p 1361-1365 doi: 10.4103/ijo.IJO_1165_21.

[8]. Bhanuprasad K, Manesh A, Devasagayam E, Varghese L, Cherian LM, Kurien R, Karthik R, Deodhar D, Vanjare H, Peter J, Michael JS, Thomas M, Samuel P, Varghese GM. Risk factors associated with the mucormycosis epidemic during the COVID-19 pandemic. Int J Infect Dis. 2021 Oct;111:267-270. doi: 10.1016/j.ijid.2021.08.037. Epub 2021 Aug 24. PMID: 34450284; PMCID: PMC8383616.

[9]. Selarka, L, Sharma, S, Saini, D, et al. Mucormycosis and COVID-19: An epidemic within a pandemic in India. Mycoses. 2021; 64: 1253– 1260. https://doi.org/10.1111/myc.13353

[10]. Ibrahim A.S., Spellberg B., Edwards J., Jr. Iron acquisition: a novel perspective on mucormycosis pathogenesis and treatment. Curr. Opin. Infect. Dis. 2008;21(6):620–625. doi: 10.1097/QCO.0b013e3283165fd1. [PMC free article] [PubMed] [CrossRef] [Google Scholar].

[11]. Boelaert JR, Van Cutsem J, de Locht M, Schneider YJ, Crichton RR. Deferoxamine augments growth and pathogenicity of Rhizopus, while hydroxypyridinone chelators have no effect. Kidney Int. 1994 Mar;45(3):667-71. doi: 10.1038/ki.1994.89. PMID: 8196268.

[12]. Alekseyev K, Didenko L, Chaudhry B. Rhinocerebral Mucormycosis and COVID-19 Pneumonia. J Med Cases. 2021 Mar;12(3):85-89. doi: 10.14740/jmc3637. Epub 2021 Jan 19. PMID: 33984095; PMCID: PMC8040444.

[13]. Revannavar SM, P S S, Samaga L, et al COVID-19 triggering mucormycosis in a susceptible patient: a new phenomenon in the developing world? BMJ Case Reports CP 2021;14:e241663.

Dr Asam Bhavana, et. al. "Role of inflammatory markers in assessing the severity of Mucormycosis in covid 19 patients." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(01), 2022, pp. 25-30.