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Original Research Article

Inflammatory Markers Associated with Adverse Outcome among Covid-19 Patients: An Observational Study

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Abstract

Background: Ever since the beginning of the Covid-19 pandemic, pathologists started investigating the laboratory markers that can assist in predicting the outcome among Covid-19 patients presenting to the emergency/inpatients department of hospital.

Aim: This study aimed to investigate the association between the blood/serum levels of various inflammatory biomarkers at the time of admission to hospital and mortality among COVID-19 patients.

Material and Methods: This was a single centre, hospital (inpatient) based prospective cohort study involving 508 Covid-19 patients admitted to the study institute. We collected data on CRP, D-dimer, LDH, Ferritin, and IL-13 levels at the time of admission. We also assessed the correlation between CT Severity score and the inflammatory markers.

Result: Among 508 included patients: 53 (10.4%) patients died, 73 (14.4%) patients required admission to Intensive Care Unit studied, 39 (7.7%) patients required mechanical ventilation, 23 (4.5%) had Coma and 328 (64.6%) patients were discharged from hospital without any complications. The levels of all measured inflammatory markers were significantly higher (worse) ($p < 0.05$) among patients suffered adverse complications (including death) during treatment. In addition, the level of several inflammatory markers strongly and positively correlated with CT scan findings.

Conclusion: The level of all inflammatory markers was significantly higher among Covid-19 patients who died during the treatment. However, more research is needed to identify the upper cut-off levels of inflammatory markers to identify patients who are at increased risk of complications including death.

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Introduction

It was immediately discovered during the Covid-19 pandemic that the majority of patients recover without experiencing any consequences or long-term damage[1]. The fact that only a small percentage of Covid-19 positive individuals had a severe illness characterised by abrupt respiratory failure necessitating assisted ventilation was also realised at the same time[2]. Saturation of hospital beds especially Intensive Care Units (ICU) beds, was a defining characteristic of every Covid-19's waves[3]. Physicians faced a moral conundrum when prioritising patients for admission to hospitals and ICU during the abrupt rise in the number of Covid-19

cases, especially during the peak of a Covid-19 wave and the restricted number of hospital beds, especially the ICU beds[4,5]. Physicians, pathologists, and radiologists started looking for clinical cues that may help predict the prognosis among hospitalised Covid-19 patients sometime after the epidemic started. The clinical traits, analytical markers, radiographic features, and outcomes of hospitalised Covid-19 patients were described in several extensive hospital-based research[6,7]. These investigations provided evidence that Covid-19 is linked to severe organ system inflammation, which manifests as neurological, cardiovascular, coagulation, and other end-organ symptoms[8,9]. Based on these factors, several prognostic models were developed to forecast the severity and course of patients' conditions. On three different fronts—clinical characteristics, laboratory markers, and radiological aspects—the prognostic models were created.

Numerous research conducted in hospitals found that a cytokine storm and broad systemic inflammation make up the main pathophysiology of Covid-19[11,12]. It soon became clear that vasculitis, which causes the shutdown of important organs in severe instances of Covid-19, is a secondary cause of the multi-organ damage. As was already indicated, one of the characteristics of Covid-19 is substantial pulmonary system involvement that culminates in ARDS, which causes respiratory failure and, sadly, mortality in a major number of all hospitalised patients[13,14]. It is currently unclear, though, whether specific biomarkers COVID-19 patients may rely on to indicate a poorer result, and even fewer molecules show the capacity to forecast treatment outcomes. The therapeutic value of these numerous indicators for risk-stratification and evaluating prognosis among patients with COVID-19 is developing and yet ill-defined. In order to investigate the relationship between the levels of several inflammatory markers at the time of admission and the clinical outcome among hospitalised Covid-19 patients, we designed this hospital-based prospective study. The goal of this study was to determine the range of inflammatory marker values upon admission in order to improve clinical judgement, develop a prompt treatment plan, and lower mortality.

Material and Methods

Study Design: This was a hospital-based, single-centre, prospective, observational study.

Study Settings: The present study was conducted at the Department of Pathology, Sukh Sagar, Medical College, Jabalpur, Madhya Pradesh. It is a tertiary care institute.

Study Duration: The total duration of the study was 18 months.

Study Outcomes: Primary outcome parameters were the blood/serum levels of the C-Reactive Protein, D-Dimers, Lactase Dehydrogenase (LDH), Ferritin, and Interleukin-13 (IL-13) among the patients who did and did not survive the hospital treatment among the Covid-19 patients admitted to hospital. The secondary objective of the study was to assess the degree of correlation between the lab and the radiological parameters[15,16].

Sample Size Calculation: We enrolled all participants fulfilling the selection criteria in the present study. Following this approach, we recruited 508 participants for the present study.

Case Definition: A patient presenting with signs and symptoms suggestive of Covid-19 and fulfilling the below-mentioned selection criteria.

Inclusion Criteria:

1. Age>= 18years
2. Patients tested Covid-19 positive on RT PCR.
3. The patient admitted to the inpatient department.

4. Patients whose laboratory parameters were measured within 24 hours of admission to hospital.
5. Patients who had CT scan within 24 hours of admission to hospital.
6. Patients who gave consent to participate in the study.

Exclusion Criteria:

1. Women patients who were pregnant.
2. Patients without the known outcomes i.e., LAMA, referred out etc.
3. Contraindications to CT scan.
4. Patients who refused to participate in the study.

Informed Consent: A bi-lingual (Hindi & English) consent form was drafted following the prescribed guidelines for research on human participants. The contents of the consent form were explained to all the prospective participants. All the questions from participants about the study, procedure, follow-up, and data privacy were answered. The participants were informed and explained that they have the right to withdraw from the study at any point in time.

Data Collection: The data were collected in a paper-based proforma. The proforma had three parts as follows: (i) Clinical details. (ii) Laboratory findings and (iii) Radiological findings.

Source of Data: There were two sources of data. First was the interview with the participants/relative/guardians containing details about the demographic details, clinical history, symptoms, signs, and previous treatments (if any). The second source of the data was clinical records containing details about the clinical examination, laboratory & radiographic findings.

Statistical Analysis Plan: The primary outcome was the laboratory parameters among laboratory-confirmed Covid-19 patients admitted to our institute. We aimed to identify from the collected data the blood/serum levels of laboratory parameters among patients having adverse/terminal outcomes. The data were analysed using Stata 17.1 version. For the interval and ratio data types, the author calculated the mean, median, mode, and standard deviation[17]. For the nominal and ordinal data, the author calculated the frequency, percentage, and proportion. The interval and the ratio data variables were analysed using a student's t-test test. Categorical variables were analysed using chi-square (χ^2) tests[18]. A P-value < 0.05 was considered statistically significant.

Results

During the period of the study, a total of 734 Covid-19 patients came to the emergency/inpatient department of the institute. After clinical and laboratory examination, 167 Covid-19 patients were recommended home isolation and 567 patients were admitted to the hospital. Out of 567 admitted patients, 23 patients were excluded using the selection criteria and 544 covid-19 patients were enrolled in the present study. Of the 544 enrolled participants, the clinical outcome wasn't available (referred out/LAMA) for 36 patients. Thus, data on only 508 patients were included in the present study.

Among 508 included patients: 53 (10.4%) patients died because of Covid-19-related complications, 73 (14.4%) patients required admission to Intensive Care Unit studied, 39 (7.7%) patients were on mechanical ventilation, 23 had Coma (4.5%) and 328 (64.6%) patients were discharged from hospital without any complications. Overall, the mean age of the participants was 49.7 (±7.3) years. Further, of the total participants, 219 (43.1%) were female and 288 (56.9%) were male. Among the study participants: 165 (32.4%) had diabetes, 98 (19.3%) had hypertension, 142 (28.1%) were smokers, 93 (18.4%) had pre-existing pulmonary disease, and 113 (22.2%) had a history of CVD. Collectively, about 75 (14.7%) of participants had multiple (>3) morbidities (Table 1).

Table 1: Descriptive Characteristics of Study Participants (n=508)

Variable	Discharged Alive (n=455)	Died (n=53)	P-value
Age			
Mean (±SD)	44.8	63.7	0.006
Gender			
Male	255 (93.4%)	33 (57.6)	0.298
Female	200 (93.8%)	20 (42.3)	
Co-Morbidity			
History of CVD	96 (21.1%)	17 (32.7%)	0.057
H/O Pulmonary Disease	70 (15.4%)	23 (43.4%)	<0.0001
Diabetes	130 (28.6%)	35 (66.0%)	<0.0001
Hypertension	70 (15.4%)	28 (52.8%)	<0.0001
BMI	23.3	28.1	0.008
Obese	85 (18.7%)	29 (54.7%)	<0.0001

Table 1 illustrates the descriptive characteristics of the participants. The mean age of the participants who had favourable and adverse outcomes were 44.8 and 63.7 years, respectively (p=0.006). Most of the patients with unfavourable outcomes were multimorbid: 39 (73.9%) with an unfavourable outcome and 36 (7.9%) with favourable outcome (p=0.003). The mean BMI among the participants with favourable and unfavourable outcomes was 23.3 and 28.1 Kg/sqm (p=0.008).

Table 2: Distribution of Inflammatory marker levels among participants (n= 508)

Markers	Discharged Alive (n=455)	Died (n=53)	P-value
LDH (U/Litres)	293	489	<0.001
LDH >250 (U/Litres) n, (%)	**72 (15.8%)**	**31 (58.4%)**	**<0.001**
Ferritin (ng/ml)	543	991	0.013
Ferritin level >500 ng/ml n, (%)	**94 (20.6%)**	**42 (79.2%)**	**0.003**
D-Dimer (ng/ml)	552	964	0.009
D Dimer>500 (ng/ml) n, (%)	**105 (23.7%)**	**38 (71.7%)**	**0.03**
CRP (mg/dl)	53	184	0.039
CRP >50 mg/dl n, (%)	**110 (24.2%)**	**32 (74.7%)**	**0.032**
NLR	4.7	10.3	0.002
IL-13 (pg/ml)	19	43	<0.0001
IL-13 >25 (pg/ml)	**59 (12.9%)**	**42 (79.2%)**	**<0.001**

Table 2 illustrates the levels of various inflammatory markers on admission among the Covid-19 patients who did and did not survive hospital treatment. All measured inflammatory parameters viz. LDH, Ferritin, CRP, D-dimer, NLR, and IL-13 were significantly higher (and worse) among the patients who died during the treatment (p<0.05).

Table 3: Correlation Coefficient between inflammatory markers and CT- Severity Score (n= 508)

Markers	Correlation Coefficient
LDH	+0.68
Ferritin	+0.71
D-Dimer	+0.55
CRP	+0.81
NLR	+0.76
IL-13	+0.83

Table 3 illustrates the correlation coefficient between the CT Severity score and the various inflammatory markers. The strongest correlation was observed between the IL-13 (+0.83) followed by CRP (+0.81) and the weakest correlation was observed for D-dimer (+0.55).

Discussion

Theoretically, some biomarkers can predict a worse outcome during any disease or condition, supporting clinical management. Practically, the clinical usefulness of a given biomarker is not proven until it helps clinicians manage patients and make treatment decisions. The biomarker pipeline involves many steps; thus, the evaluation and validation of a certain molecule require rigorous studies with faultless methods and homogeneous features. Although the usefulness of biomarkers for the prediction of disease severity and treatment response in COVID-19 patients is a fascinating prospect, at present, their applicability in clinical practice remains conceptual. The differences in cut-offs and outcomes limit the possibility of drawing definitive conclusions about the usefulness of a certain biomarker in predicting prognosis.

At the time this study was conceptualized, there were insufficient data to prove the usefulness of a certain biomarker for therapy guidance and appropriate patient management. Based on these considerations, promising findings have been reported on the potential usefulness of CRP, LDH, Ferritin, IL-13, and D-dimer levels as biomarkers of COVID-19 severity. However, the clinical usefulness of these biomarkers remained to be established. Further, the data on the efficacy of these biomarkers in predicting the treatment response are sparse, necessitating confirmatory studies. In the present study, among 508 included patients: 53 (10.4%) patients died because of Covid-19-related complications, 73 (14.4%) patients required admission to Intensive Care Unit studied, 39 (7.7%) patients were on mechanical ventilation, 23 had Coma (4.5%) and 328 (64.6%) patients were discharged from hospital without any complications.

C-Reactive Protein:

In the present study, the mean CRP Levels among those with favourable and poor outcome was 53 mg/dl and 184 mg/dl, respectively ($p<0.05$). Further, a CRP level > 50 mg/dl was seen among 24% and 74% of COVID-19 patients having good and poor clinical outcome ($p<0.05$). In the present study, the levels of CRP correlated linearly with the CSS ($r = + 0.81$). The association of higher CRP with worse outcomes may be due to the severity of the disease consistent with the 'cytokine storm' theory of COVID-19, where the innate immune system is activated releasing TNF-alpha, IL-6, IL-1, and IL-13. Elshazli *et al*. found CRP to be a valid biomarker of death from COVID-19 when examining a range of haematological and immunological markers[19]. In their study, IL-6 was found to be most predictive (OR = 13.87) of death, and CRP was the next best inflammatory biomarker (OR = 7.09). However, IL-6 is not routinely available to clinicians, but being linked to CRP as a trigger for its transcription makes CRP a better candidate tool for front-line hospital usage. In addition, an elevated CRP may not be attributable to COVID-19 alone and may represent concomitant pathology such as secondary bacterial pneumonia. Similar to our findings, Sharma S et al. (2022), showed that mean of CRP was significantly higher in severe group (11.7) as compared to mild (5.3) and moderate (5.2) group ($p = 0.045$)[20]. Wang D et al., (2020), reported that the mean CRP found significantly higher among severe cases 54.8 mg/dl vs mild/moderate cases 8.6 mg/dl with ($p = 0.000$)[21]. Chen T et al. (2021) revealed that mean of CRP was 22.4 mg/dl in non-severe group of patients, 73.6 mg/dl in severe group[22]. On comparison with disease severity as per CTSS, mean of CRP shows increasing trend with increasing disease severity, with positive

statistical correlation (p = 0.001). Ali N et al., (2020) reported that a significant increase of CRP was found with levels on average 20 to 50 mg/L in patients with COVID-19[23]. Elevated levels of CRP were observed up to 86% in severe COVID-19 patients. Patients with severe disease courses had a far elevated level of CRP than mild or non-severe patients. Wang G et al., (2020) reported that compared with non-severe patients, the aggravated patients had much higher levels of CRP (median 43.8 vs 22.1 mg/L; *P* = .000)[24]. Similar to oud fidings they observed that CRP was significantly associated with aggravation of no severe COVID-19 patients. They concluded that CRP could be a valuable marker to anticipate the possibility of aggravation of nonsevere adult COVID-19 patients, with an optimal threshold value of 26.9 mg/L.

D-Dimer

In the present study, the mean d-dimer levels among those with favourable and poor outcome was 552 units and 964 unit, respectively (p<0.05). Further, a D-Dimer level > 500 unit was seen among 20% and 81% of COVID-19 patients having good and poor clinical outcome (p<0.05). Furthermore, the mean D-dimer level was significantly higher who had high CT severity score. Sharma S et al., (2022), showed that mean of D-dimer values was 861.16 in mild group of patients, 1035.19 in moderate group, and 2476.85 in severe group [20]. On comparison with disease severity as per CTSS, mean of D-dimer shows increasing trend with increasing disease severity, with positive statistical correlation (p = 0.002). Wang D et al. (2020), reported that, mean D-Dimer found significantly higher among severe cases 1200 vs mild/moderate cases 400 with (p = 0.000) [21]. Yao Y et al., reported that very high levels of D-dimer at admission was the only variable associated with increased odds of mortality (OR 10.17). D-dimer elevation was seen in 74.6% (185/248) of the patients [25]. D-dimer levels significantly increased with increasing severity of COVID-19 as determined by clinical staging and chest CT staging. Poudel A et al., studied 182 patients and reported that the mean admission D-dimer among surviving patients was significantly greater among patients who died. The authors concluded that D-dimer value on admission is an accurate biomarker for predicting mortality in patients with COVID-19[26]. Quolim S et al., reported that the optimum cutoff value to predict mortality in patient using D-dimer levels on admission was 668 ng/ml. As for D-dimer levels on day 5, it was 1360 ng/ml[27]. They observed that D-dimer greater than 1360 ng/ml on day 5 could help clinicians identify patients with poor prognosis at an early stage of COVID-19. Duz ME et al., conducted a metanalysis and concluded that the D-dimer concentrations were significantly higher in patients with more severe COVID-19.

LDH

In the present study, the mean LDH levels among those with favourable and poor outcome was 293 unit/l and 489 units/l, respectively (p<0.05). Further, serum LDH levels of > 250 unit was seen among 15% and 58% of COVID-19 patients having good and poor clinical outcome (p<0.05).

Wang D et al. (2020), revealed that, mean lactate dehydrogenase (LDH) found significantly higher among severe cases 321 vs mild/moderate cases 256 with (p = 0.000)[21]. Fialek B et al., (2020) from a meta-analysis of 28 studies reported that a statistically significant higher level of LDH was observed among those with good and poor outcome, ICU vs. Non-ICU, mechanical ventilation versus non mechanical ventilated and in nonsurvival patients vs. survival patients[28]. Martha JW et al., (2021) conducted a study involving 10,399 patients and found that elevated LDH was present in 44% (34%–53%) of the patients(100). The data analysis showed that elevated LDH was associated with composite poor outcome (OR

5.33;p<0.001). In their study, elevated LDH has a sensitivity of 0.74 (95% CI 0.60 to 0.85), specificity of 0.6. They concluded that LDH was associated with poor prognosis in patients with COVID-19. Henry BM et al., conducted analysis of 1,532 COVID-19 patients) and reported that elevated LDH levels were associated with a ~6-fold increase in odds of developing severe disease and a ~16-fold increase in odds of mortality in patients with COVID-19[29].

Serum Ferritin

In the present study, the mean serum ferritin levels among those with favourable and poor outcome was 543 units and 951 units, respectively (p<0.05). Further, a serum ferritin levels > 500 unit was seen among 20% and 79% of COVID-19 patients having good and poor clinical outcome (p<0.05). Moreover, the serum ferrtin level correlated linearly with the CSS and number of lung segments involvement. Sharma S et al. (2022), showed that mean of serum ferritin was 202.31 in mild group of patients, 571.25 in moderate group, and 746.21 in severe group. On comparison with disease severity as per CTSS, mean of serum ferritin shows increasing trend with increasing disease severity, with positive statistical correlation (p = 0.001)[20]. Kaushal K et al., reported that severe to critical COVID-19 patients showed higher ferritin levels compared to mild to moderate COVID-19 patients[30]. Further they reported that non-survivors had higher serum ferritin level compared to survivors. Patients requiring ICU and mechanical ventilation had higher serum ferritin levels compared to those who didn't. They concluded that serum ferritin level may serve as an important biomarker which can aid in COVID-19 management. Alroomi M et al., (2021) reported that high ferritin level (odds ratio (OR) = 0.36) had significance in predicting in-hospital mortality[31]. Kaplan–Meier survival probability plot showed a higher mortality rate among patients with ferritin levels > 1000. Hulkoti VS et al., (2022) reported that serum ferritin was found to be a potent marker for clinical outcome in intensive care unit patients in terms of death versus treated. HRCT Score and N:L ratio were found to be correlated with serum ferritin. Serum ferritin may determine the severity of COVID-19 infection and it can be used as a marker for Clinical Outcome[32]. Carubbi et al., reported that that ferritin levels over the 25th percentile were associated with the involvement of all 5 pulmonary lobes, the presence of septal thickening and the presence of mediastinal lymph node enlargement independently of age and gender[33]. They observed that ferritin levels over the 25th percentile are associated with a more severe pulmonary involvement, and associated with disease outcomes.

IL-13:

In the present study, the mean IL-13 levels among those with favourable and poor outcome was 19 pg/ml and 43 pg/ml, respectively (p<0.05). Further, a serum IL-13 levels >25 (pg/ml) was seen among 12% and 79% of COVID-19 patients having good and poor clinical outcome (p<0.05). Moreover, the serum IL-13 level correlated linearly with the CSS and number of lung segments involvement[12]. Laboratory studies indicate that severe COVID-19 is related to type-2 cytokine storm including rise in the levels of IL-13[34]. Moreover, neutralisation of IL-13 in mice infected with SARS-CoV-2 protects from mortality, in part by preventing hyaluronan production and excessive deposition, in COVID-19 patients using the IL-13 blocking medication Dupilumab[35]. It is doubtful that IL-13 inhibition will be effective in all patients given the substantial variation in immunological responses to COVID-19[35].

It is difficult to pinpoint the exact mechanism by which IL-13 caused severe illness since it had so little of an effect on the IL-13 downstream effectors that are frequently seen during allergy or asthmatic

inflammation[36]. However, given the low degree of their induction compared to previous models of type 2 immunity in the lung, their biological importance. IL-13 actively participates in the Th2 pathway, since both interleukins share the same receptor (IL-4Ra). IL-13 works with IL-4 to induce alternative activation of M2 macrophages (Sphingosine-1), promoting the release of TGF-β and platelet-derived factor[34]. This phase is characterized by the transient expansion of resident fibroblasts and the formation of a temporary matrix and the proliferation of airway progenitor cells and type 2 pneumocytes.

Donlal AN et al., reported that among the Covid-19 patients who required mechanical ventilation; Seven cytokines were found to be elevated in COVID-19 patients that ultimately required ventilation[37]. These included FGF basic (bFGF) (p=0.0006), IL-7 (p=0.0006), IFN-γ (p=0.0102), MIP-1α (p=0.0195), IL-13 (p=0.0159), G-CSF (p=0.0368), and IL-17 (p=0.0448). IL-1ra (p=0.0554) and IL-1β (p=0.0707) were also trending towards being significantly higher in the ventilated patients. Additionally, IL-13 levels were predictive of ventilation, with the highest odds ratio of 1.58. IL-7, which is an important for induction and maintenance of lymphocytes was also predictive of ventilation (OR: 1.04) and patients in the top three percentiles for IL-7 expression were significantly more likely to require ventilation (p = .015)[38]. Of the cytokines tested, IFN-γ (P=0.0011) and IL-1ra (p=0.0042) were significantly higher in patients with elevated glucose levels. In contrast to our study, Ozger HS et al., did not find any difference in the IL-13 levels among those who survived and did not survive the covid-19[39].

Conclusion

Patients who had an unfavourable treatment outcome- whether it was morality, admission to the ICU, the requirement for mechanical ventilation, or who went into coma, had considerably higher levels of all of the assessed inflammatory biomarkers. However, we need more research to develop a sensitive upper level cut off levels that can be reliably used to assess whether covid-19 patients should be admitted to the hospital and the intensive care unit. Further, the level of biomarkers should always be correlated with clinical as well as radiological findings to increase their reliability and utility.

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