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Original Article

Biochemistry Section

Association of Inflammatory Biomarkers with COVID-19 Disease Severity at Tertiary Care Hospital, Mumbai, India

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ABSTRACT

Introduction: After originating from China in 2019, the novel Coronavirus Disease 2019 (COVID-19) pandemic has badly affected most of the world and now India is witnessing second wave of COVID-19 cases posing a severe threat to public health in the country. The single-stranded Ribonucleic Acid (RNA) virus belongs to beta coronavirus family and is defined as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The inflammatory response caused by COVID-19 infection can play a critical role in progression of disease to severe level.

Aim: This study aimed to explore the association of inflammatory biomarkers with COVID-19 disease severity.

Materials and Methods: In this single centre retrospective observational study, 103 confirmed COVID-19 patients were included and divided into non severe and severe groups based upon their clinical features. Information about age, gender, co-morbidity and inflammatory biomarkers (serum interleukin-6, serum ferritin and plasma D-dimer) was collected for patients admitted between 1st June 2020 to 30th June 2020. The study was conducted at the Department of Biochemistry, Seth GS Medical College and King Edward Memorial (KEM) Hospital, Mumbai, Maharashtra, India. Comparison of biochemical parameters between non severe and severe group was done using independent t-test and correlation analysis was done using Pearson's correlation test.

Results: Out of total 103 patients, 67 (65%) had one or more co-morbidities. Total 53 (51.4%) patients were on various types of oxygen support. Mean serum IL-6, serum ferritin and plasma D-dimer levels were significantly higher in severe COVID-19 group (IL-6-1561.0±6015.0, ferritin- 2386±5051, and D-dimer-4.1±4.4) ($p<0.0001$) compared to non severe COVID-19 group (IL-6-26.7±46.6, ferritin- 513.0±419.9, and D-dimer-1.1±1.4). The best cut-off point for serum IL-6 was 20.2 pg/mL; sensitivity 91% and specificity 70.2%). For serum ferritin best cut-off point was 566.5 ng/mL; sensitivity 78.5% and specificity 70.2%. For plasma D-dimer best cut-off point was 0.9 pg/mL; sensitivity 72.2% and specificity 70.2%.

Conclusion: Serum IL-6, serum ferritin and plasma D-dimer can be helpful in monitoring of COVID-19 patients for early identification of severe cases and reducing mortality.

Keywords: Coronavirus disease 2019, D-dimer, Ferritin, Interleukin-6

INTRODUCTION

After originating from Wuhan, China in December, 2019, the novel COVID-19 has badly affected most of the world posing a severe threat to public health worldwide. COVID-19 belongs to beta coronavirus, a member of the coronaviridae family. It is a form of respiratory and systemic zoonosis caused by a new viral outbreak, finally defined as SARS-CoV-2 [1].

The COVID-19 is affecting 213 countries and territories around the world and two international conveyances. United states, Brazil, Russia are the worse hit countries and India has come on 4th position in the list of countries worse hit by this disease [2].

India witnessed a very high rise in COVID-19 cases during first wave of pandemic. By 15th June 2020, there were total 3,32,434 cases in India. Out of total cases, 1,53,106 were active cases, 1,69,797 patients had been cured and 9,520 had died due to COVID-19 [3]. Now India is witnessing rapidly spreading second wave of COVID-19 infection which again is posing a severe threat to public health and increased burden on healthcare system in the country. The COVID-19 cases declined in January-February 2021 to the lowest level (10,000-13,000 per day new cases) and then from March 2021, cases started to spike again. On March 31st, there were 72,330 new cases in India, highest surge since October last year.

During first wave, Maharashtra was the most affected state of India having 32.5% (1,07,958/3,32,434) of total cases in India contributing 41.5% (3,950/9,520) deaths out of total death in India due to COVID-19. In second wave also, Maharashtra is becoming the most affected state, contributing to around 50% of total cases of India. On March 31st, Maharashtra reported 39,544 new cases [3].

World Health Organisation (WHO) has recommended diagnostic modality for COVID-19 where Nucleic Acid Amplification Tests (NAAT), such as Reverse Transcription Polymerase Chain Reaction (RT-PCR) has been suggested and is used as a gold standard for the confirmation of COVID-19. RT-PCR detects viral genome envelope (E), RNA-dependant RNA polymerase genes and N gene which encode viral nucleocapsid phosphoprotein in clinical samples [4]. Other tests include radiological pulmonary imaging by Computed Tomography (CT), while various serum biochemical markers are being used for supporting diagnosis, monitoring and prognosis of the disease [5].

Several researches have been published recently on the newly emerged SARS-CoV-2 to elucidate the pathogenic mechanisms, epidemiological characteristics, diagnostic-prognostic biochemical markers and to identify potential drug targets, which will contribute to the development of effective prevention and treatment strategies [1,6,7]. Inflammation plays a role in pathogenesis of COVID-19 where release of pro-inflammatory cytokines followed by inflammasome activation produce lung injury [8].

Serum CRP, LDH, Interleukins, CK-MB, Troponins and coagulation marker D-dimer have been found to be useful to identify severe cases and predictor of mortality revealing important role of routine and inflammatory biochemical markers in the treatment and management of COVID-19 patients [9-11]. Studies had been published on Indian population for identifying potential inflammatory biomarkers, their

diagnostic/prognostic role and assessing their usefulness in Indian population. Those studies included sample population with different demographic and geographical characteristics and evaluated different serum inflammatory markers [12-14].

Conducting similar studies by taking sample subjects with different demographic and geographical characteristics will provide more evidences to verify the association of various inflammatory biomarkers with COVID-19 disease severity in Indian subjects.

Therefore, it makes sense to study inflammatory biomarkers which are useful for reflecting severity of COVID-19 in Indian setup and population. So, the present study was designed to evaluate association of inflammatory biochemical markers with severity of COVID-19. It may help in early identification of severe cases so that timely clinical interventions can by initiated and may also help in monitoring the effect of treatment on COVID-19 patients.

MATERIALS AND METHODS

Present retrospective observational study was conducted at the Department of Biochemistry, Seth GS Medical College and King Edward Memorial (KEM) Hospital, Mumbai, Maharashtra, India. KEM hospital is one of the hospitals responsible for the treatment for COVID-19 assigned by the government. The study protocol was approved by the Institutional Ethics Committee (IEC-II/OUT/459/2020).

Inclusion and Exclusion criteria: Data of patients with confirmed COVID-19 disease admitted to the centre from July 1st, 2020 to July 30th 2020, were collected using convenient sampling method. The posteriori (retrospective) strategy was used for data abstraction. All males and females admitted with COVID-19 disease having age >20 years were included in the study. COVID-19 patients with age <20 years were not included in the study.

Patients were diagnosed for COVID-19 based on the results of real time RT-PCR conducted at the centre. Clinical classification was according to the protocol developed by COVID Core Committee of Seth GS Medical College and KEM Hospital, Mumbai.

The clinical classification is as follows: (1) Mild (Early Infection)- It included asymptomatic but positive for COVID-19 patients and symptomatic patients with or without co-morbidity. Symptomatic patients were those having at least three symptoms out of four symptoms (fever, dry cough, shortness of breath and myalgia). (2) Moderate- It included patients having pneumonia with or without respiratory failure. (3) Severe- It included patients having pneumonia with respiratory failure, multiorgan dysfunction syndrome and systemic hyper inflammation with cytokine storm.

Data Collection

Electronic medical records were evaluated to identify mild, moderate and severe COVID-19 cases. Total 103 patients' data was used for analysis. Information about age, gender, co-morbidity, O_2 status mortality and values of biochemical parameters were noted and used for data analysis.

The values of biochemical parameters of COVID-19 patients were noted from laboratory reports of patients. For serum IL-6 and ferritin estimation, blood samples were collected in plain vacutainer and for plasma D-dimer estimation, blood samples were collected in citrate vacutainer. Serum IL-6 and serum ferritin were estimated by sandwich electrochemiluminescence immunoassay [15,16]. Plasma D-dimer was estimated by immunoturbidimetric assay [17].

STATISTICAL ANALYSIS

Statistics analyses were performed using Graph Pad Prism software. Categorical variables were displayed as frequency and percentage while continuous variables were expressed as mean and Standard Deviation (SD). Normality test of continuous variables was done using Kolmogorov-Smirnov test. Between groups comparison of categorical variables was done using Chi-square test. Independent t-test was used for Inter group comparisons of continuous variables. Pearson correlation coefficient was used for the variables of normal distribution. The p-value of <0.05 was considered statistically significant.

The ROC curve analysis was conducted to determine the optimal cut-off of parameters for identifying severe COVID-19 cases. For ROC curve analysis, mild and moderate COVID-19 patients were taken as control group and severe COVID-19 patients were taken as test group. An Area Under the Curve (AUC) value of 0.9-1.0 signified a perfect biomarker with excellent accuracy, 0.8-0.9 as very good, 0.6-0.7 as sufficient and a value of 0.5 signified it was no better than what would be expected by chance. The optimal cut-off value was the value that had the highest combined sensitivity and specificity.

RESULTS

Total 103 confirmed COVID-19 patients hospitalised at the centre were included in the study. Of the 103 patients, 67 (65.0%) had one or more co-morbidities and 53 (51.4%) were on various types of oxygen support. The study subjects were divided into two groups: Mild and moderate COVID-19 patients group (n=47) and severe COVID-19 patients group (n=56).

[Table/Fig-1] presents the comparison of demographic and clinical characteristics in both the groups. In mild-moderate COVID-19 group, 59.5% were male while in severe COVID-19 group 73.2% were male. Severe COVID-19 group patients were significantly older compared to mild-moderate COVID-19 group (p<0.0009) [Table/Fig-1].

Characteristics		COVID-19 positive patients		p-value (t-test)
		Mild-moderate (n=47)	Severe (n=56)	
Gender male/female		28/19	41/15	0.14
Age (Years)	Mean±SD	46.4±12.5	53.4±14.1	0.0009***
	Range	(27-72 years)	(20-83 years)	
	(11-20)	0	1	
	(21-30)	8	3	
	(31-40)	10	7	
	(41-50)	12	10	
	(51-60)	10	18	
	(61-70)	6	13	
	(71-80)	1	3	
	(81-90)	0	1	
Hypertension (n, %)			24 (42.8)	-------
Diabetes mellitus (n, %)			23 (41)	-------
Chronic Kidney disease (n, %)			9 (16)	-------
NP/FM/NRBM/NIV/IV (n)			4/3/11/23/1	-------
Mortality (N, %)			23 (41%)	-------

[Table/Fig-1]: Baseline demographic and clinical characteristics of study population. *p<0.05; **p<0.01; ***p<0.001; NS: Not significant; NP: Nasal prongs; FM: Face mask; NRBM: Non rebreather mask; NIV: Non invasive ventilation; IV: Invasive ventilation; Chi-square test; Age (years) (Mean±SD) and range

No significant difference was observed with regard to gender in both the groups. In both mild-moderate and severe groups, diabetes, hypertension and Chronic Kidney Disease (CKD) were the most common co-morbid conditions but severe COVID-19 group was having more co-morbidity; hypertension (42.8%) vs (36.1%); diabetes mellitus (41.0%) vs (36.1%) and CKD (16.0%) vs (8.51%).

Patients with severe COVID-19 disease required oxygen support more compared to mild-moderate group (75% vs 19.1%). The mortality rate reported in severe COVID-19 group was 41% while no death reported in non severe COVID-19 group.

Mean serum IL-6, serum ferritin and plasma D-dimer levels were significantly higher in severe COVID-19 group compared to non severe COVID-19 group (p<0.0001) [Table/Fig-2].

Characteristics	Normal range	COVID-19 positive patients				p-value (t-Test)
		Mild-moderate (N=47)		Severe (N=56)		
		(Mean±SD)	N%=Patients having value above normal range	(Mean±SD)	N%=Patients having value above normal range	
Interleukin-6	<7.0 pg/mL	26.7±46.6	31 (65.9)	1561.0±6015.0	53 (94.6)	<0.0001***
Ferritin	30-400 ng/mL	513.0±419.9	24 (51.0)	2386± 5051	48 (85.7)	<0.0001***
D-dimer	0.50 µg/mL FEU	1.1±1.4	19 (40.4)	4.1±4.4	44 (78.5)	<0.0001***

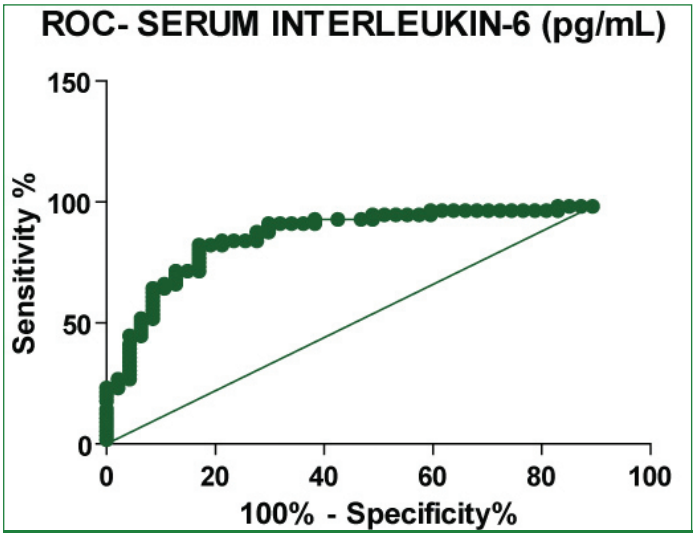
[Table/Fig-2]: Comparison of biochemical parameters in study population.
*p<0.05; **p<0.01; *** p<0.001

No significant correlation (p>0.05) could be observed between inflammatory parameters and age in total (n=103) COVID-19 patients group (IL-6 r=-0.04, ferritin r=-0.004 and D-dimmer r=0.11).

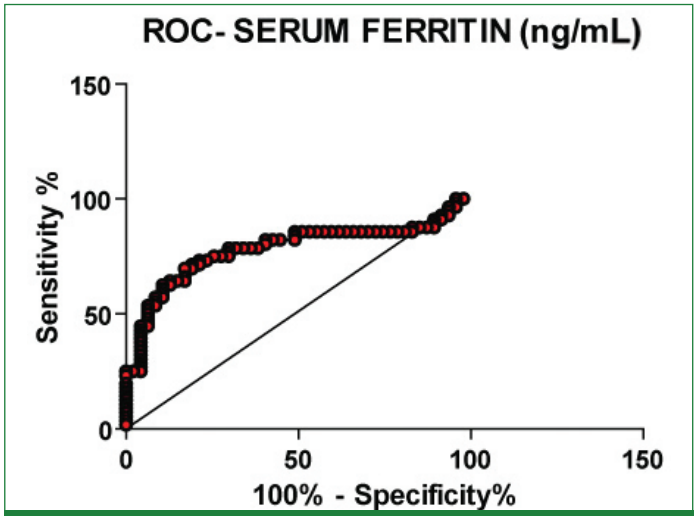
The ROC curve analysis was conducted for serum IL-6, serum ferritin and plasma D-dimer to detect severe COVID-19 positive cases. The best cut-off point for serum IL-6 was 20.2 pg/mL (sensitivity 91% and specificity 70.2%), for serum ferritin best cut-off point was 566.5 ng/mL; sensitivity 78.5% and specificity 70.2% and for plasma D-dimer best cut-off point was 0.9 pg/mL; sensitivity 72.2% and specificity 70.2% [Table/Fig-3-6].

Parameters	Cut-off	Area under the curve	Sensitivity	95% CI	Specificity	p-value (t-test)
Interleukin-6 pg/mL	>20.2	0.867	91%	0.7955 to 0.9390	70.2%	<0.0001
Ferritin ng/mL	>566.5	0.781	78.5%	0.6883 to 0.8740	70.2%	<0.0001
D-dimer ug/mL	>0.9	0.773	72.2%	0.6831 to 0.8638	70.2%	<0.0001

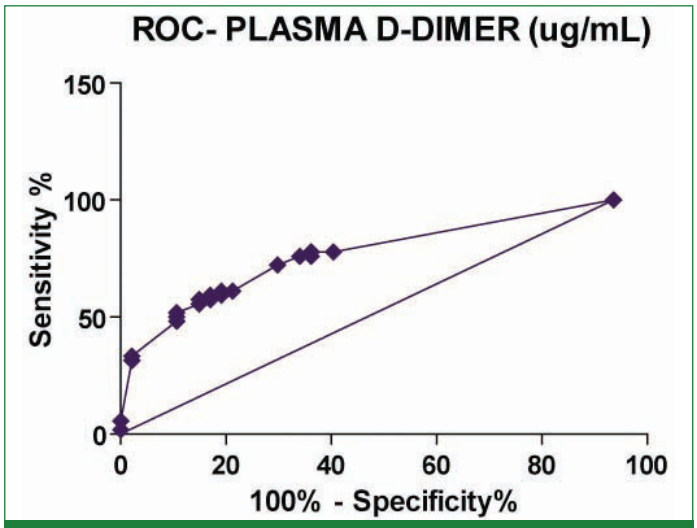
[Table/Fig-3]: ROC analysis of serum IL-6, serum ferritin and plasma D-dimer for indication of severe COVID-19 condition.
*p<0.05; **p<0.01; ***p<0.001; NS: Not significant; CI: Confidence interval



[Table/Fig-4]: ROC curve of serum IL-6 for indication between non severe and severe COVID-19 cases.



[Table/Fig-5]: ROC curve of serum ferritin for indication between non severe and severe COVID-19 cases.



[Table/Fig-6]: ROC curve of plasma D-dimer for indication between non severe and severe COVID-19 cases.

DISCUSSION

This study was an attempt to assess role of serum inflammatory markers to identify severe COVID-19 cases. For this, result of serum interleukin-6, serum ferritin and plasma D-dimer were compared between non severe and severe COVID-19 patient groups.

It was found that serum interleukin-6, serum ferritin and plasma D-dimer levels were significantly elevated in severe group compared to non severe group.

Aziz M et al., conducted meta-analysis for comparison of mean serum IL-6 in severe COVID-19 and non severe COVID-19 patients groups using seven studies and observed that the mean serum IL-6 was 56.8 (41.4±72.3 pg/mL) and 17.3 pg/mL (13.5±21.1 pg/mL) for severe and non severe COVID-19 group, respectively. This was statistically significant (mean difference: 38.6 pg/mL; p<0.001). The author suggested that IL-6 can guide the clinicians in recognising patients with severe COVID-19 early before appearing of clinical symptoms in the disease course [18].

Bhandari S et al., determined the serum IL-6 levels and its association with clinical presentation, severity, radiological imaging in 132 COVID-19 patients. They observed that patients with higher IL-6 levels (p<0.05) showed severe lung involvement in radiological findings (in terms of a digital chest radiograph), were on non invasive ventilation (p<0.001), and under intensive care unit (p=0.009) which was associated with greater mortality (p=0.046) [19].

Interleukins, a type of cytokine is not only produced by leukocytes but are produced by various other cells of the body. Interleukins perform important roles in immune cell's activation and differentiation. They are also essential for immune cell maturation, proliferation, migration, and cell adhesion. They are synthesised by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. However, in some infection diseases, excessive

inflammation activates cytokine storm, lead to serious pathological changes, even is responsible for multiorgan dysfunction [20].

The IL-6, a chemokine, is an important biomarker of inflammation and has been shown as an important predictor of severe COVID-19 [21]. It is responsible for elevation of acute phase reactants, such as C-reactive protein, serum amyloid A, fibrinogen and inhibition of albumin synthesis.

In another study conducted by Yao Y et al., where they assessed the use of D-dimer as a biomarker for COVID-19 severity and clinical outcome and observed significant higher median D-dimer levels in patients with mortality (n=17) than in survivors (n=231) {6.21 (3.79-16.01) mg/L versus 1.02 (0.47-2.66) mg/L, p=0.0001} indicating its association with increased odds of mortality [22]. D-dimer is a marker of fibrinolytic activity and is a degradation product of fibrin. It is produced when fibrin is cleaved by plasmin to break down blood clots. Plasma level of D-dimer is increased in various pathogenic or non pathogenic conditions when production of fibrin or its breakdown is increased [23]. Yu HH et al., in their meta-analysis found that severe COVID-19 patients had higher D-dimer levels than those with non severe COVID-19 patients {1.8 (0.9-4.6) vs 0.5 (0.3-1.1) µg/mL, p<0.001}, and the odds of COVID-19 severity was associated with D-dimer more than 0.5 µg/mL. The observed D-dimer elevation in COVID-19 patients might be associated with hyper fibrinolysis state, viral infection induced sepsis, coagulation dysfunction and increased inflammatory burden induced in 2019-nCoV infection [24].

High ferritin levels may indicate severe COVID-19 and predict poor outcome. Ferritin causes macrophages activation. When activated, macrophages begin to secrete cytokines. Elevated ferritin levels are reported to be due to secondary haemophagocytic lymphohistiocytosis and cytokine storm syndrome in severe COVID-19 patients [25].

Kirtana J et al., retrospectively studied the association between inflammatory bio markers and clinical presentation with progression of COVID-19 disease and concluded that patients with moderate disease were older {mean (SD): 57.33 (10.21) vs 36.13 (14.05); p=0.014} and had significantly higher inflammatory markers level (C-Reactive Protein 2.46 vs 0.20 (p=0.024), and Ferritin 306.15 vs 72.53 (p=0.023) compared to mild disease group [26].

Study by Ruan Q et al., identified predictors of fatality from a retrospective, multicentre study of 150 confirmed COVID-19 cases in Wuhan, China, and observed that ferritin was elevated in non survivors (mean 1297·6 ng/mL) compared to survivors (614·0 ng/mL) p<0·01 [27].

In this study, we assessed the association of inflammatory markers with age in COVID-19 patients and observed that although no correlation of inflammatory biomarkers could be observed with age but the mean age of severe COVID-19 group was significantly higher compared to non severe group and there was 41% mortality in severe group which indicates that higher age plays important role in disease severity and mortality. This can be explained by the age-dependent defects in T-cell and B-cell function. Poor outcome in older patients can be associated with excess type 2 cytokines production in older patients which could lead to a poor viral replication control and extended proinflammatory responses [9]. It is important to identify critically ill patients even earlier, aiming to reduce mortality and improve the recovery rate.

Co-morbidities such as diabetes, hypertension may play an important role in disease severity and poor prognosis of COVID-19 patients. In our study, diabetes and hypertension were present in both non severe and severe groups but the incidence were higher in severe group {diabetes mellitus (41.0%) vs (36.1%); hypertension (42.8%) vs (36.1%)}. Mishra Y et al., compared D-dimer levels of diabetes patients with non diabetic patients of COVID-19 infection and observed that diabetic patients had significantly higher D-dimer levels compared to non diabetic COVID-19 patients (1509±2420 ng/mL vs 515±624 ng/mL) [28].

It is likely that severe COVID-19 infection in diabetic patients make them more prone to develop coagulopathy as prolonged hyperglycemia induces endothelial dysfunction and inflammation which in turn can lead to thrombosis.

Limitation(s)

In the present study, though sufficient number of patients were included but patient data was collected retrospectively using convenient sampling method which may not be fully representative of the overall COVID-19 disease status.

CONCLUSION(S)

This study suggested that, serum IL-6, serum ferritin and plasma D-dimer can be helpful in monitoring of COVID-19 patients for early identification of severe cases which can be useful to reduce or prevent progression towards critical stage and mortality by using early clinical and therapeutical interventions based upon these biomarkers.

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