

Original Article

Serum D-dimer and C-reactive Protein Levels in Mild, Moderate and Severe COVID-19 Patients

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Abstract

Background: The COVID-19 pandemic, caused by severe acute respiratory syndrome corona virus 2 (SARS-COV-2), typically spreads via respiratory droplets and during close contact. The main clinical manifestation is lung injury. Most of the patients have favorable prognosis, but in some cases it rapidly progresses to severe and critical forms with respiratory distress syndrome, coagulation dysfunction, septic shock, metabolic acidosis, multiorgan failure etc. Therefore, early detection of the severity is very important for the clinical management and treatment of COVID-19 patients. Commonly used clinical laboratory coagulation indices are D-dimer and CRP (C-reactive protein) to assess ongoing inflammation. **Objective:** To investigate any alteration of serum D-dimer and CRP levels in mild, moderate and severe COVID-19 patients. **Materials and Methods:** This cross sectional study was carried out in the Department of Biochemistry, Dhaka Medical College & Hospital, Dhaka during the period of January 2020 to December 2020. A total of 80 subjects were selected, who were diagnosed as COVID-19 confirmed by RT-PCR with the age ranging from 18–65 years (both male and female) from the COVID unit of Dhaka Medical College Hospital, Dhaka. These diagnosed COVID-19 patients were divided into three groups—mild (Group A1), moderate (Group A2) and severe (Group A3). Forty age and gender matched non-COVID admitted individuals in medicine department of Dhaka Medical College Hospital, Dhaka were enrolled as comparison group (Group B). Informed written consents were taken from the study subjects, relevant physical examinations were done and venous blood samples were collected for estimation of serum D-dimer and CRP levels. The results were compared statistically among groups. **Results:** There were increase in the serum D-dimer and CRP levels in COVID-19 patients but the serum D-dimer and CRP levels were significantly increased in severe COVID-19 patients when compared with mild and moderate groups. **Conclusion:** The findings suggest that D-dimer and CRP levels can be used to detect the severity of COVID-19 patients.

Key words: COVID-19; D-dimer; C-reactive protein

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Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by a novel coronavirus, severe acute respiratory syndrome virus 2 (SARS-CoV-2). SARS-CoV-2 is an enveloped, single stranded RNA virus belonging to the Coronaviridae family. When a person with this infection coughs, sneezes or talks can infect another person if it makes direct contact with the mucous membrane via respiratory droplets. Infection can also occur if a person touches an infected surface and then touches his or her eyes, nose or mouth. Droplets typically do not travel more than six feet.¹ The COVID-19 outbreak started in Wuhan, China in late 2019 and rapidly spread to the rest of the world.² It has contributed to significant mortality in several countries.³ Case fatality rate is extremely higher in countries with older-aged populations.⁴ This novel member of human coronavirus is officially named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by International Committee on Taxonomy of Viruses.⁵ On 11 March 2020, the World Health Organization declared COVID-19 outbreak as pandemic.²

Coronavirus was first isolated from human airway epithelial cells.³ COVID-19 is usually characterized by lower respiratory tract symptoms with fever, dry cough and dyspnea, a manifestation similar to those of two other diseases caused by coronavirus: severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome.^{6,7}

Beyond isolated pulmonary disease, COVID-19 has been associated with significant inflammation leading to neurologic, cardiovascular, coagulation, and other end-organ manifestations. Identifying markers of disease severity may therefore help to identify patients at risk of death or prolonged intensive care.⁸

D-dimers are one of the fragments produced when plasmin cleaves fibrin to breakdown clots. Healthy individuals have low levels of circulating D-dimer whereas elevated levels are found in condition associated with thrombosis. D-dimers provide global marker of activation of the coagulation and fibrinolytic system.⁹ About 50% COVID-19 patients have increased D-dimer levels associated with poor prognosis.⁶

It is particularly noteworthy that some severe, critical and deceased patients have significant coagulation dysfunction. Upon SARS-CoV-2 entering the body through angiotensin converting enzyme 2 (ACE 2) receptor located on the surface of mucosal epithelial cells, its pathogen-associated molecular pattern (PAMP) can be quickly recognized by immune system and immune system gets activated to clear the virus. However overacted immune response can cause a cytokine storm, causes vascular endothelial damage which activates the coagulation system and inhibits the fibrinolytic and anticoagulation systems. So excessive thrombosis in the microvascular system leads to DIC (disseminated intravascular coagulation) and ultimately, microcirculatory disorder and serious multiorgan dysfunction. So D-dimer is commonly used as a laboratory coagulation indicator.¹⁰

C-reactive protein (CRP) is a widely used diagnostic marker which is used to assess ongoing inflammation. It is a key protein of acute phase response, synthesized from liver, appears in blood within 6-10 hours of any tissue damaging event, has a plasma half life of 19 hours and is produced without a memory response.¹¹ Its concentration decreases when inflammatory stages end and the patient is healing.¹²

Approximately 20% of patients infected with SARS-CoV-2 (COVID-19) develop potentially life threatening pathologies involving acute inflammation, cytokine storm specially elevated level of TNF- α (tumor necrosis factor- α), IL-1 (interleukin-1) and IL-6 (interleukin-6). Elevated IL-6 levels, among its many activities, signals the liver to increase the synthesis and secretion of CRP.¹¹ The elevated levels of CRP are linked to the overproduction of inflammatory cytokines in severe patients with COVID-19. Cytokines fight against the microbes but when the immune system becomes hyperactive, it can damage lung tissue. Higher CRP indicates more severe disease course-linked to lung injury and worst prognosis. It suggests that CRP can be a serum marker of disease aggravation in COVID-19 patients. If there is one unit increase in CRP level, there is risk of developing severe events about 5%.¹³

Coagulation indicator D-dimer should be monitored as early as possible in order to detect thromboembolic complication and should be used as a biomarker of disease severity to decrease the morbidity and mortality of COVID-19 infected patients. Elevated CRP level can also be a valuable marker to predict the possibility of aggravation of nonsevere COVID-19 patients.

Materials and Methods

This cross-sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka during the period of January 2020 to December 2020. For this study, a total number of 80 COVID-19 patients from COVID unit of Dhaka Medical College Hospital were selected. They were divided into three groups—25 were mild (Group A₁), 25 were moderate (Group A₂) and 30 were severe (Group A₃). COVID-19 infection means a person with laboratory confirmation of COVID-19 infections, irrespective of clinical signs and symptoms. Forty age and gender matched non COVID admitted individuals in medicine department of Dhaka Medical College Hospital, Dhaka were enrolled as comparison group (group B). **Mild cases:** The clinical symptoms are mild and there is no sign of pneumonia on imaging. Symptoms may be fever, cough, sore throat, malaise, headache, muscle pain without shortness of breath or abnormal imaging. **Moderate cases:** Fever and respiratory symptoms with radiological findings of pneumonia. Respiratory distress with <30 breaths/min and Pulse oxymetry showing saturation >93% at ambient air. **Severe cases:** Cases meeting any of the following criteria: i) Respiratory distress (≥ 30 breaths/min); ii) Finger oxygen saturation $\leq 93\%$ at rest.¹⁴ For collection of specimen, recommended personal protective equipment (PPE), which includes an N95 mask or higher level of respirator (or facemask if a respirator not available), goggles for eye protection, gloves and gown were used. After all aseptic precautions 5 mL of venous random blood sample was collected from each study subject in a disposable plastic syringe and immediately transferred to a dry clean test tube which was allowed to clot at room temperature and clear serum was separated into a sterile microcentrifuge tube. Samples were separated after centrifuging at

3000 rpm for 10 minutes and the separated sera were used for biochemical assay or were stored at -200C if the analysis was delayed. Serum D-dimer was estimated by immunofluorescence assay and serum C-reactive protein (CRP) level was estimated by immunoturbidimetric method. Patients with positive polymerase chain reaction (PCR) test result of SARS-CoV-2 of both gender were included in the study. Patients aged below 18 years, COVID-19 patients admitted in ICU, patients with history of malignancy, lupus, MI, rheumatoid arthritis, liver failure, cirrhosis and pregnant women were excluded. Continuous variables were expressed as median and IQR (Interquartile range) and compared among groups of subjects by ANOVA test or Kruskal Wallis test as appropriate. Level of significance was defined as 'p' value < 0.05 at 95% confidence interval (CI). All analysis was done using the SPSS 20.0 (Statistical Package for Social Science).

Results

The cross sectional study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka. The study was aimed to estimate serum D-dimer and CRP levels in mild, moderate and severe COVID-19 patients. For this study, a total number of 120 subjects were selected, among which 80 were COVID-19 patients divided into three groups—25 were mild (Group A₁), 25 were moderate (Group A₂) and 30 were severe (Group A₃) COVID-19 patients from COVID unit of Dhaka Medical College Hospital, Dhaka). Forty age and gender matched non-COVID admitted individuals in medicine department of Dhaka Medical College Hospital, Dhaka were enrolled as comparison group (group B). Outcome variables (serum D-dimer and serum CRP) were estimated. Results are presented by tables and figures.

Table I shows distribution of the study subjects according to age and gender. Male gender was predominant among the groups. Table II shows physical examination findings of the study subjects including temperature, respiratory rate and oxygen saturation among the groups. Significant differences were found among the groups in respect of temperature, respiratory rate and oxygen saturation.

Table I: Distribution of the study subjects according to age and gender

	Group A ₁ n=25	Group A ₂ n=25	Group A ₃ n=30	Group B n=40	p values
Age (years)					
≤30	2 (8.0)	1 (4.0)	1 (3.3)	2 (5.0)	^a 0.598
31 - 40	6 (24.0)	4 (16.0)	1 (3.3)	5 (12.5)	
41 - 50	4 (16.0)	6 (24.0)	4 (13.3)	9 (22.5)	
51 - 60	10 (40.0)	9 (36.0)	14 (46.7)	17 (42.5)	
>60	3 (12.0)	5 (20.0)	10 (33.3)	7 (17.5)	
Mean ± SD	48.12 ± 10.97	50.64 ± 9.94	54.16 ± 12.21	51.72 ± 9.89	^b 0.221
Gender	Male	18 (72.0)	17 (68.0)	22 (73.3)	^a 0.975
	Female	7 (28.0)	8 (32.0)	8 (26.7)	

Figures within parentheses indicate percentage. ANOVA test was done to measure the level of significance.

Table II: Physical examination findings of the study subjects

General physical examination	Group A ₁ n=25	Group A ₂ n=25	Group A ₃ n=30	Group B n=40	p values
Temperature (°F)	100.56 ± 0.82 (99–102)	100.52 ± 1.12 (98–102)	100.63 ± 1.16 (98–103)	98.00 ± 0.00 (98–98)	<0.001
Respiratory rate (breaths/min)	18.88 ± 2.09 (16–22)	25.32 ± 8.30 (16–38)	34.07 ± 1.95 (31–38)	16.80 ± 1.67 (14–20)	<0.001
Oxygen Saturation (%)	97.68 ± 0.75 (96–99)	93.52 ± 5.01 (83–99)	88.13 ± 2.30 (81–91)	98.03 ± 1.27 (95–100)	<0.001

ANOVA test was done to measure the level of significance.

Table III shows post Hoc test was done to find out the level of significance which revealed the significant differences in case of temperature between group B – A₁, B – A₂, and B – A₃. Respiratory rates had

significant differences between group B – A₂ and B – A₃. Oxygen saturations had significant differences in all groups except group B – A₁.

Table III: Post Hoc test of physical examination

General physical examination	B – A ₁	B – A ₂	B – A ₃	A ₁ – A ₂	A ₁ – A ₃	A ₂ – A ₃
Temperature (°F)	<0.001	<0.001	<0.001	1.000	1.000	1.000
Respiratory rate (breaths/min)	0.303	<0.001	<0.001	1.000	1.000	1.000
Oxygen Saturation (%)	1.000	<0.001	<0.001	<0.001	<0.001	<0.001

Bonferroni test was done

Table IV shows comparison of serum CRP and D-dimer among the groups. Highest median and IQR of serum CRP and D-dimer were found in group A₃. Table V shows the exact levels of

significance. Statistically significant differences of CRP levels were found in all groups except group B – A₁. Serum D-dimer levels had significant differences in all groups except B – A₁ and A₂ – A₃.

Table IV: Median values with IQR in parentheses of serum CRP and D-dimer of the study subjects (N=80)

Lab parameters	Group A ₁ n=25	Group A ₂ n=25	Group A ₃ n=30	Group B n=40	p values
C- reactive protein (mg/L)	6.00 (6–12)	12.00 (6–37.70)	22.00 (12–48)	6.00 (6–10)	<0.001
D-dimer (µg/mL)	0.30 (0.10 – 0.81)	0.94 (0.12–7.60)	4.11 (1.07–8.66)	0.21 (0.08–0.92)	<0.001

Kruskal-Wallis test was done to measure the level of significance.

Table V: Post Hoc test results of serum D-dimer and CRP

Lab parameters	B – A ₁	B – A ₂	B – A ₃	A ₁ – A ₂	A ₁ – A ₃	A ₂ – A ₃
C- reactive protein	1.000	<0.001	<0.001	0.002	<0.001	0.003
D-dimer	1.000	<0.001	<0.001	0.001	<0.001	0.062

Bonferroni test was done,

Table VI shows correlation of serum C-reactive protein (CRP) with temperature, respiratory rate and oxygen saturation. A significant positive correlation between CRP and respiratory rate (Fig 1) and a significant negative correlation between CRP and oxygen

saturation (Fig 2) was found in moderate COVID-19 patients. Table VII shows no significant correlation of serum D-dimer with temperature, respiratory rate and oxygen saturation in mild, moderate, severe COVID-19 patients.

Table VI: Correlation of serum C-reactive protein with temperature, respiratory rate and oxygen saturation

	Mild		Moderate		Severe		Controls	
	r	p values	r	p values	r	p values	r	p values
Temperature (°F)	-0.128	0.543	0.109	0.604	-0.305	0.101	0.040	0.860
Respiratory rate (breaths/min)	0.248	0.232	0.512	0.009s	0.181	0.338	-0.013	0.935
Oxygen saturation (%)	-0.214	0.303	-0.489	0.013s	0.070	0.714	0.252	0.116

Pearson's correlation test was done

Table VII: Correlation of serum D-dimer with temperature, respiratory rate and oxygen saturation

	Mild		Moderate		Severe		Controls	
	r	p values	r	p values	r	p values	r	p values
Temperature (°F)	-0.211	0.311	0.100	0.633	-0.217	0.248	0.055	0.736
Respiratory rate (breaths/min)	0.079	0.707	0.221	0.288	-0.071	0.708	-0.010	0.953
Oxygen saturation (%)	-0.036	0.866	-0.352	0.085	-0.124	0.514	0.237	0.140

Pearson's correlation test was done

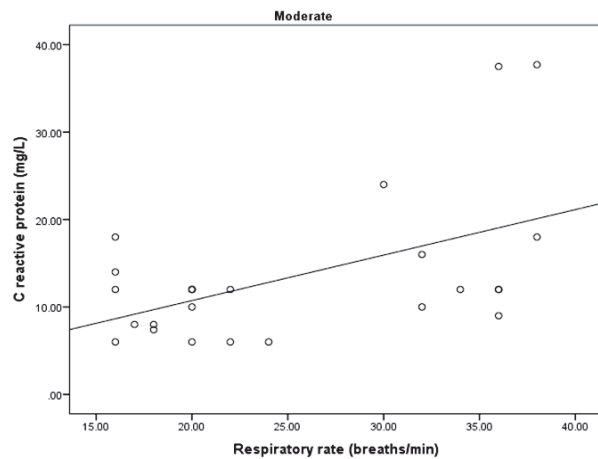


Fig 1. Scattered diagram showing positive correlation of serum C-reactive protein with respiratory rate in moderate COVID-19 patients

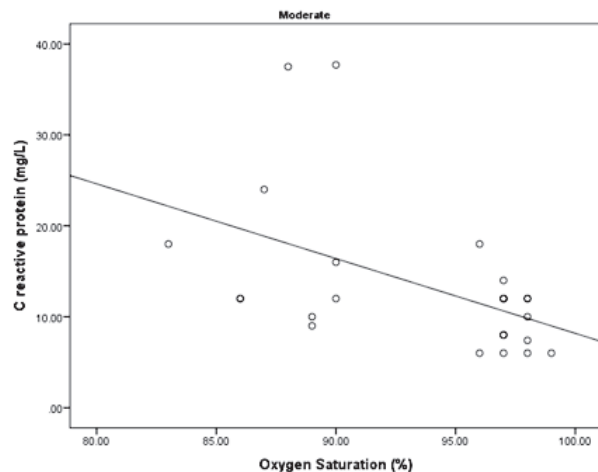


Fig 2. Scattered diagram showing negative correlation of serum C-reactive protein with oxygen saturation in moderate COVID-19 patients

Discussion

This cross-sectional study was carried out with the aim to estimate serum D-dimer and CRP levels in mild, moderate and severe COVID-19 patients. A total of 80 RT-PCR positive COVID-19 patients were selected on the basis of inclusion and exclusion criteria from the COVID unit of Dhaka Medical College Hospital, Dhaka. Among them 25 were mild, 25 were moderate and 30 were severe COVID-19 patients labeled as group A₁, A₂ and A₃ respectively. Age and sex matched 40 non-COVID adult individuals were taken as comparison group and considered as Group B.

Mean age of the study subjects in group A₁, A₂, A₃ and group B were 48.12 ± 10.97 , 50.54 ± 9.94 , 54.16 ± 12.21 and 51.72 ± 9.89 respectively. The difference was not significant. Male gender was predominant in all groups. The gender difference between groups was found to be non significant. Long et al¹⁰ found in their study that the median age of the study subjects was 63.5 ± 13.86 years. The mean age of the study subjects is far away from that of this study. This may be due to different selection criteria.

In this study, mean $\pm$ SD of temperature was significantly higher ($p < 0.001$) in group A₃ compared to group A₁ and A₂. Wang et al¹³ evaluated that fever was present significantly higher in non-severe group which is not similar to this study. Respiratory rate was significantly higher ($p < 0.001$) in group A₃ compared to group A₁ and A₂.

The mean $\pm$ SD of oxygen saturation was less in group A₃ than in groups A₁ and A₂ which was found statistically significant ($p < 0.001$). Yao et al⁵ conducted a retrospective study over 248 COVID-19 cases in China and stated that severe patients were on oxygen therapy and invasive and noninvasive mechanical ventilation due to low oxygen saturation. This finding is consistent to the present study.

Median with IQR of D-dimer were higher in group A₃ against group A₁ and A₂. This result is statistically significant ($p < 0.001$). A case control study was done by Yao et al⁵ in Wuhan, China over 248 confirmed COVID-19 patients and they found higher D-dimer levels in those with critical illness which is similar to the current study. Li et al¹⁵ conducted a study among 279 confirmed COVID-19 patients in China and found elevated D-dimer in poor groups than ordinary groups. This is also similar to current study. Garcia-Olivé¹⁶ et al also found similar type of findings.

Median with IQR of CRP were significantly higher in group A₃ compared to group A₂ and A₁. A case series done by Wang et al¹³ at Changsa, China over 209 COVID-19 patients found significantly higher level of CRP than non-severe patients which is similar to the current study. Chen et al¹⁷ and Wang et al¹³ also found elevated CRP in severe patients of COVID-19 which is similar to this study. A retrospective cohort study was done by Sharifpour

et al⁸ over 268 COVID-19 patients in Atlanta and found elevated levels of CRP in patients who died compared to those who survived which is similar to the findings of the present study.

In this study, Pearson's correlation coefficient test was done to observe the relationship of CRP and D-dimer with temperature, respiratory rate and oxygen saturation in mild, moderate, severe COVID-19 patients and in comparison group. There were significant positive correlation between CRP and respiratory rate of moderate COVID-19 patients ($r=+0.512$; $p= 0.009$) and significant negative correlation between CRP and oxygen saturation of moderate COVID-19 patients ($r = -0.489$; $p = 0.013$) . No significant correlation was found in serum D-dimer with temperature, respiratory rate and oxygen saturation in mild, moderate, severe COVID-19 patients and in comparison group.

In the present study, higher levels of serum D-dimer and CRP were found in COVID-19 patients. But the higher levels of D-dimer and CRP were more significant in severe COVID-19 patients than in the mild and moderate patients. So the D-dimer and CRP can be valuable markers to predict the severe versus mild or moderate COVID-19 patients, which can help healthcare workers to identify risk at an early stage of disease for early treatment.

Although optimal care had been taken during every steps of the study, there were some limitations. The study populations were selected from only one hospital of Dhaka and samples were taken purposively; so there was chance of bias. And because of resource limitation, the study was conducted with small sample size.

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