

## Original Research Article

# The role of inflammatory markers in COVID-19 associated rhino-orbital-cerebral mucormycosis patients

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### ABSTRACT

**Background:** India has experienced an unprecedented heave of mucormycosis (MCR) cases during second wave of COVID-19. Possible mechanisms may involve immune and inflammatory processes. The aim of the study is to estimate the role of inflammatory markers for triaging patients of COVID-19 associated rhino-orbital-cerebral mucormycosis (CAROCM) at tertiary care hospital in north India.

**Methods:** A retrospective observational study was conducted between September 2021 to December 2021, at Government medical college and hospital, Patiala (Pb), 83 CAROCM patients admitted in ENT department were evaluated for serum ferritin, D-dimer and PCT.

**Results:** The median age of the patients was 50 years. The number of male patients were 45 and female patients were 38. Based upon the medical history and associated comorbidities involved, 83 CAROCM patients were divided into three groups. 43 diabetic patients in group I, 33 patients with multiple risk factors in group II and 7 patients with no comorbidity in group III. The mean D-dimer levels were 702 ng/ml in group I, 831 ng/ml in group II and 399 ng/ml in group III, and ferritin levels were 522 ng/ml, 711 ng/ml and 426 ng/ml in group I, II and III respectively. Mean PCT levels in group I were 0.27 ng/ml, 1.32 ng/ml in group II and 0.42 ng/ml in group III.

**Conclusions:** Our study concluded that a significant association was observed between levels of inflammatory markers and susceptibility factors. Serum ferritin, D-dimer and procalcitonin, can be used for assessing the severity of infection and decreases the mortality and morbidity in CAROCM patients.

**Keywords:** COVID-19, D-dimer, Ferritin, MCR, Procalcitonin

### INTRODUCTION

Mucormycosis (MCR) is a rapidly progressive, morbid and potentially lethal fungal infection. India has experienced a detrimental heave of MCR during the second wave of coronavirus disease 2019 (COVID-19). Till July 2021, 13 million cases of MCR have been reported in India, so it was declared as epidemic and notifiable disease by central government of India.<sup>1</sup>

MCR, commonly known as 'black fungus' is caused by mucoromycetes, a group of molds with *Rhizopus* and *Mucor* as the most common species.<sup>2</sup> In humans *Rhizopus*

*oryzae* is responsible for 70% of infections.<sup>3</sup> The fungal spores enter the human body through inhalation, ingestion, or direct inoculation through wounds. The clinical presentations are based upon the anatomical sites like rhino-orbital-cerebral, pulmonary, cutaneous, gastrointestinal and disseminated.<sup>4</sup> COVID-19-associated CAROCM is the most common type observed and it is responsible for one-third to half of all MCR cases.<sup>5</sup>

In CAROCM, the fungal mould enters directly through nose and sinuses or indirectly via vascular occlusion. Under optimal conditions of temperature (36° C), *Rhizopus oryzae* proliferate very fast at 3mm per hour

and causes irreversible tissue necrosis (black fungus).<sup>6</sup> Fatal complications will occur when fungus molds enter the intra cranial space.<sup>7</sup>

Rapid progression of MCR makes early diagnosis and treatment critical and delay of even one week, doubles the mortality from 35% to 66%.<sup>8</sup>

Major risk factors in COVID-19 patients like high glucose (diabetes and steroid induced hyperglycaemia), acidic medium (metabolic acidosis and diabetes ketoacidosis), high iron levels (increased serum ferritin) which individually or when combined will increase the proliferation of mucor spores.<sup>9</sup> MCR is found to be more prevalent in patients having suppressed immune function either by immunosuppressive medications like corticosteroids, or immunocompromising illnesses like diabetes mellitus, cancer and retroviral disease.<sup>10</sup> Iron is an essential element for growth and virulence of fungus Mucorales. The organism *Rhizopus oryzae*, grows poorly in iron deprived environment while iron overload predispose individuals to MCR.<sup>10</sup> Serum ferritin levels are widely measured as an indicator of iron status.<sup>7</sup>

Mucorales is an angio invasive fungus. It has high affinity for internal elastin lamina of arteries, that will lead to endothelial damage and micro vascular thrombosis which is a nidus for fungus infection.<sup>11</sup> Thrombus degradation will produce the by-product known as D-dimer. It is widely recognized as a biomarker for thromboembolism and as a prognostic marker for MCR patients.<sup>12</sup> Procalcitonin (PCT) is 116 amino acid precursors of calcitonin. It is a marker of sepsis. Several studies have found a close association between elevated PCT levels and the severity of SARS-CoV-2 infection.<sup>13</sup>

Evidence suggested that inflammation play a critical role in progression of COVID-19 associated superadded infections. The aim of this study was to evaluate the levels of the inflammatory markers and their association with susceptibility factors in CAROCM patients and the impact of inflammatory status on clinical outcomes.

## METHODS

### Study design

A retrospective observational study was conducted between September 2021 to December 2021 for a period of four months. Study involved 83 patients, presented with sign and symptoms of CAROCM admitted in emergency ward or in wards of E.N.T department at government medical college and hospital, Patiala. Study was initiated after obtaining approval from institutional ethical and research committee.

### Inclusion criteria

Patients with history of COVID-19 within 4-5 weeks of onset of symptoms of rhino-orbito-cerebral

mucormycosis, age more than 18 years were included in the study.

### Exclusion criteria

Patients with active COVID-19 infection, severe anemia (Hb<7 gm/dl) were excluded from the study.

Detailed demographic history, medical history pertaining to diabetes mellitus or any comorbidity, use of steroids and oxygen support during COVID-19 active period was collected. Pre-operative nasal swabs were sent for 10% KOH (potassium hydroxide) mount study, Giemsa stains and fungal microscopy for confirmation of mucor.

The CAROCM patients were divided into three groups: Group I, II and III, based upon the medical history and susceptibility factors (Table 3).

### Sample collection

Venous sample was drawn under aseptic conditions, 3 ml sample was collected in red top vacutainer and assayed for blood urea, serum creatinine, serum ferritin and procalcitonin (PCT). 2 ml was collected in sodium citrate vial and assayed for plasma D-dimer estimation and 2ml sample was used for estimation of fasting or random blood sugar in grey top vacutainer. Serum ferritin and PCT samples were analysed by ELFA (enzyme linked fluorescent assay) technique on VIDAS instrument. D-dimer was measured by fully automated coagulation system using latex turbidimetry method. Routine biochemistry parameters like FBS/RBS, blood urea, serum creatinine was done on fully automatic analyser (XL-1000) by using commercially available kits.

### Statistical analysis

The data analysis was carried out by using SPSS (Ver.22.0). Results were expressed as Mean  $\pm$  Standard Deviation (SD). The statistical significance of difference between the various groups was determined by using the student's t-test. A probability value of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

A 83 patients of CAROCM were included in this study, of which 45 (54%) were males and 38 (46%) were females. The median age of patients was 50 years, youngest was 25 years and oldest was 80 years. Various characteristic of patients was described in Table 1.

The mean levels of routine biochemical parameters like RBS, blood urea and serum creatinine and inflammatory markers were described in Table 2.

Based upon the medical history and associated comorbidities involved the CAROCM patients were divided into three groups; group I, II and III (Table 3).

**Table 1: Characteristics of the 83 CAROCM patients.**

Patient's characteristics	N (%)
<b>Age (in years)</b>	Median 50
≤40	14 (16.8)
41-59	48 (57.8)
≥60	21 (25.3)
<b>Gender</b>	
Male	45 (54)
Female	38 (46)
<b>History of steroid use</b>	
Present	41 (49.3)
Absent	29 (34.9)
No history	13 (15.6)
<b>Oxygen support</b>	14 (17)
<b>ICU admissions</b>	22 (26.5)
<b>Hospital stay</b>	2-3 weeks (average)

**Table 2: Mean levels of RBS, Blood urea, serum creatinine and inflammatory markers in 83 CAROCM patients.**

Variables	Mean	Reference range
<b>RBS</b>	237	<140 mg/dl
<b>Blood urea</b>	52.7	20-40 mg/dl
<b>S. creatinine</b>	1.84	0.7-1.3 mg/dl
<b>D-dimer</b>	663	500 ng/ml
<b>Ferritin</b>	546	70-435ng/ml (males)
		10-160 ng/ml (pre-menopausal females) 25-280 ng/ml (post-menopausal females)
<b>PCT</b>	0.67	<0.5 ng/ml

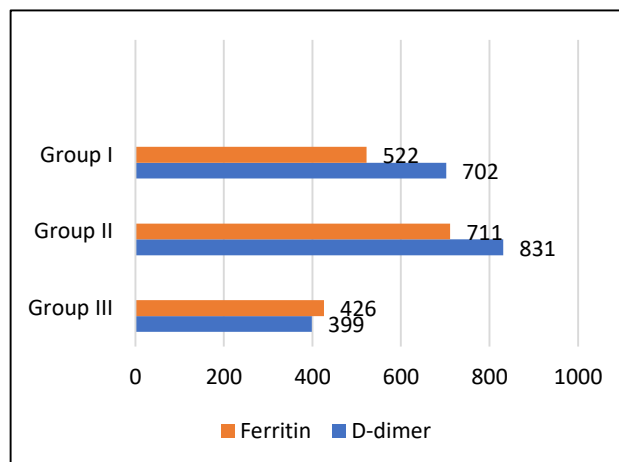
**Table 3: Distribution of 83CAROCM patients with history of comorbidities under group I, II and III.**

Groups	Comorbidities	N (83)	Male	Female
<b>Group I</b>	DM	43	21	22
	DM and HTN	22	14	08
	DM, HTN and CAD/MI	05	03	02
	DM, hypothyroidism and dyslipidaemia	02	01	01
<b>Group II (N-33)</b>	DM, HTN and CKD	02	02	00
	DM and HIV	01	00	01
	DM and HCV	01	00	01
<b>Group III</b>	No comorbidities	07	04	03

N: Number of patients, DM: diabetes mellitus, HTN: hypertension, CAD: Coronary artery disease, MI- Myocardial infarction, CKD-Chronic kidney disease.

The mean levels of serum ferritin and D-dimer of patients in group I, II and III has been described in Figure 1. The

mean serum ferritin levels were found to be higher than normal range in all the three groups. There has been no change in D-dimer levels in subjects belonged to group III, however the levels were markedly high in group I and II.

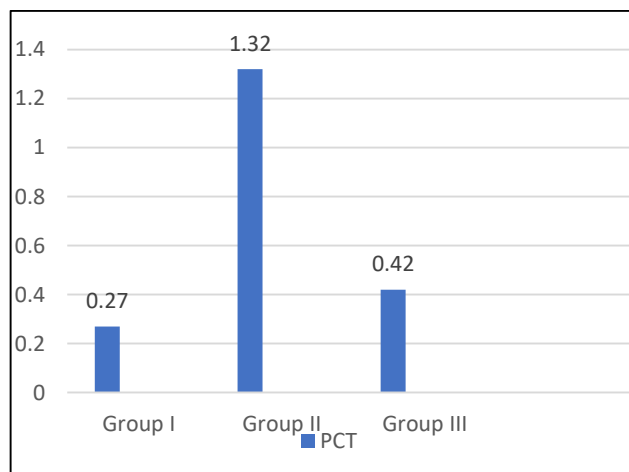


**Figure 1: Distribution of mean levels of serum ferritin and D-dimer in group I, II and III.**

The mean±SD of serum ferritin was higher in male patients as compared to female patients in all groups, but statistically significant variation was observed only in group I (p 0.02) (Table 4).

The mean±SD levels of D-dimer compared between male and female patients and was observed statistically insignificant in group I, II and III (Table 5).

The mean levels of serum PCT were found normal in group I and III but significant high levels were observed in group II patients (Figure 2).



**Figure 2: Distribution of mean levels of PCT in group I, II and III.**

The mean±SD of PCT levels in female patients were significantly higher than male patients in group I and II (Table 6).

After radiological assessment, FESS surgery or maxillectomy was being done according to involvement of anatomical site in CAROCM patients. Orbital decompression and orbital exenteration were performed in 28 patients. Postoperative management included administration of injection liposomal amphotericin B (5-10 mg/kg) for 14 days. Patients were discharged with stable or improved conditions. Out of total 83 patients, 11 patients were not survived and 6 were not being followed up in the hospital.

**Table 4: Comparison of serum ferritin levels between male and female patients of group I, II and III.**

Gender distribution	Group I, (Mean±SD)	Group II, (Mean±SD)	Group III, (Mean±SD)
Male (45)	654±415	789±346.6	653±565
Female (38)	409±140.7	734±367.2	459±54.8
P value	0.02	0.71	0.84

**Table 5: Comparison of D-dimer levels between male and female patients of group I, II and III.**

Gender distribution	Group I, (Mean±SD)	Group II, (Mean±SD)	Group III, (Mean±SD)
Male (45)	712±150.3	826±180.1	424±281
Female (38)	698±380.7	849±444.8	376±102
P value	0.82	0.75	0.32

**Table 6: Comparison of PCT levels between male and female patients of group I, II and III.**

Gender distribution	Group I, (Mean±SD)	Group II, (Mean±SD)	Group III, (Mean±SD)
Male (45)	0.13±0.08	0.73±57.5	0.39±0.35
Female (38)	0.42±0.41	1.90±27.4	0.45±0.40
P value	<0.01	<0.001	0.84

**DISCUSSION**

India has 61.3 million (2011) diabetics and number is estimated to rise to 101.2 million by 2030.<sup>14</sup> Commonly affected diabetes patients are adults belong to 20-79 years of age group.<sup>15</sup> Present study also observed that the patients suffered from diabetes mellitus were belong to age group 20-80 years.

Diabetes has been reported as a risk factor for MCR in 73.5% of cases in India.<sup>16</sup> The present study observed that CAROCM was more prevalent in diabetic patients. Out of total 83 patients, 76 (91.5%) were suffered from diabetes mellitus (alone or with comorbidities). It could be due to the repression of normal immunological response of the body due to diabetes. Diabetes stimulates fungal proliferation, decreases the chemotaxis and phagocyte efficiency and weakened the intra cellular killing of Mucorales.<sup>17</sup> Our study findings are in

consistent with published studies reporting a higher risk of MCR among COVID-19 diabetic patients.<sup>18,19</sup> Hariprasath Prakash et al done a comparative study between north and south India in 2019, they reported that MCR cases were higher in uncontrolled diabetes patients in north Indians.<sup>20</sup>

COVID-19 is a prevalent hyperinflammatory syndrome where ferritin is not only an acute phase reactant but also an inflammatory mediator.<sup>21</sup> We observed that serum ferritin levels were significantly higher in patients of group II as compared to others. This can be attributed to the fact that hyperglycaemia along with other co-morbid conditions like hypertension, dyslipidaemia, CAD, steroid therapy etc is known to cause glycosylation of protein ferritin which reduces the iron binding and thus causing elevated free iron which serves as an ideal source for mucor infection.<sup>22</sup> Our observation was consistent with previous studies, which observed that iron overload predisposes the individual to MCR.<sup>23-25</sup> Present study observed that male patients had higher serum ferritin levels than female patients’ similar observations was noticed by study conducted Selarka et al they also reported male preponderance in their study.<sup>26</sup> Studies done relates hyper-ferritinemia with high mortality. In present study also, four non survived male patients had serum ferritin >1200 ng/ml.<sup>27,28</sup>

D-dimer is a marker of hypercoagulability, high levels of which reflects the process of active clotting.<sup>29</sup> In our study, the standard treatment protocol used for CAROCM patients was surgical debridement of necrosed tissue and endoscopic sinus surgery (ESS) followed by systemic antifungal therapy. Intraoperatively very unusual and frequent clotting of blood gave the suspicion of abnormal thromboembolism, so D-dimer and PCT were routinely done. The present study observed high levels of D-dimer in group I and II. It may be explained by Matteo et al that in diabetic patients, there is an imbalance between clotting factors and fibrinolysis so they were at higher risk for thrombotic events.<sup>30</sup> Study done by Litao et al demonstrated that elevated levels of D-dimer was associated with increase fatality in COVID-19.<sup>31</sup> In our study, three patients who couldn’t survived belonged to group II and had D-dimer levels >1000 ng/ml.

Serum PCT may help to identify secondary infection in patients of COVID-19. Our study observed significantly high levels of serum PCT in group II patients. Similar to our findings, the study conducted by Torres et al explained that in COVID-19 infection, dysregulated immune response by different cytokines and TNF-alpha activates PCT production.<sup>32,33</sup>

Present study observed that maximum CAROCM patients were belong to peripheral or rural areas near Patiala (Punjab) These patients did not hospitalize in first wave of COVID-19. They took over the counter cheap drugs and poor adherence to the guidelines on steroid use for COVID-19 has been noticed. Substantial use of easily

available steroid drugs has been noticed among 41 (49.3%) CAROCM patients. Corticosteroids not only condense the activity of the immune system but also cause drug-induced hyperglycaemia and both were considered as major risk factors in the development of MCR in COVID-19 patients.<sup>34</sup>

### Limitations

A baseline levels of HbA1c in these patients needs to be considered. History of associated treatment that might have effect on the levels of D-dimer, ferritin and PCT should also be considered.

### CONCLUSION

Present study concluded that serum ferritin, D-dimer and PCT levels were positively correlated in CAROCM patients with pre-existing comorbidities. Injudicious use of steroid therapy and SARS-COV-2 virus induced vulnerability could be the cause of fulminant MCR infection in patients without any predisposing factor. Estimation of these levels may assist the clinician to monitor and predict risk assessment in CAROCM patients. Public awareness and education about the use of on the counter drugs, are the requisites for management of COVID-19 and MCR in North India.

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