Original Article

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The study of serum hsCRP, ferritin, IL-6 and plasma D-dimer in COVID-19: a retrospective study

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Abstract

Objectives: The cut off values for serum high sensitivity C-reactive protein (hsCRP), ferritin, interleukin 6 (IL-6) and plasma D-dimer could be of profound help in detecting COVID-19 patients at risk of adverse outcomes. Therefore, the aim of the present study is to determine the cut off values of the serum hsCRP, ferritin, IL-6 and plasma D-dimer in predicting mortality in COVID-19 patients.
Methods: Four hundred RT-PCR confirmed cases of COVID-19 were sub divided into two groups based on their outcome during hospitalisation. Group I consisted of survivors and Group II consisted of non-survivors. The survivors were further divided into three sub-groups: mild, moderate and severe based on the severity of infection. The laboratory data of serum hsCRP, ferritin, IL-6 and plasma D-dimer for all these patients was retrieved from the Medical Record Section of the Hospital.
Results: Mean serum hsCRP, ferritin, IL-6 and plasma D-dimer levels were significantly higher in non-survivors as compared to survivors of COVID-19. The levels of these biomarkers correlated with the severity of COVID-19 illness. ROC curve analysis revealed that plasma D-dimer is having a better predictive value as compared to other parameters in predicting mortality in COVID-19.
Conclusions: The serum hsCRP, ferritin, IL-6 and plasma D-dimer levels could be used in risk stratification of COVID-19 patients. The optimum cut off given by the current study could be considered in predicting adverse outcome in these patients. Amongst the many studied biomarkers, plasma D-dimer might be the best early biomarker to predict mortality in COVID-19 patients.

Keywords: COVID-19; cut off; D-dimer; ferritin; hsCRP; IL-6.

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Introduction

COVID-19 pandemic has created a havoc across the globe affecting every aspect of life, be it medical, socio-economic or political. By March 25th 2022, a total of 474,659,674 confirmed cases of COVID-19, including 6,103,355 deaths have been reported to WHO [1]. Furthermore, no reliable prognostic biomarkers for predicting disease severity, progression and mortality exist till date for COVID-19 [2]. Some biomarkers such as serum high sensitivity C-reactive protein (hsCRP), ferritin, interleukin 6 (IL-6) and plasma D-dimer have been reported to identify COVID-19 patients at risk of developing severe illness and death [3]. However, the cut off values of these biomarkers in prediction of mortality has not been established yet [2]. These cut off values could be of profound help in identifying the patients at risk of adverse outcome and therefore aid in proper triaging, timely intervention and prognostication of such critical patients. Therefore, the aim of the present study is to determine the cut off values of the serum biomarkers of COVID-19 such as hsCRP, ferritin, IL-6 and D-dimer in predicting the severity of COVID-19 including death.

Materials and methods

Study design

This hospital based retrospective study was carried out from January 2021 to March 2021, in the Department of Biochemistry in collaboration with the Department of Medicine, AIIMS Nagpur after Institutional Ethical Committee (IEC) approval. The present study was according to declaration of Helsinki as involving human subjects. As per study design, the informed and written consent from the study subject had been waived and same had been approved by IEC.

Sample size calculation and sampling technique

Assuming the accuracy of serum hsCRP in predicting mortality by area under curve (AUC) value of 0.8, α error of 0.05, precision of 0.05, the sample size estimated was 368. Considering the missing information of approximately 10% from the records, the final sample size estimated was 400. The consideration of hsCRP for sample size calculation was based on the fact that serum hsCRP was being estimated in almost all the admitted patients in comparison to other biochemical parameters. The patients included in the study were randomly selected from amongst those admitted in the COVID wards at AIIMS, Nagpur, fulfilling the following inclusion and exclusion criteria.

Inclusion criteria: Diagnosed cases of COVID-19 by real time PCR (RT-PCR).

Exclusion criteria: Individuals with history of any chronic illnesses such as tuberculosis, chronic hepatic or renal disease, pelvic inflammatory disease, inflammatory bowel disease, connective tissue disorders, autoimmune disorders such as systemic lupus erythematosus, rheumatic disease etc. or with any history of debilitating illnesses such as cardiovascular or cerebrovascular, neurological disease or malignant conditions were excluded from the study.

The study population was further divided into three sub-groups – mild, moderate and severe, according to the severity of COVID-19 illness as given below [4].

(1) Mild cases: (i) mild symptoms of upper respiratory infection, vital parameters – stable, SpO2 – more than 96%; (ii) no clinical and/or radiological evidence of lower respiratory tract infection/ pneumonia.
(2) Moderate cases: (i) severe symptoms like shortness of breath, (ii) SpO2 – less than 96%, clinical and/or radiological evidence of lower respiratory tract infection/pneumonia present.
(3) Severe cases: (i) evidence of Acute Respiratory Distress syndrome (ARDS), respiratory failure requiring assisted ventilation, (ii) multi organ dysfunction syndrome (MODS).

Data collection

The main outcomes measured in the study were severity of the illness and/or death of COVID-19 patients during hospitalisation.

All clinical and laboratory data, including the outcome data was retrieved from the medical records section of the hospital. The collected data consisted of the demographic profile and blood investigations including levels of serum hsCRP, ferritin, IL-6 and plasma D-dimer.

Measurement of serum hsCRP, ferritin, IL-6 and plasma D-dimer levels

The analyser used by the Clinical Biochemistry Laboratory of the Hospital for the estimation of serum hsCRP, ferritin and IL-6 was the Cobas® 6000 modular (c501 and e601) Biochemistry and Immunoassay analyser. All reagent kits used belonged to Roche Diagnostics, Basel, Switzerland. Estimation of serum hsCRP was based on particle enhanced immunoturbidimetric assay and its biological reference interval was taken as <5 mg/L. Estimation of serum ferritin was based on electrochemiluminescence immunoassay (ECLIA) and its biological reference interval was considered as 30–400 µg/L in 20–60 years of male and 13–150 µg/L in 17–60 years of female. Estimation of serum IL-6 was also based on ECLIA and its biological reference interval was considered as <7 pg/mL. Plasma D-dimer was estimated on STAR Max® haemostasis analyser from Diagnostica StagoInc, Asnières-sur-Seine, France and was based on immune-turbidimetric assay. Its biological reference interval was considered as <0.5 µg/mL. The biological reference intervals in consideration were as provided by the manufacturer, mentioned in the pack inserts of the respective diagnostic kits. Quality control assessment was done using internal quality control material "PreciControl" provided by Cobas, Roche Diagnostics, Basel, Switzerland.

Statistical analysis

The data was analysed by appropriate statistical methods using Statistical Package for Social Sciences (SSPS) 20th version, IBM, USA and GraphPad prism, Ilniosis, USA. Data was analysed for test of normality. Continuous variables were expressed as mean ± SD in parametric distribution of data and as median and interquartile range (25th percentile and 75th percentile) in non-parametric distribution of data. Correlation analysis was done among study variables by Pearson's and Spearman's correlation analysis. ROC curve was used to define sensitivity, specificity and optimum cut off of serum hsCRP, ferritin, IL-6 and plasma D-dimer to predict the mortality. Logistic regression analysis was carried out to assess the independent effect of study variables on the occurrence of adverse outcome in COVID-19. The observed frequencies of the categorical variables were compared by Chi-square test. A p value of <0.05 was considered as significant.

Results

The demographical and biochemical characteristics of the study populations (Group I – survivors and Group II – non-survivors) are shown in Table 1. The present study was sex matched. A total of 4.7% (18/400) of the patients died due to COVID-19 in the present study. Of the 382 survivors,

Table 1: Distribution of demographic and biochemical profile of the study population.

Serum parameters	Survivors (Group I) (n=382)	Non-survivors (Group II) (n=18)	p-Value
Age, years	42 ± 17	65.7 ± 13	<0.001[a]
Sex (male/female)	236/146	13/5	0.367
RBS, mg/dL	125 ± 63 (n=372)	248 ± 99 (n=18)	<0.001[a]
HbA_{1c} (%)	5.7 ± 1.3 (n=372)	10.7 ± 2 (n=18)	<0.001[a]
Na^+, mmol/L	140, 138–141 (n=140)	130, 126–130 (130)	0.370
K^+, mmol/L	4.1 ± 0.6 (n=372)	6 ± 1 (n=18)	<0.001[a]
Urea, mg/dL	20, 16–26 (n=372)	76, 42–124 (n=16)	<0.001[a]
Creatinine, mg/dL	1.3 ± 0.6 (n=372)	1.6 ± 0.8 (n=16)	0.852
Total bilirubin, mg/dL	0.43, 0.31–0.62 (n=381)	0.22, 0–0.025 (n=17)	0.813
Direct bilirubin, mg/dL	0.20, 0.14–0.28 (n=381)	0.00, 0–0.36 (n=17)	0.752
ALT, U/L	20, 13–30 (n=365)	45, 33–52 (n=18)	<0.001[a]
AST, U/L	23, 18–33 (n=365)	70, 32–176 (n=4)	<0.001[a]
ALP, U/mL	77, 61–108 (n=363)	140, 80–284 (n=4)	0.065
Total protein, g/dL	7.3 ± 0.9 (n=357)	5.7 ± 0.5 (n=4)	<0.001[a]
Albumin, g/dL	4.2 ± 0.5 (n=369)	2.4 ± 0.5 (n=7)	<0.001[a]
hsCRP, mg/L	4, 1–20 (n=140)	150, 93–1,755 (n=17)	<0.001[a]
Ferritin, ng/mL	120, 48–282 (n=365)	874, 667–1,372 (n=17)	<0.001[a]
IL-6, pg/mL	10, 3–30 (n=35)	95, 51–190 (n=18)	<0.001[a]
D-dimer, µg/mL	0.1, 0–0.12 (n=381)	2,940, 548–3,935 (n=18)	<0.001[a]

[a]p<0.05 is considered as statistically significant. hsCRP, high sensitivity C-reactive protein, ALT, alanine aminotransferase, AST, aspartate aminotransferase, ALP, alkaline phosphatase.

83.5% (319/382) patients presented with mild illness, 13.8% (53/382) patients presented with moderate illness and 2% (9/382) patients presented with severe illness during hospitalisation. Mean serum hsCRP, ferritin, IL-6 and plasma D-dimer levels were significantly higher in the non-survivors (Group II) as compared to survivors (Group I) as shown in Table 1 and Figure 1. The mean serum hsCRP, ferritin, IL-6 and plasma D-dimer levels were increased in severe cases of COVID-19 as compared to moderate and mild cases of COVID-19, although the difference was not

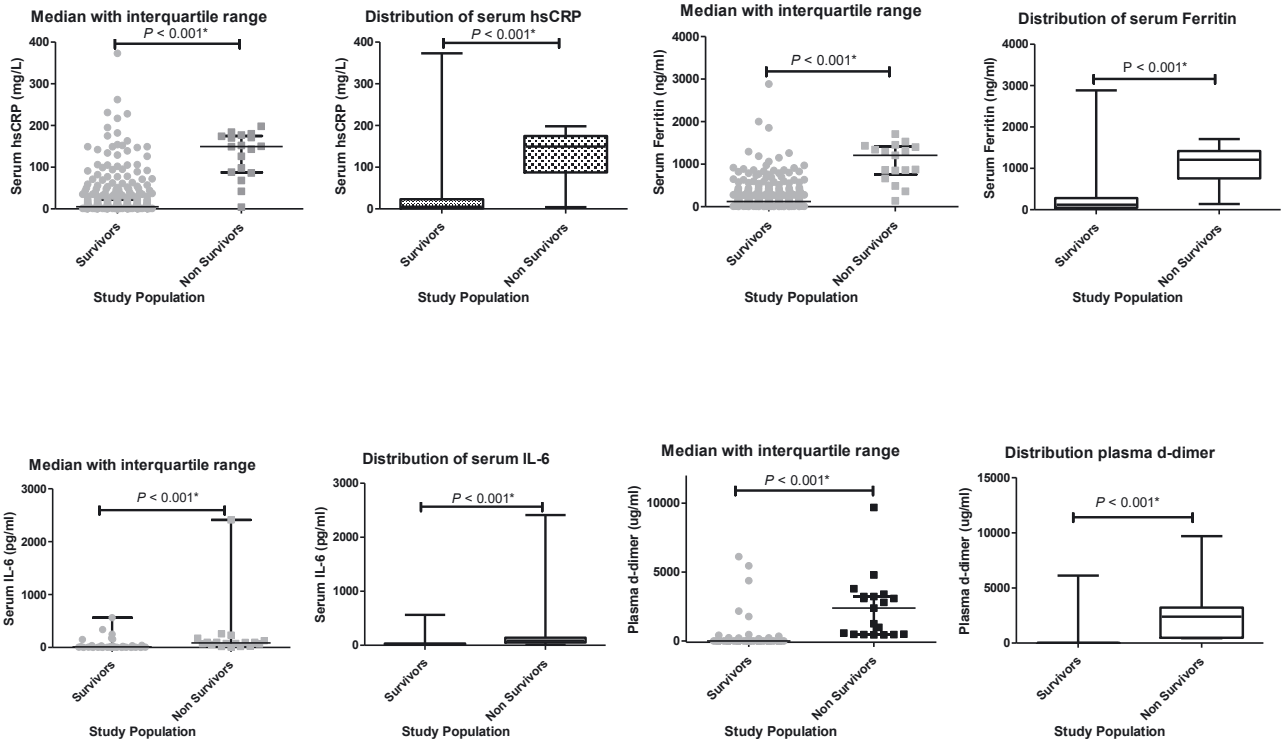

Figure 1: Serum hsCRP, ferritin, IL-6 and plasma D-dimer in the study population.

statistically significant as shown in Table 2 and Figure 2. The mean serum hsCRP, ferritin, IL-6 and plasma D-dimer levels were increased in non-survivors of COVID-19 as compared to moderate and severe cases of COVID-19 as shown in Table 3. The Spearman's correlation analysis showed that serum hsCRP, ferritin, IL-6 and plasma D-dimer correlated with each other and other biochemical parameters as shown in Table 4 and Figure 3. The receiver operating characteristic curve (ROC curve) analysis showed that plasma D-dimer had the highest probability of predicting mortality in COVID-19 as shown in Table 5 and Figure 4. ROC curve analysis showed that AUC is maximum (0.989) for plasma D-dimer as compared to serum hsCRP, ferritin and IL-6 (Table 5). Plasma D-dimer at 368 µg/mL cut off, predicted

Table 2: The median and interquartile range of serum hsCRP, ferritin, IL-6 and plasma D-dimer in mild, moderate and severe cases of COVID-19 infection in survivor group.

Biochemical parameters	Patients with mild COVID-19 infection	Patients with moderate COVID-19 infection	Patients with severe COVID-19 infection	Overall p-Value	Pair wise comparison
hsCRP, mg/L	3, 1–10 (n=302)	39, 8–89 (n=51)	153, 124–203 (n=10)	<0.001[a]	P^1=<0.001[a]
					P^2=<0.001[a]
					P^3=<0.001[a]
Ferritin, ng/mL	50, 42–223, n=276	282, 115–581 (n=46)	635, 418–756 (n=9)	<0.001[a]	P^1=<0.001[a]
					P^2=<0.001[a]
					P^3=0.236
IL-6, pg/mL	0.1, 0.01–0.1 (n=315)	0.1, 0.01–0.27 (n=54)	0.085, 0.00–0.085 (n=10)	<0.001[a]	P^1=0.824
					P^2=0.997
					P^3=0.941
D-dimer, µg/mL	7, 2–14.2 (n=26)	40, 18–154 (n=5)	250, 176 – NC (n=3)	0.115	P^1=0.976
					P^2=0.622
					P^3=0.882

P^1, mild COVID cases vs. moderate COVID cases; P^2, mild COVID cases vs. severe COVID cases; P^3, moderate COVID cases vs. severe COVID cases; NC, not calculated as the no. of study subjects were three; hsCRP, high sensitivity C-reactive protein. [a]p<0.05 is considered as statistically significant.

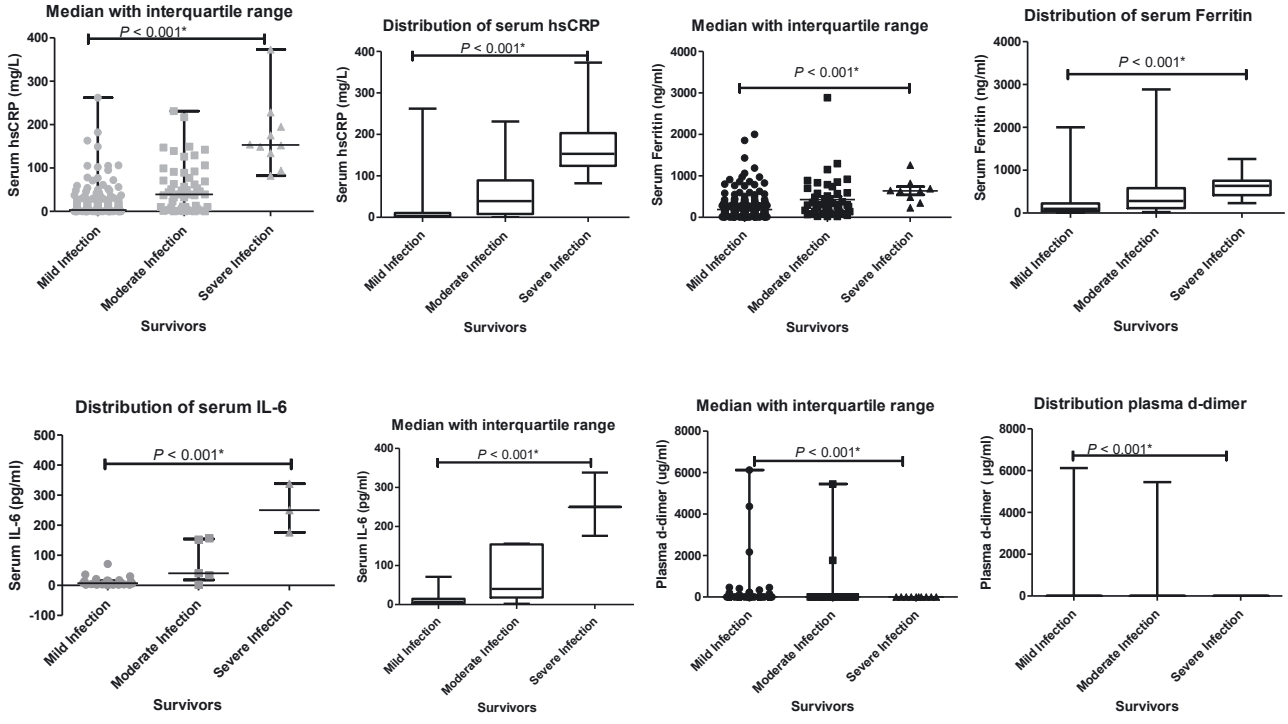

Figure 2: Serum hsCRP, ferritin, IL-6 and plasma D-dimer in mild, moderate and severe cases of COVID-19 infection.

Table 3: The median and interquartile range of serum hsCRP, ferritin, IL-6 and plasma D-dimer in moderate and severe cases of COVID-19 infection in survivor group and Non survivor group.

Biochemical parameters	Patients with moderate COVID-19 infection	Patients with severe COVID-19 infection	Non-survivors	Overall p-Value	Pair wise comparison
hsCRP, mg/L	39, 8–89 (n=51)	153, 124–203 (n=10)	3, 1–10 (n=302)	<0.001[a]	P^1=<0.001[a] P^2=0.051
Ferritin, ng/mL	282, 115–581 (n=46)	635, 418–756 (n=9)	50, 42–223, n=276	<0.001[a]	P^1=<0.001[a] P^2=0.02[a]
IL-6, pg/mL	0.1, 0.01–0.27 (n=54)	0.085, 0.00–0.085 (n=10)	0.1, 0.01–0.1 (n=315)	<0.001[a]	P^1=0.728 P^2=1.000
D-dimer, µg/mL	40, 18–154 (n=5)	250, 176 – NC (n=3)	7, 2–14.2 (n=26)	0.115	P^1=<0.001[a] P^2=<0.001[a]

P^1, moderate COVID cases vs. non-survivors; P^2, severe COVID cases vs. non-survivors; NC, not calculated as the no. of study subjects were three. [a]p<0.05 is considered as statistically significant. hsCRP, high sensitivity C-reactive protein.

Table 4: Spearman correlation analysis of serum hsCRP, ferritin, IL-6 and plasma D-dimer with various biochemical variables.

Parameters	Serum hsCRP		Serum ferritin		Serum IL-6		Plasma D-dimer	
	r value	p-Value	r value	p-Value	r value	p-Value	r value	p-Value
hsCRP	1	–	0.587	<0.001[a]	0.860	<0.001[a]	–0.014	0.782
Ferritin	0.587	<0.001[a]	1	–	0.617	<0.001[a]	0.091	0.088[a]
IL-6	0.860	0.001[a]	0.617	<0.001[a]	1	–	0.383	0.004[a]
D-dimer	–0.014	0.782	0.091	0.088	0.383	0.004	1	–
RBS	0.449	<0.001[a]	0.350	<0.001[a]	0.449	<0.001[a]	0.038	0.449
HbA_{1c}	0.324	<0.001[a]	0.264	<0.001[a]	0.539	<0.001[a]	0.096	0.059
Sodium	–0.375	<0.001[a]	–0.330	<0.001[a]	–0.165	0.399	0.027	0.613
Potassium	0.213	<0.001[a]	0.191	0.001	0.126	0.514	–0.071	0.171
Urea	0.341	<0.001[a]	0.372	<0.001[a]	0.348	0.573	0.030	0.55
Creatinine	0.319	<0.001[a]	0.300	<0.001[a]	–0.031	0.220	0.127	0.013
Total bilirubin	–0.009	0.841	–0.009	0.870	0.318	0.022	0.030	0.550
Direct bilirubin	–0.010	0.841	–0.099	0.54	–0.221	0.116	0.020	0.974
ALT	0.385	<0.001[a]	0.518	<0.001[a]	0.329	0.017	0.053	0.300
AST	0.534	<0.001[a]	0.545	<0.001[a]	0.557	<0.001[a]	–0.053	0.306
ALP	–0.241	<0.001[a]	–0.268	<0.001[a]	0.323	0.045	0.084	0.108
Total protein	–0.327	<0.001[a]	–0.203	<0.001[a]	–0.368	0.021[a]	0.017	0.751
Albumin	–0.467	<0.001[a]	–0.221	<0.001[a]	–0.4	0.001	0.012	0.816

[a]p<0.05 is considered as statistically significant. hsCRP, high sensitivity C-reactive protein; IL-6, interleukin 6, ALT, alanine aminotransferase, AST, aspartate aminotransferase, ALP, alkaline phosphatase.

mortality with 100% sensitivity and 98% specificity as shown in Table 6. On binary logistic regression analysis, serum ferritin and plasma D-dimer show statistically significant predictability of mortality in COVID-19 infection as shown in Table 7.

Discussion

Retrospective data of four hundred COVID-19 patients hospitalised at a tertiary care hospital was analysed in the present study. This study shows that mean serum hsCRP, ferritin, IL-6 and plasma D-dimer were significantly higher in the non-survivors (Group II) as compared to survivors (Group I) of COVID-19. The levels of these biomarkers also correlated with the severity of COVID-19 illness. Area under ROC curve analysis predicted that plasma D-dimer had the highest predictability of mortality in COVID-19 as compared to serum hsCRP, ferritin and IL-6. The present study proposes an optimal cut off of serum hsCRP, ferritin, IL-6 and plasma D-dimer to predict mortality in COVID-19 as shown in Table 5.

The overall mortality of COVID-19 ranges from 0.01% to 40% depending upon the presence or absence of various risk factors like old age, comorbidities etc. [5]. Majority of the COVID-19 patients are asymptomatic or have mild symptoms, but a small fraction of these patients may develop serious complications such as ARDS, disseminated intravascular

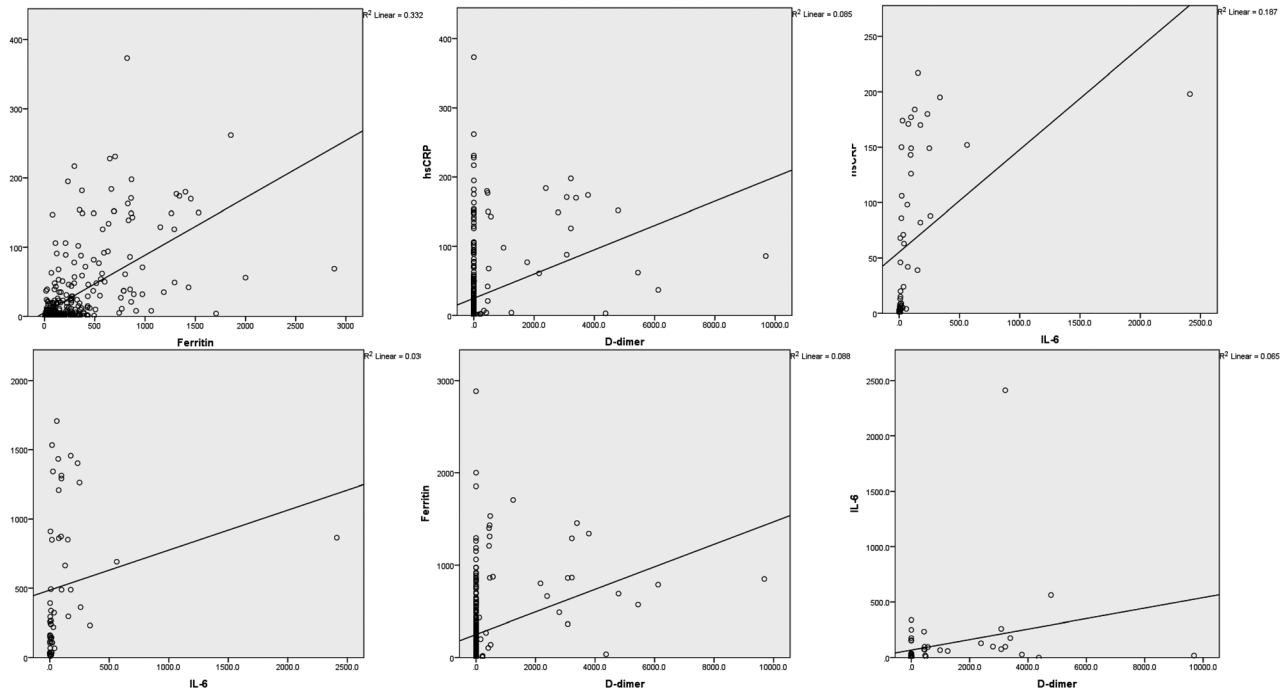

Figure 3: Scatter plot showing correlation among serum hsCRP, ferritin, IL-6 and plasma D-dimer in study population.

Table 5: Area under ROC curve of various biochemical markers with 95% CI of biochemical variables.

S. no	Variables	AUC	95% CI	p-Value
1	Serum hsCRP, mg/L	0.891	0.788–0.993	<0.001[a]
2	Serum ferritin, ng/mL	0.916	0.83–1.000	<0.001[a]
3	Plasma D-dimer, µg/mL	0.989	0.966–1.000	<0.001[a]
4	Serum IL-6, pg/mL	0.846	0.737–0.954	<0.001[a]

[a]p<0.05 is considered as statistically significant. AUC, area under curve; ROC, receiver operating characteristic; hsCRP, high sensitivity C-reactive protein.

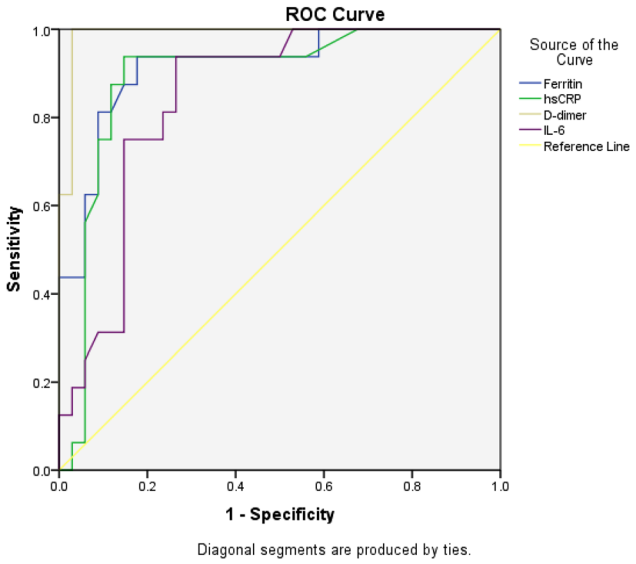

Figure 4: ROC curve analysis of various biochemical markers in predicting the hospital mortality in COVID-19 with the grouping outcome of being survivors and non-survivors at the end of the study.

coagulation (DIC), multi organ failure (MOF), sepsis and can eventually be fatal for the patient [6]. Many hypotheses have been put forward to explain the factors responsible for the varied clinical outcomes, but the puzzle still remains unsolved for researchers. Long hospitalisation, unpredicted course of the disease and associated mortality drive the search for biomarkers which can accurately, rapidly and effectively predict the mortality in COVID patients.

SARS-CoV-2 infection leads to activation of various inflammatory cells such as macrophages. These cells in turn release various cytokines aiding in combating infection, provided the cytokine secretion is regulated. When cytokines secretion is dysregulated as in the COVID-19 "cytokine storm", it may prove lethal for the patients especially those with comorbidities and the elderly. Therefore "cytokines storm" has been considered as one of the major mechanisms in the pathogenesis of COVID-19 associated complications [7]. Hence, the inflammatory mediators associated with cytokine storm and its products such as hsCRP, ferritin, IL-6 and D-dimer have been used to predict the severity and outcome in COVID-19. However,

Table 6: Cut off level, sensitivity, specificity of various biomarkers to predict mortality in COVID-19.

Biochemical parameters	Cut off levels	Sensitivity (%)	Specificity (%)
Serum hsCRP, mg/L	65.5	93.8	85.3
Serum ferritin, ng/mL	349	93.8	82
Serum IL-6, pg/mL	17.5	93.8	84
Plasma D-dimer, µg/mL	368	100	98

hsCRP, high sensitivity C-reactive protein.

Table 7: Binary logistic regression analysis of biochemical markers.

Biochemical parameters	Odd ratio	p-Value	95% CI
Serum hsCRP, mg/L	1.018	0.076	0.998–1.039
Serum ferritin, ng/mL	1.003	0.018[a]	1.001–1.006
Serum IL-6, pg/mL	0.999	0.650	0.995–1.003
Plasma D-dimer, µg/mL	1.001	0.008[a]	1.000–1.002

[a]$p<0.05$ is considered as statistically significant. hsCRP, high sensitivity C-reactive protein.

the cut off of these biomarkers to predict mortality has not been established yet.

C-reactive protein (CRP) is a positive acute phase protein produced by liver in various acute and chronic inflammatory conditions and is often useful as an indirect biomarker of generalised inflammation. It has an important role in immunity, clearance of damaged tissue and prevention of autoimmunity. It also regulates inflammatory response by pro- and anti-inflammatory action similar to IL-6. The blood CRP levels increase by varying amounts in response to a variety of inflammatory conditions such as bacterial infections or due to intracellular antigens, such as in viral infection of COVID-19 [8].

Serum hsCRP has been shown to be associated with disease severity and adverse outcome in COVID-19. The prognostic value of serum hsCRP can be assessed with the help of ROC curve as shown in Figure 4. Liu et al. [9] and Lau ES et al. [10] reported significantly higher serum hsCRP in severe COVID-19 infection in comparison to non-severe infection. Shabrawy ME et al. [11] proposed that a cut off of serum hsCRP of more than 33.9 ng/L has a sensitivity of 76.5% and specificity of 88.9% to predict the mortality in COVID-19, and is comparable to the cut off predicted in the present study as shown in Table 5.

Ferritin is an intracellular iron binding protein and involved in iron metabolism. It is an acute phase protein which is released from hepatocytes in inflammation. Hyperferritinemia causes immune dysregulation by pro-inflammatory and immune suppressive effect. Hyperferritinemia activates macrophages to release cytokines causing cytokine storm. Therefore, hyperferritinemia has a direct link with cytokine storm and serious outcome in COVID-19. Ferritin synthesis is increased by pro-inflammatory cytokines such as TNFα, IL-6 and IL-1β in COVID-19 infection. These cytokines cause inflammation and damage to cell resulting in release of ferritin. Chen N et al. [12] found that serum ferritin was significantly higher in severe COVID-19 cases as compared to the non-severe cases. They showed that higher level of serum ferritin is associated with critical and life threatening COVID-19 infection. VV Met et al. proposed a possible strategy to reduce the serum ferritin by iron chelators in COVID-19. According to this research group, decrease dietary intake of iron may reduce the exacerbation of COVID-19, especially in diabetic individuals where increased ferritin is the part of natural course of the diabetes [13].

Lino K et al. [14] devised that serum ferritin at a cut off of 1873 ng/mL has a sensitivity of 68.4% and specificity of 79.3% to predict mortality in COVID-19, which is comparable to the cut off predicted in the present study as shown in Table 5. Ahmad S et al. [15] also proposed that serum ferritin at a cut off of 574.5 ng/mL has a sensitivity of 80% and a specificity of 50% in predicting mortality in COVID-19. This result is comparable to the cut off predicted in the present study (Table 5).

D-dimer is a fibrin degradation product, widely used in diagnosing coagulation and thrombotic disorders. Cytokine storm associated with COVID-19 leads to activation of coagulation cascades, resulting in thrombotic complications and coagulopathies including DIC. Poudel A et al. in their study found that mean plasma D-dimer was higher in non-survivors (3.2 ± 2.6 µg/mL) as compared to survivors (1.067 ± 1.7 µg/mL) [16]. The research group gave the cut off for plasma D-dimer of 1.5 µg/mL with a sensitivity of 70.6% and specificity of 78.4% in predicting mortality [16]. Zhang et al. also gave the cut off of 2 µg/mL with a sensitivity of 92.3% and a specificity of 83.3% in predicting mortality [17]. The cut off level of plasma D-dimer to predict mortality is higher in the present study as shown in Table 5.

IL-6 is an interleukin which has both pro-inflammatory and anti-inflammatory activity. It is an important mediator of humoural immunity and is known to be associated with respiratory distress, MOF, shock and death in COVID-19. Many studies have shown that serum IL-6 could be used as an inflammatory biomarker for predicting poor prognosis in COVID-19 infection. Shabrawy ME et al. [11] proposed the cut off of serum IL-6 of more than 32.3 pg/mL with a sensitivity of 82.4% and specificity of 94.4% in predicting mortality in COVID-19, which is comparable to the cut off predicted in the present study (Table 5).

We therefore conclude that the cut off values of serum hsCRP, ferritin, IL-6 and plasma D-dimer proposed in the present study can be used as biomarkers to suggest adverse outcome in COVID-19. The measurement of these biomarkers

could provide valuable information to clinicians, as patients infected with SARS-CoV-2 display different disease severities. Increased biomarkers in present study correlated with increased disease severity and mortality. The cut off as shown in Table 5 may offer benefit to clinicians in making clinical and patient-management decisions for patients with COVID-19.

The biochemical parameters were measured by standardised and validated methods. However, there may be lack of generalisability of findings of the current research depending on the geographical location of the subjects, the virus variant, presence of comorbidities etc. Also, the present study was a cross-sectional study, hence cause and effect relationship cannot be established through this study. Data was collected retrospectively and missing clinical history may be the limitation of the present study. Follow up study with a larger sample size and/or a multicentric study should be conducted to confirm the findings of this study.

Conclusions

The current study suggests that serum hsCRP, ferritin, IL-6 and plasma D-dimer could be used in risk stratification of COVID-19 patients. The optimum cut off given by the current study should be considered in predicting adverse outcomes in COVID-19. Amongst the studied biomarkers, plasma D-dimer might be the best early biomarker to predict mortality in COVID-19. Patients with a higher level of these biomarkers beyond the cut off values should be carefully monitored throughout the natural course of the disease. Thus, plasma D-dimer could be used alone during monitoring the disease progression or when the resources for performing other serum biomarkers are limited.

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