

# Serum Levels of Interleukin-10 and D-Dimer as Biomarkers of Coronavirus Disease

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## Abstract

**Background:** Coronavirus 2019 is one of the most remembered pandemics, and it is regarded as one of the most significant occurrences of the twenty-first century. Clinical signs and symptoms, as well as reverse transcription-polymerase chain reaction (RT-PCR) test, are used to diagnose COVID-19. Several biomarker molecules are being researched for their potential utility in evaluating the severity and prognosis of severe acute respiratory syndrome corona virus-2 (SARS-CoV-2). This study was designed to assess the level of IL-10 and D-Dimer in patients who were suffering from COVID-19. **Materials and Methods:** This prospective study included 168 participants who were attending Imam Hussein Hospital in Karbala. They were divided into two groups (I & II) based on the result of RT-PCR test; IL-10 and D-Dimer were analyzed for studied groups. **Results:** The median age of 41.6 years, 86 males and 82 females. A noteworthy increase was observed in the level of IL-10 ( $9.803 \pm 0.44$ ) and D-Dimer ( $1511.534 \pm 192.56$ ) in the positive PCR group as compare with negative PCR group. **Conclusion:** The levels of IL-10 and D-Dimer can be utilized to estimate the severity and prognosis of COVID-19 patients, according to our findings. D-dimer levels, among these biomarkers, correspond more closely with severity and can be used as a solid prognostic indicator.

**Keywords:** COVID-19, SARS-CoV-2, IL-10, D-Dimer, RT-PCR.

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## INTRODUCTION

The world now infected with Corona Virus Disease 2019 (COVID-19), a virus produced by the beta-coronavirus SARS COV-2, which is one with pandemic potential, following the SARS and MERS epidemics. The illness broke out around the end of December in the Central Chinese province of Wuhan, and it's assumed that the marine food market was to cause, as it was abruptly shut down. [1]. On February 11, 2020, WHO stated SARS-CoV-2-related sickness Corona Virus Disease 2019. (COVID-19) [2]. Fever, cough, myalgia phlegm production, headache, hemoptysis, and diarrhea are all symptoms [3], in the early stages of a respiratory infection,

patients experienced rapid respiratory distress condition, acute respiratory failure, and other effects. [4] COVID-19 patient death processes are yet unclear, making it difficult to establish the appropriate treatment choices for patients. COVID-19 symptoms are induced by an inflammatory cytokine storm and a condition of hypercoagulability, both of which have different treatments. The cytokine storm phenomenon can be seen in a variety of viral and non-infectious disorders. It's thought to be the cause of COVID-19's acute lung injury. [5]. Lymphopenia, neutrophilia, dysregulation of monocytes, decreased type I interferon (IFN-I) response, and most significantly, cytokine storm are all thought to play a role in

pathophysiology of this disease. Excessive production of proinflammatory cytokines, which is connected to a poor prognosis, is known as a cytokine storm, and these condition activate numerous inflammatory signaling ways by their receptors on immune cells, leading to a variety of medical symptoms such as fever, vessel leakage disorder, disseminated intravascular clotting, acute respiratory distress pattern, and multi organs failure, that lead to death in severe cases [6].

Some of the cytokines found in severe cases of COVID-19 patients are interleukin-1 (IL-1), IL-2, IL-6, IL-10, tumor necrosis factor alpha (TNF), Interferon; granulocyte colony-stimulating factor (G-CSF), and monocyte chemo attractant protein-1 (MCP-1) [7, 8, 9]. These cytokines and chemokines then beginner more immune cells such as dendritic cells, monocytes, macrophages, neutrophils, and natural killer cells from the peripheral tissues, as well as activate adaptive immune cells (CD4+ and CD8+T cells), resulting in myelopoiesis and emergency granulopoiesis, aggravating lung and epithelial inflammation. Excessive production of systemic cytokines, such as IL-2, IFN-, and TNF-, stimulates macrophage activation and erythrophagocytosis, which leads to anemia. [10], Capillary leak syndrome and thrombosis can occur as a result of coagulation and vascular hemostasis abnormalities. [11].

IL-10 is a multifunctional cytokine that has the ability to both motivate and suppress the immune system. Macrophages, T-helper 2 cells, and B-cells all contribute to its growth. A number of immune responses have been found to be suppressed by IL-10 [12], and the levels of it were shown to be highly associated with IL-6 levels and other inflammatory indicators including C-reactive protein [13]. High levels of IL-10, like IL-6, have been related to poor outcomes in SARS COV-2 patients [14]. IL-10 is higher earlier in COVID-19 patients than IL-6 [14]. During long-term infection, anti-inflammatory molecules like IL-10 are produced to keep inflammation in check and maintain immunological homeostasis [15]. D-dimer is formed when fibrin is broken down by fibrinolytic activities, and high levels indicate a hypercoagulable condition and secondary fibrinolysis in the body, which is useful for thrombotic disease diagnosis. COVID-19 patients have been reported to be hypercoagulable [16]. In addition, people with severe COVID-19 had a 25% likelihood of getting venous thromboembolism (VTE), and 30% of COVID-19 patients had pulmonary embolism. [17, 18]. SARS COV-2 patients with ischemic stroke had higher D-dimer

levels in their blood [19]. In light of the foregoing, our research focuses on determining the levels of IL-10 and D-Dimer in both positive and negative PCR (SARS-CoV-2) people. To get a clearer idea of their involvement in Corona virus disease-19 development and severity.

## PATIENTS AND METHODS

### Patient Selection

The study included 168 individuals (86 males and 82 females) who were admitted to the Imam Hussein Hospital in Karbala between December 2020 and June 2021 and were all anticipated having COVID-19. The participants in this study were all admitted to the hospital with signs and symptoms of COVID-19, and were confirmed in the lab using RT-PCR, using a method outlined by the manufacturing businesses in the accompanying booklet, and the findings of PCR, they were classify into two groups. Where PCR was positive in Group I (patients with COVID-19), but negative in Group II (patients without COVID-19) (patients with another respiratory diseases).

### Laboratory Testing

All participants in this study gave blood samples to be tested for IL-10 and D-dimer levels. After signing the informed consent form, whole blood sample was drawn via clean venipuncture. Serum was obtained by blood centrifugation 3500 rpm for ten minutes at room temperature, then freezing it until it was used for biomarker analysis. The Maglomi device used for D-Dimer and ELISA methods (Elabscience Company, china) were used to assessment IL-10 levels in both groups.

### Statistical Analysis

Mean and standard deviation were determined. The letter n was used to denote categorical variables (percent). An independent two-sample T-test was used to analyze the obtained data. The receiver operator (ROC) curve was used to estimate the correctness of IL-10 and D-dimer as predictors of mortality. The ROC curve was used to compute the area under the curve, sensitivity, and specificity, so (SPSS v.25) was used for bio statistical analysis at p-value equal or less than 0.05.

## RESULTS

This study included 168 patients (86 males and 82 females) with a median age of 41.6 years. The patients were divided into two categories based on PCR results: group I had positive PCR and group II had negative PCR. Group I had a median age of 43.7 years, while group II had a median age of 39.2 years, and the (20-40) age group is the largest in both groups. In terms of sex ratio, group I has 45 (52.3%) males and 41 (47.7%) females, while group II has 44 (53.7%) males and 38 (46.3%) females, and there is no significant differences between them as seen in table (1).

Table 1. Illustrate age and gender distribution in studied groups

Characters		PCR		P-value
		Positive	Negative	
	Median age (year)	43.7	39.2	
Age groups	<20	6	9	0.4
	20-40	37	31	
	40-60	25	26	
	>60	14	20	
Gender	Male	45 (52.3%)	44 (53.7%)	0.5
	Female	41 (47.7%)	38 (46.3%)	
Total		86 (50.6%)	82(49.4%)	

Table 3. Levels of IL-10 in COVID-19 patients according to the age groups and sex

Variable		IL10		p-value
		Normal	Abnormal	
Age groups	<20	0	15	0.08
	20-40	19	49	
	40-60	16	35	
	>60	7	27	
SEX	F	18	65	0.3
	M	24	61	

Coronavirus disease manifests itself in a variety of ways, depending on the patient's age and the existence of co-morbidities. Biomarkers will help with early suspicion, diagnosis, screening, and identification of issues, as well as patient treatment and disposal. Each of these factors has the potential to have a considerable impact on the healthcare system and administrative machinery, directly influencing patient care [20]. Through the results obtained from this study, it appeared that patients with confirmed corona virus disease (positive PCR) had a significant rise in IL-10 and D-dimer levels in

As shown in table (2), D-dimer increased significantly in group I (1511.534± 192.56 ng/ml) compared to group II (282.085± 42.65 ng/ml), and IL-10 increased significantly in group I (9.803± 0.44 ng/ml) compared to group II (3.770± 0.44 ng/ml).

We found no significant differences in the levels of biomarkers (IL-10 and D-dimer) according to age group or gender in our findings (Table 3 and 4).

Table 2. Show means levels of D-dimer and IL-10 both in groups of COVID-19 patients

Characters	Group I	Group II	p-value
D-dimer (ng/ml)	1511.534±192.56	282.085 ± 42.65	0.0001
IL-10(ng/ml)	9.803 ± 0.44	3.770 ±0.44	0.01

The area under the curve for IL-10 was 0.895 (95 percent CI 0. 843–0. 946, P0.0001), whereas the area under the curve for D-dimer was 0.937 (95 percent CI 0. 898–0. 976, P0.001). D-dimer has a higher sensitivity than IL-10, according to these studies, and it indicates that D-dimer is better at predicting COVID-19 severity.

## DISCUSSION

serum of these patients as compared with patients without corona virus (negative PCR). According to numerous lines of evidence, COVID-19 degradation is associated to immunological pathogenic damage. Multiple studies have found that when epithelial and immune cells interact in COVID-19, extremely high quantities of pro-inflammatory cytokines are released, showing a relationship between the CS and the infection's severe consequences and poor outcomes [21, 22]. "C-reactive protein (CRP), interleukin 6, 8, 10, ferritin and the erythrocyte sedimentation rate (E.S.R) were all much higher than the upper of reference value in COVID-19 patients, and this was particularly higher in individuals with poor clinical outcomes" [23]. IL-10 inhibits inflammation by either directly reducing macrophage and DC innate immunological functions or indirectly by improving Treg formation, also can trigger mast cell and improvement the activity of cytotoxic T-cells, B-cells, and natural killer cells [24].

Patients with a critical illness (n = 17) exhibited significantly higher serum IL-10 levels than those with a moderate (n = 42) or severe (n

= 43) illness, according to a clinical study involving 102 COVID-19 patients and 45 controls from Wuhan, China. IL-10 levels were connected to serum CRP levels in a similar way, suggesting that IL-10 might be used to determine the severity of an illness [25]. Those patients admitted to the ICU showed significantly greater plasma levels of IL-10 than those who were not. Huang et al [3]. Also, a follow-up experimental study involving 71 COVID-19 patients from Beijing, China (53 moderate cases and 18 severe cases.) and 18 controls discovered that IL-10 formation in the early stages was significantly linked to disease severity [26]. Excessive IL-10 production could be a negative response mechanism aimed to counterbalance the immune system's hyperactivity [27]. In the early stage of SARS-CoV-2 infection, IL-10 may be harmful by generating T cell weariness, according to a recent research. Delaying IL-10 with a counteracting antibody in the early stages of SARS-CoV-2 infection, according to the researchers, might be a viable therapeutic option [28].

Table 4: D-dimer levels in patients with COVID-19 according to the age groups and sex

Variable		D-Dimer		p-value
		Normal	Abnormal	
Age groups	<20	6	9	0.2
	20-40	45	23	
	40-60	30	21	
	>60	18	16	
SEX	F	48	35	0.7
	M	51	34	

According to the findings of this study, the value of interleukin 10 increase in confirmed Covid-19 patients, cytokine play an important part in the advancement of these individuals' medical disorders, according to the findings. In my opinion the activation of T- cell regular may be aided by an increase in IL-10 in very ill patients. The characteristics of host immunity in really severe cases, on the other hand, have not always been this way. Lymphocytic energy will develop in the later stages of infection as a result of the constant and overwhelming inflammatory reactions, which will eventually cause lymphocyte death. As a result, lymphocyte function might vary dramatically depending on the stage of infection. This could explain why the number of lymphocytes in covid-19 patients reduced. The current study's findings are in line with those of a number of others [25, 29], those who believe that cytokines, particularly interleukin

10 and 6, are important in the development of Covid-19. To rule out pulmonary embolism, the plasma D-dimer assay was utilized in conjunction with clinical prediction scores in patients with low pretest likelihood for more than two decades, having this illness without having to resort to more expensive and intrusive treatments [30]. This indicates that the dimer plays important role in the process of infection and its severity for these patients, if the comparison is made with those people who do not have a confirmed infection with Covid-19. There are many studies [16, 17] conducted in this field that agree with the results of current study. A viral infection can cause sepsis and coagulation problems, which are prevalent in catastrophic illness development. Furthermore, a rise in D-dimer might be an indirect indication of an inflammatory response, since inflammatory cytokines may induce an imbalance in coagulation and fibrinolysis in the alveoli, activating the fibrinolysis system, and increasing D-dimer levels. The cause of high blood D-dimer levels is complex, and best threshold value for increased D-dimer in COVID-19 patients has yet to be determined COVID-19-related coagulopathy, it is obvious, requires specific attention and therapy. The International Society of Thrombosis and Hemostasis (ISTH) has published a guideline, a significantly increased blood D-dimer level (which is still inadequately definite as a three to four fold rise) predicts amplified thrombin generation. [18]. Severe patients have substantially greater D-dimer levels than non-severe individuals, according to a research by Zhang et al. [31], so it has been linked to clinical categorization and can be used to predict prognosis and mortality in COVID-19 patients, according to recent research [32, 33].

The current study concludes the levels of IL-10 and D-Dimer can be utilized to estimate the severity and prognosis of COVID-19 patients, according to our findings. D-dimer levels, among these biomarkers, correspond more closely with severity and can be used as a solid prognostic indicator.

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None.

#### AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual

contribution to the work, and approved it for publication.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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None.

## DATA AVAILABILITY

All datasets obtained or studied during this study are incorporated in the manuscript.

## ETHICS STATEMENT

Not applicable.

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