A Descriptive Study of C-Reactive Protein (CRP) and Interleukin-6 (IL-6) in COVID-19 Patients

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Abstract: _Background: In December 2019, Wuhan, China, announced the first COVID-19 case. The World Health Organization proclaimed it to be a pandemic because of its intensified outbreak. According to reports, the presentation symptoms included severe respiratory diseases with cold, cough, and nasal congestion. Serious illnesses arose in elderly individuals with debilitating medical conditions. Within a week after infection, symptoms began to appear in the affected patients. Pneumonia followed in the second or third week. Numerous biochemical parameters were changed. Pro-inflammatory indicators such as CRP and IL-6 were found to be increased, suggesting a potential for severe inflammation and cytokine storm. Materials and methods: This study analyzed the IL-6 and CRP levels in COVID-19 patients, to assess the prognosis of the disease in a tertiary care hospital in Bangalore. CRP & IL-6 levels of 106 COVID-positive patients were studied for a period of one 1year. Based on their levels, the subjects were categorized into 3 groups- Mild, Moderate & Severe. These levels were further correlated with the severity and prognosis of the disease. Result: The level of IL-6 and CRP in COVID-19 patients were monitored on admission. There was no significant difference in gender and age among the three patient groups. Levels of CRP and IL-6 were determined at the time of admission. All the patients showed high levels of CRP and IL-6. The level of IL-6 was comparatively higher than of CRP. Conclusions: Both IL-6 and CRP is widely obtaining marker that correlates with disease severity and mortality. However, IL-6 shows significantly higher. Our finding supports the utility of daily IL-6 and CRP value in hospitalization COVID-19 patients and provides early detection before admission that may facilitate risk stratification and prognostication._

Keywords: C-Reactive Protein; cytokine release syndrome; interleukin-6; intensive care unit; severe acute respiratory syndrome

1. Introduction

Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread worldwide and was declared a pandemic in early 2020.COVID-19 destroyed people's mental and physical health and staggered global economic growth. SARS-CoV-2 invades the host by angiotensin-converting enzyme 2 (ACE2) receptors broadly distributed on various tissues and immune cells. Viruses cause a wide range of clinical manifestations, from mild forms such as fever, cough, and myalgia to moderate forms requiring hospitalization (pneumonia and localized inflammation) to severe/ critical forms with fatal outcomes. Older people, those with medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer, developed serious illnesses. Severe or critical infection often manifests as pneumonia, disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), low blood pressure, and multiorgan failure. In symptomatic patients, with infection clinical manifestation of the disease started after less than a week, consisting of fever (body temp 37° C to 38° C), cough, nasal congestion, and fatigue. Followed by pneumonia in the second or third week of symptomatic infection, [1,2,3]. Many biochemical parameters were altered especially, IL-6 & CRP.

Interleukin 6 (IL-6) was recognized as a key mediator of cytokines release syndrome (CRS), a form hyperinflammation leading the to use of IL-6 receptor inhibitor [4]. Interleukin acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL 6 gene. IL-6 has widespread actions on the immune system, hemopoiesis, and inflammation. In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine [5]. IL-6's role as an inflammatory myokine is mediated through its inhibitory effects in TNF-alpha and IL-1 and its activation of IL-1 and IL-10. There is some early evidence that IL-6 can be used as an inflammatory marker for severe COVID-19 infection with a poor prognosis, in the context of the wider coronavirus pandemic.The plasma cytokine interleukin-6 (IL-6) plays an important role in mediating inflammation and is the central stimulus for acute phase response. IL-6 induces the hepatic synthesis of C-Reactive Protein (CRP), which is known as the pro-inflammatory marker [6].

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CRP is produced by the liver. Level rises when there is inflammation in your body. This damage causes inflammation that the body tries to heal by sending a "response team" of protein called "acute phase reactants". CRP level is a better indicator of cardiovascular disease (CVD) than the LDL test[7]. Elevated levels of IL-6 may be an early indicator that a patient is at risk of cytokine storm and acute respiratory distress. Measuring IL-6 levels may help assess disease progression and help develop appropriate treatment plans. CRP serum levels can predict the severity and progression of illness in patients with COVID-19[8]. The objective of the study was to determine CRP & IL 6 Levels and assess the usefulness of these in the early stages of COVID 19 by correlating them with lung lesions and progression of severity of disease in patients.

2. Materials and Methods

Study Design: A descriptive study was conducted using the medical record data.

Sample Size: Medical records data of 100 COVID-infected patients admitted in Tertiary care Hospitals analyzed for a period of one year.

Study Period: One year, January 2021 to January 2022.

Source of Data: Reports of blood samples received for the measurement of various biochemical parameters in the clinical biochemistry laboratory of St. John's Medical College Hospital during treatment for COVID 19. LIS data of RT PCR positive patients were retrieved and CRP & IL-6 values along with CT Score and other biochemical values during the study period of at least 1yr was analyzed

Inclusion Criteria:
RT PCR, Rapid Antigen test (RAT), Gene Xpert, POCT positive patient in the age group of 18 to 65 admitted to St. John's Hospital. All the patients admitted to St John's Hospital, Bangalore with a PCR- positive pharyngeal swab test for SARS-CoV-2 infection were included in the study. Blood samples were drawn at admission and for routine monitoring and treatment purposes on the remaining days for hospitalization. Blood samples were centrifuged at 2,500 rpm at 37°C for 5min and residual EDTA-plasma were stored at minus 80°C and thawed before analysis.

Exclusion Criteria: RT-PCR Negative & and patients with no symptoms of COVID.

Study Design:
Lab data of patients with confirmed SARS-CoV-2 infection are retrieved and evaluated. CRP & IL-6 and CT scores were measured during admission. Based on these patients were categorized into Mild, Moderate and Severe. Mild patients showed fever, cough, myalgia, Moderate patients showed fever, respiratory symptoms, imaging findings of pneumonia. Severe patients showed respiratory distress, required mechanical ventilation, shock, combing other organ distress. Each category compared and correlates the IL-6, CRP, and CT findings, and timely monitored over the period of admission. The necessary data were obtained from the

MRD- data includes demographic data (age, gender), laboratory values (IL-6, CRP), past medical history (hypertension, diabetes mellitus, coronary heart disease, heart failure, obesity chronic kidney disease) was obtained from participants. All subjects with less than 50% of missing data were collected. The samples were analyzed on ARCHITECT c 8000, Roche based on Immunochemical methods. Samples received during the study period was reviewed in the Department of Clinical Biochemistry, St John's Medical College Hospital, Bangalore.

Statistical Analysis:
Data were analyzed using STATA software version 16. All categorical data was presented using frequency and percentages, all continuous data were described using median and inter quartile ranges based on the distribution. To assess the relationship of CRP and IL-6 with COVID severity, the Kruskal Wallis test was applied after checking the normality assumption. The P-value was considered significant at a 5% level of significance for all comparisons.

3. Result

A total of 100 COVID-19 patients were admitted during the study period January 2020 to January 2021. Out of a total 100 of patients, 75 patients were Male and 25 were Female. Most of the patients were between the age of 18 and 65 years. Most cases experienced influenza-like symptoms such as fever, cough, and myalgia during their time at the hospital. Diabetes mellitus present in 11 out of 100 patients, was the most common comorbidity followed by hypertension present in 13 patients and both diabetes mellitus and hypertension present in 13 patients. All patients were discharged following recovery of clinical symptoms.

The baseline characteristics are shown in Table 1.

Table 1: For Baseline Characteristics

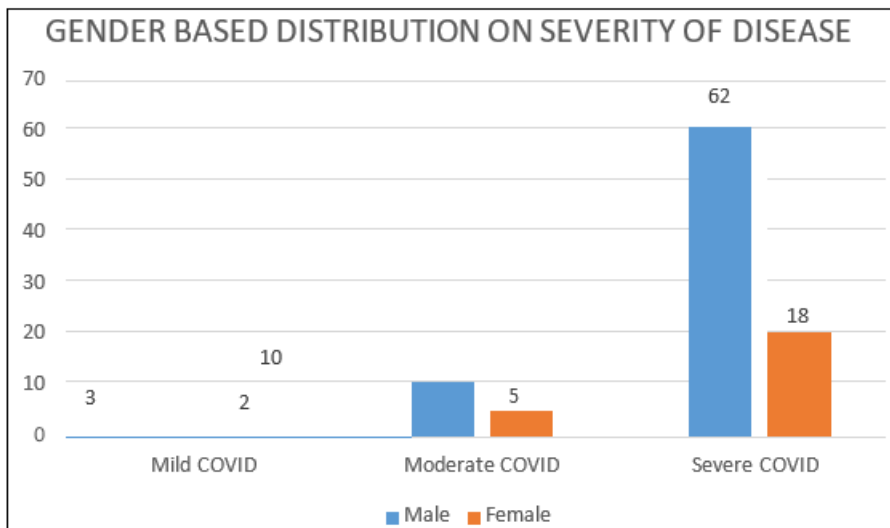| Base Line characteristics	N	Percentage (%)
Gender		
Male	75	75%
Female	25	25%
Comorbidities		
Diabetes Mellitus		
Present	11	11%
Absent	89	89%
Hypertension		
Present	12	12%
Absent	87	87%
DM & Hypertension		
Present	12	12%
Absent	87	87%
Discharge Status		
Expired	43	43%
Improved	44	44%
Recovered	8	8%
Unchanged	5	5%
Final Diagnosis		
Mild COVID	5	5%
Moderate COVID	15	15%
Severe COVID	80	80%

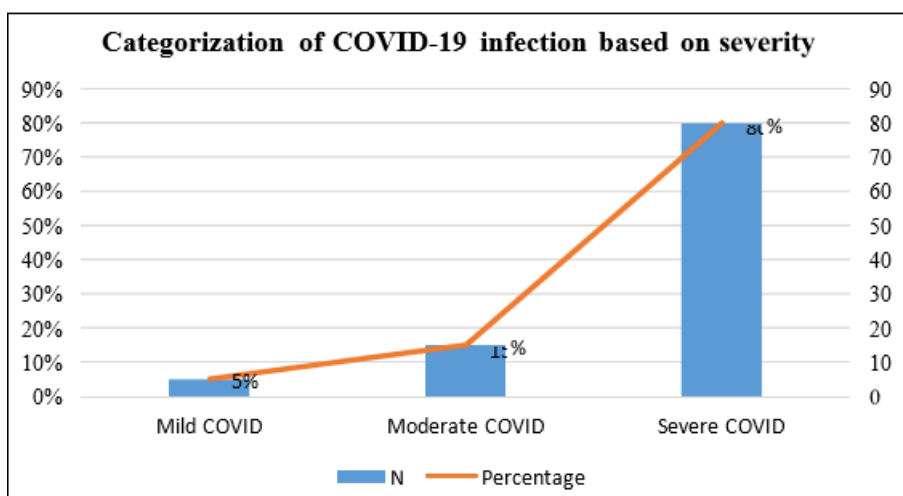**Figure 1:** Gender-Based distribution on Severity of disease

Figure 2: Categorization of COVID-19 infection based on the severity

Table 2: Findings in COVID-19 patients

Baseline	Mean/Median	SD/IQR
Age (Mean)	47.56	13.12
CRP	8.48	(4.22,12.22)
IL-6	36.175	(17.9,110.94)
CT Score	15.88	5.59

Table 3: Association between gender and COVID 19 status

Gender	Mild COVID (n%)	Moderate COVID (n%)	Severe COVID (n%)	p-value
Male	3 (4%)	10 (13.33%)	62 (82.67)	0.436
Female	2(8%)	5 (20.00%)	18 (72.00%)	

p-value was obtained using chi-square/fisher exact test.

There was no association between gender and covid-19 status found as per the data collected.

Table 4: Association between biochemical parameters indifferent categories of covid (CRP, IL-6, CT score)

	Mild COVID Mean (SD) Median (IQR)	Moderate COVID Mean (SD) Median (IQR)	Severe COVID Mean (SD) Median (IQR)	p-value
Age	45.8 (13.498)	53.33(13.890)	46.58(12.849)	0.412
CT Score	7.5(0.707)	11.77(3.456)	16.65(5.487)	0.065
CRP	4.72(4.72,12.86)	1.71(0.5,8.26)	8.81(5.58,12.35)	0.043**
IL-6	7.9(2.94,25.5)	34.68(1.5,58.12)	8.81(5.58,12.35)	0.039**

p value was obtained using Anova/Kruskal Wallis test.

There was a significance in CRP and IL-6 with covid-19 status.
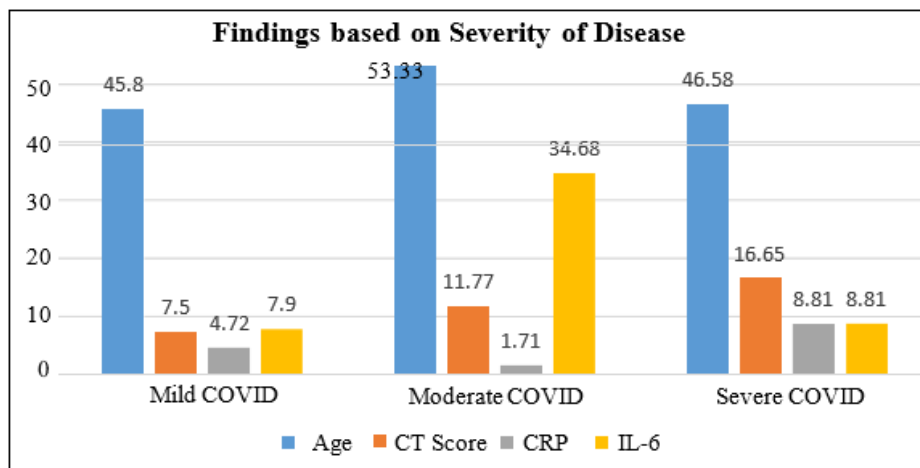
Graph 3: Association between biochemical parameters in different categories of COVID 19(CRP, IL-6&CT SCORE)

Table 5: Correlation of CRP and IL-6 with CT score

	Status	N	Correlation value	P value
CRP	Moderate	9	-0.330	0.385
	Severe	70	0.052	0.669
IL-6	Moderate	9	0.271	0.480
	Severe	70	0.605	<0.001

p-value obtained using Spearman correlation.

There was negative mild correlation between CRP and Moderate COVID status which is statistically not significant. There was Mild correlation between CRP and Severe COVID status which is statistically not significant. There was Mild correlation between IL-6 and Moderate COVID status which is statistically not significant. There was Moderate correlation between IL-6 and Severe COVID status which is statistically significant.

4. Discussion

A total of 100 COVID-19 patients were admitted to Tertiary Care Hospital, Bengaluru from January 2020 to January 2021. Cases were between the age of 18 and 65 years. Approximately 25 of the patients were female and 75 were male. Most cases experienced influenza-like symptoms such as fever, cough, and myalgia during their time at the hospital. All patients were discharged following recovery of clinical symptoms. Diabetes mellitus, present in 11 out of 100 patients, was the most common comorbidity, followed by hypertension present in 13 patients and both diabetes mellitus and hypertension present in 13 patients.

We found that the severe and critical COVID-19 patients had significant, IL-6 levels that could effectively discriminate COVID-19 severity. The level of IL-6 increased during COVID infection indicates an acute phase inflammatory cytokine in serum level and it reflects the degree of lung inflammation. Several pieces of evidence indicated IL-6 as one major cytokine that takes hyperinflammation in COVID-19 infection. CRP was an independent risk factor rate in patients with COVID-19. According to a study by Herold T et al. elevation of IL-6 was found very strongly with the need for mechanical ventilation as well as a comparison of the levels observed in patients with septic shock predicts that elevated IL-6 level above 80pg/ml are sufficient to identify COVID-19 with risk of respiratory failure and to be represented as a biomarker of severe COVID-19 parameter and helps physicians to correctly allocate patients at early stage.[9]

Study conducted by Wang M et.al showed that serum concentrations of IL- 6 and CRP are higher in patients with depressive SARS-CoV-2 can directly attack the heart, leading to myocardial cell apoptosis and necrosis. For critical patients- the systemic viral load was larger, the direct damage to myocardium was greater. CRP and IL-6 in the critically ill group were higher than those in the severe group, the lung damage usually more serious for critical patients, the inflammatory response and myocardial hypoxia were greater for critical ill patients. [10].

Chen X et.al during a study conducted at Wuhan, China observed that increased IL- 6 level were observed in critical ill patients were more severe patients and all deaths where extremely high IL-6 value and which suggesting that IL-6 shows an important biomarker to progress the poor prognosis of COVID-19 patients and high level of IL- 6 is hallmark and important force of cytokine storm which cause multiple organ dysfunction. The laboratory data analysis strongly suggests that level of inflammatory cytokine IL-6 critically ill patients was significant elevated [12].

Study coounducted by Wolszczak-Biedrzycka et al concluded that IL6 is a significant predictor of mortality in COVID-19 patients with acute respiratory failure. Increased Th1 lymphocytes secreting GM-CSF and IFN-γ, along with elevated levels of CD14+ and CD16+ monocytes secreting excessive IL6, are associated with the cytokine storm.[30]

Jensen et.al performed an international, prospective cohort study examining the trajectories of virological and immunological biomarkerss in the hospitalized COVID 19 patients. They found that unfavorable 5 day biomarkers trajectories particularly for plasma nucleocapsid antigen CRP & IL-6 were associated with worse clinical outcomes and increased 90 days mortality.[31]

Current study aimed to evaluate the role of laboratory markers at the time of diagnosis in estimating the severity and predicting the prognosis of COVID-19. IL-6 showed it to be a useful marker for early recognition of the severe disease, it showed significant predictive power even after adjustment to different models that include clinical laboratory significant parameters. So, IL-6 can have considered as an independent predictor of COVID-19 severity. There are some limitations as it was single-center design and retrospective nature at a tertiary care hospital, where severe cases were more compared to mild and

moderate, along with data obtaining being a difficult task from the elderly. We observed a more severe course of COVID-19 disease and a higher mortality rate of a higher percentage of patients at the beginning of the pandemic than present.

5. Conclusion

The present study suggests that CRP and IL-6 could be routinely used as biomarkers for progressive and severity assessment of COVID-19. Both IL-6 and CRP is widely obtaining marker that correlates with disease severity and mortality. However, IL-6 shows significantly higher. Our finding supports the utility of daily IL-6 and CRP value in hospitalization COVID-19 patients and provides early detection before admission that may facilitate risk stratification and prognostication.

Ethics: The project was approved by the Institutional Ethics Committee (I.E.C), SJMC. Study Ref No.239/2022.

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Declaration Of Competing Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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