Is It Possible to Anticipate the Severity of a Coronavirus Infection Using an Interlukin-1 Receptor Antagonist?

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is a contagious illness brought on by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and spread mostly by touch and droplets. Numerous laboratory markers have been connected to sickness and fatality since the first case was reported in Wuhan, China, in December 2019. The outbreak has steadily expanded across the country. **Objective**: This study investigated the impact of serum IL-1 receptor antagonist (IL-1Ra) levels on the clinical course and prognosis of COVID19. **Materials** and **Methods**: The study included 120 patients with COVID-19. The patients with COVID-19 were divided into three groups according to disease severity as critical disease (n=23), severe disease (n=37), and mild/moderate disease (n=60) compared with (60) healthy volunteers as control group. All basic demographic and clinical data of the patients were recorded, and blood samples were collected. **Results**: IL-1Ra levels were significantly higher in the all cases of patients with COVID-19 ($p<0.0001$). IL-1Ra levels were correlated with SpO2 and Lymphocyte negatively ($r = -0.798$ and -0.509 respectively; $p < 0.01$), ($r=0.32$, $p=0.002$ and $r=0.25$, $p=0.019$, respectively), and correlated with age, SBP, DBP, WBCs, CRP, D-Dimer, Ferritin, FBG, ALT, AST, and ALP ($r = 0.294, 0.525, 0.290, 0.656, 0.703, 0.724, 0.778, 0.660, 0.659, 0.703$, and 0.620 respectively; $p<0.01$) positively. A cutoff value of 27.525 pg/ml for IL-1Ra predicted severe COVID-19 with a sensitivity of 90% and a specificity of 83.3% (AUC: 0.951, 95%CI 0.923–0.978; $p<0.0001$). **Conclusion**: In COVID-19, interlukin-1 receptor antagonist could be useful as a promising predictive biomarker for assessing disease severity.

Keywords: COVID-19, Severe COVID-19, Interlukin-1 receptor antagonist (IL-1Ra).

1. Introduction

In Wuhan, China, in December 2019, many individuals who had recently had viral pneumonia were discovered to have severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Most COVID-19 infections are minor, with clinical signs that often include fever and cough, and a recovery time of two to three weeks. Acute respiratory distress syndrome, septic shock, refractory metabolic acidosis, coagulation issues, multiorgan failure, and death are all signs of severe infections [2]. Although the reason why only a small percentage of patients experience severe sickness is still unknown, it has been proposed that this is related to both an excessive adaptive immune response and virally generated lung pathology [3].

Low molecular weight signaling proteins known as cytokines are generated by immune and nonimmune cells in response to a variety of stimuli, such as pathogens like viruses and bacteria, to control innate and adaptive immune responses [4]. The host's immune system is stimulated by SARS-CoV-2 infection, resulting in the activation of genes linked to inflammation and the generation of cytokines from target cells [5]. Particularly in situations of ARDS, where the risk of mortality is raised, upregulated production of proinflammatory cytokines and the subsequent recruitment of immune cells to the site of infection might result in tissue damage. Investigation of the methods by which the immune system (proinflammatory and anti-inflammatory cytokines) activity in the human body is balanced to permit a suitable response in virus clearance is therefore a crucial problem [6].

A naturally occurring anti-inflammatory protein called interleukin-1 receptor antagonist (IL-1Ra) reduces inflammation by inhibiting the interleukin-1 (IL-1) inflammatory pathway[7]. The prototypical proteins of the IL-1 superfamily, interleukin-1 (IL-1), and interleukin-1 (IL-1), have pleiotropic proinflammatory effects on a variety of cell types throughout the body. IL-1 receptor type 1 (IL-1RI) and type 2 are the two IL-1 receptors that bind to IL-1 superfamily members (IL-1RII). IL-1RII is regarded as a decoy receptor since binding to it has no effect on cell signaling [8]. Several pro-inflammatory processes are triggered when IL-1 binds to its receptor. Nitric oxide and other highly inflammatory chemicals are released by activated cells [9]. Additionally, when IL-1 binds to its receptor, chemokines are produced and released, attracting neutrophils, macrophages, and lymphocytes and causing tissue inflammation [10]. No such events are started when IL-1Ra binds to the IL-1 receptor, and IL-1Ra blocks the binding of IL-1, which does not transmit signals [11]. There are several types of cell, including immune cells [10], Kristensen et al. [12], [13], endothelial cells [14], adipocytes [15], epithelial cells, keratinocytes and hepatocytes which produce IL-1Ra [16-

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18].

The cytokine storm syndrome is greatly influenced by IL1, which is antagonistically activated by IL1Ra. It is now possible to provide an attenuated version of this protein to those who have the cytokine storm syndrome linked to COVID-19 [19].

2. Materials and Methods

This case control study of 180 subjects was conducted age 35 to 65 years. Between September and November 2021, 120 confirmed COVID-19 patients were admitted to Al-Amal Specialized Hospital for infectious diseases in an Najaf governorate, Iraq. Patients with COVID-19 were diagnosed based on positive quantitative RT-PCR and chest x-ray or chest computed tomography (CT) scan findings, with 60 healthy participants providing as a control group with similar ages ranges to the patients. This study excluded participants with diabetes, liver illness, chronic renal disease, pulmonary disease, pregnant women, and smokers to prevent the impact of additional comorbidities. Before participating in this study, all controls and patients provided written informed permission.

The COVID-19 patients were divided into three groups upon admission to the hospital based on the clinical findings, respiration rates, oxygen saturation (SpO2) levels, and low-dose chest CT results. The classification's specifics are as follows:

Mild /Moderate illness: SpO2 94% on room air, mild clinical symptoms, mild respiratory symptoms, positive pneumonial signs on low-dose CT.

Severe illness: Those who fit one or more of the following descriptions:

mild clinical symptoms, mild respiratory symptoms, positive pneumonial findings on low-dose CT, and room air SpO2 ≥ 94%.

Severe illness: Those who meet any of the following criteria:

1. Respiratory rate >30 times per min.
2. SpO2 <94% at room air.
3. Lung infiltrates >50% on low-dose CT.

Critical disease: Acute respiratory distress syndrome (ARDS) patients may have septic shock, numerous organ failure, coagulation issues, and possibly pass away [20].

The research was carried out in accordance with Iraqi and international ethical and privacy rules, as well as the declaration of helsinki of the world medical association.

Samples of venous blood were taken from both the patient and control groups. We used two tubes to collect blood samples. Prior to centrifuging 3 ml at 3000 Xg for 10 minutes to extract serum, allow the sample to clot at room temperature for 10 to 15 minutes. Following that, the serum samples were separated into tubes and stored in the refrigerator at -20°C until they were ready for analysis. The rest of the blood (2 ml) was used to calculate the complete blood count. Using Biolabo® kits from Maizy, France, the levels of serum ALT, AST, ALP, and FBG were measured spectrophotometrically. Fluorescence immunoassay was used to determine serum ferritin, C-reactive protein, and D-dimer levels were measured by (ichromaTM). An autohematology analyzer was used to determine the whole blood count (linear, Spain). We used Melsin Medical Co. (Jilin, China) ELISA kits to measure serum IL-1Ra. Furthermore, the detection range of IL-1Ra was 10–160 pg/ml.

Statistical Analyses

The statistical studies were conducted using IBM SPSS Statistics 26 software. The analyses' findings were presented as mean standard deviation. The cutoff point for statistical significance was p0.05. Using the student's t-test, two independent samples were compared. The Pearson's correlation analysis was used to assess the parametric variables. Analysis of variance (ANOVA) was employed in the study to examine any variations in scale variables between categories. To establish the cutoff value for IL-1Ra, the receiver operating characteristic (ROC) analysis approach was used. The area under curve (AUC) value was calculated using the ROC curve.

3. Results

The 120 patients enrolled in this study were categorized according to severity of COVID-19. Fifty patients had moderate disease, while remaining patients experienced either severe or critical illness (37 and 23 patients, respectively). The latter two groups of patients had a significantly higher mean age compared to patients with moderate disease (57.09±4.89, 50.66±2.57 and 45.98±2.92 years, respectively; p = 0.05). These differences were significant (p = 0.011). Distributions of BMI subgroups in the three disease severity groups showed no significant differences. Patients were also defined by the laboratory parameters listed in Table 1. Means of these parameters showed significant differences between the three disease severity groups except Hb. The mean serum IL-1Ra levels of the patients with critical, severe and mild/moderate COVID-19 were 76.83±16.02, 44.33±12.68 and 37.51±8.86, respectively (p<0.0001). Pearson's correlation analysis was performed to calculate correlation coefficients (r) between IL-1Ra and laboratory parameters in COVID-19 patients. IL-1Ra showed significant negative correlation with SpO2 and Lymphocyte (r = -0.798 and -0.509 respectively; p < 0.01), while it was significant positively correlated with age, SBP, DBP, WBCs, CRP, D-Dimer, Ferritin, FBG, ALT, AST, and ALP (r=0.294, 0.525, 0.290, 0.656, 0.703, 0.724, 0.778, 0.660, 0.659, 0.703, and 0.620 respectively; p < 0.01) (Table 2 and Figure 1).

As it was shown in Figure 2, IL-1Ra significantly increase in the critical group in comparison with another groups (severe, moderate, and healthy control).

The efficacy of IL-1Ra for the prediction of severe disease was evaluated by ROC analysis. AUC of IL-1Ra was found as 0.951 (95%CI 0.923–0.978; p<0.0001). When the cutoff value for IL-1Ra in predicting of severe disease was determined to be 27.525, the sensitivity was determined as 90%, whereas the specificity was 83.3% (Figure 3).

Table 1. Comparison of the demographical and laboratory data of patients with COVID-19 and control groups.					
Variables	COVID-19 cases; n = 120			Healthy control (n=60)	p-value
	Critical (n=23)	Severe (n=37)	Mild/Moderate (n=60)		
Age (year)	57.09±4.89	50.66±2.57	45.98±2.92	49.99±5.78	0.05
Gender M/F	15/8	20/17	40/20	40/20	----
BMI (kg/m^2)	23.73±0.55	24.07±1.01	24.61±1.07	24.04±0.86	0.105
SBP (mmHg)	140.04±4.76	133.64±5.68	129.11±4.75	127.8±3.27	0.00
DBP (mmHg)	81.61±3.65	75.89±3.11	77.48±3.22	78.06±2.31	0.00
SpO_2	67.79±9.66	87.82±6.38	95.38±1.09	98.96±0.47	0.00
Hb (g/dl)	12.48±1.20	12.38±1.42	12.73±1.41	12.46±1.28	0.590
WBCs ×10^9/L	13.47±1.05	11.86±1.14	10.06±1.47	8.78±0.99	0.000
Lymphocyte ×10^9/L	2.41±0.55	2.95±0.88	3.93±0.83	4.24±0.51	0.000
Platelet ×10^9/L	311.55±38.73	264.64±49.67	243.79±41.14	304.88±34.45	0.000
CRP (mg/L)	41.39±8.38	32.86±10.17	28.71±7.69	3.49±1.47	0.000
D-Dimer (ng/ml)	3873.76±870.17	3029.83±833.57	1125.28±400.82	304.81±123.72	0.000
Ferritin (ng/ml)	731.56±87.71	503.72±71.22	438.3±74.03	135.06±47.87	0.000
FBG (mg/dl)	274.86±41.81	219.05±49.41	203.06±50.91	96.28±8.06	0.000
ALT (U/L)	45.61±4.31	33.56±6.54	21.33±7.03	17.46±5.83	0.000
AST (U/L)	57.26±7.14	46.83±5.92	30.26±10.13	19.35±6.25	0.000
ALP (U/L)	93.47±7.95	81.35±10.77	69.21±12.71	54.81±14.33	0.000
IL-1Ra (pg/ml)	76.83±16.02	44.33±12.68	37.51±8.86	20.79±7.18	0.000

Abbreviations: M/F, Male/Female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure ; SpO_2, saturation oxygen percentages ; Hb, hemoglobin; WBCs, white blood cells; CRP, C-reactive protein; FBG, fasting blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; IL-1Ra, Interlukin-1 receptor antagonist. Values are given as mean ± standard deviation.

Table 2. The correlation between clinical parameters and serum IL-1Ra level in COVID-19 patients	
Variables	r
Age (year)	0.294*
BMI (kg/m^2)	-0.104
SBP (mmHg)	0.525*
DBP (mmHg)	0.290*
SpO_2	-0.798*
Hb (g/dl)	-0.064
WBCs ×10^9/L	0.656*
Lymphocyte ×10^9/L	-0.509*
Platelet ×10^9/L	0.020
CRP (mg/L)	0.703*
D-Dimer (ng/ml)	0.724*
Ferritin (ng/ml)	0.778*
FBG (mg/dl)	0.660*
ALT (U/L)	0.659*
AST (U/L)	0.703*
ALP (U/L)	0.620*

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; SpO_2, saturation oxygen percentages ; Hb, hemoglobin; WBCs, white blood cells; CRP, C-reactive protein; FBG, fasting blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; r, Pearson's correlation coefficient; *, p<0.01.

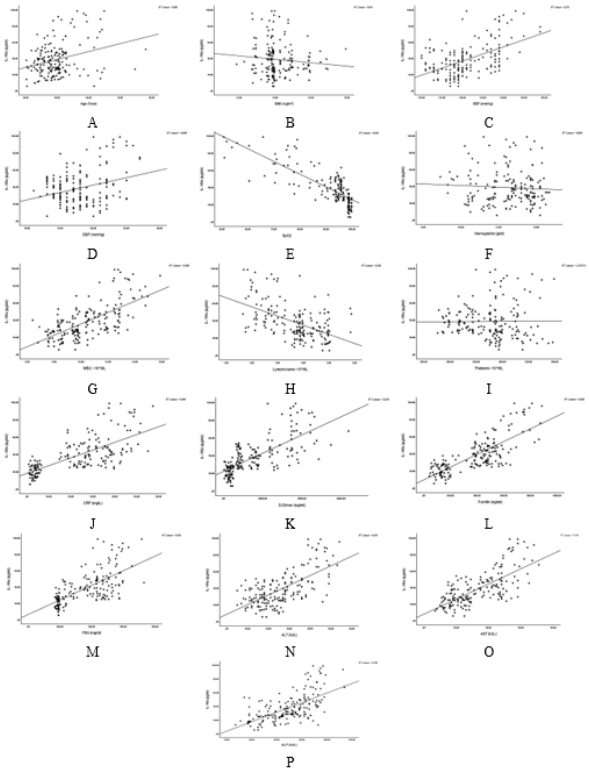

Figure 1. Correlation between serum IL-1Ra levels and (A) Age, (B) BMI, (C) SBP (D) DBP (E) SpO2, (F) Hemoglobin, (G) WBCs (H) Lymphocytes, (I) Platelets, (J) CRP, (K) D-Dimer, (L) Ferritin, (M) FBG, (N) ALT, (O) AST, and (P) ALP.

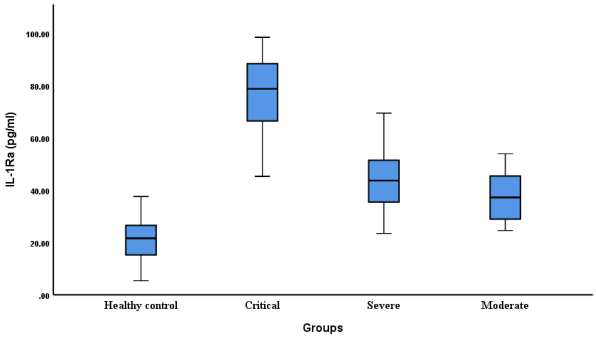

Figure 2. Comparison between groups (COVID-19 cases and healthy control) of IL-1Ra level

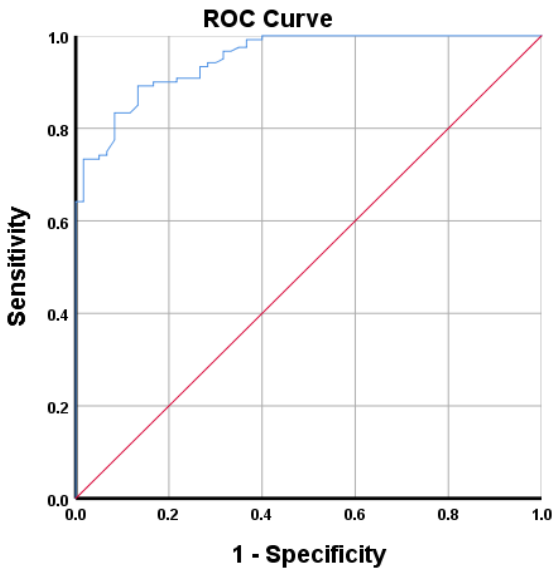

Figure 3. Receiver operating characteristic curve analysis of IL-1Ra for diagnosis of COVID-19.

4. Discussion

In this study, we determined that IL-1Ra levels was elevated in all cases patients with COVID-19. IL-1Ra, levels was positively correlated with CRP, D-dimer and ferritin but negatively correlated with the Lymphocyte and SpO_2. A serious threat to world health, the coronavirus disease 2019 (COVID-19) pandemic is brought on by coronavirus 2 that causes severe acute respiratory syndrome (SARS-CoV-2). Despite the fact that most patients only have minor flu-like symptoms, some individuals go on to develop severe illness that is commonly linked to the clinical presentation of pneumonia. Acute respiratory distress syndrome and multiorgan failure can follow pulmonary infection, which contributes to the patients with SARS-CoV-2 infection's high fatality rate [21]. Age, environmental variables like smoking, and comorbidities like diabetes, hypertension, or lung and heart illnesses all have a significant impact on mortality [22].

In patients with severe COVID-19, higher levels of proinflammatory cytokines, C-reactive protein (CRP), and ferritin are associated with poorer outcomes [23, 24]. There is mounting evidence that these individuals experience a hyperinflammatory state similar to cytokine storm syndromes, which may be treated with immunomodulators. Early in the pandemic, indicators for a hyperinflammatory state and illness severity have been identified as CRP, D-dimer, and ferritin [25], and COVID-19 recommendations suggested that assessing those levels may be useful for prognosis [26]. Pulmonary macrophages that have been stimulated may produce ferritin, and due to the fact that severe COVID-19 patients had much higher ferritin levels than non-severe COVID-19 patients, systemic inflammation from COVID-19 is currently thought to fall within the category of hyperferritinemic syndromes [27].

As crucial mediators of the inflammatory response during a viral infection, cytokines play a vital role. The initial line of protection against viruses must be a sufficient, quick, and coordinated innate immune response [28]. SARS-CoV-2 stimulates the adaptive immune system, which causes T and B cells to generate inflammatory cytokines, in addition to the innate immune response that releases cytokines and chemokines [29]. Particularly in severe and critical patients, this strong cytokine release takes place, playing a crucial role in the development of the illness and the eventual passing of SARS-CoV-2-infected individuals [30].Liu et al. [31] reported that IL-1RA, IL-1α, IL-2, IL-4, IL-7, IL-10, IL-12, and IL-17 were linked to lung damage and the severity of the disease.[32] further discovered IP10, MCP-3, and IL-1RA as biomarkers for disease severity and worse outcomes. IL-1Ra is a protein that inhibits inflammation and has been found to be considerably greater in COVID-19 individuals with severe symptoms[34]. Previous study study showed that critical cases compared to moderate or severe patients had considerably higher levels of the anti-inflammatory cytokines IL-1Ra and IL-10 [33]. The inflammatory response is controlled by IL-1Ra, which is generated by activated macrophages. IL-1Ra is a competitive antagonist for IL-1 and regulates the production of other inflammatory cytokines. IL-1Ra has been examined in the past in a number of modest investigations, and elevated levels have been discovered in serious clinical situations [34, 35], confirming its significance as a key indicator of disease severity. An important aspect of COVID-19's pathogenesis is inflammation. According to early observations, patients had significantly increased levels of many circulating pro-inflammatory cytokines and chemokines [36]. Additionally, compared to moderate instances, severely sick COVID-19 patients have increased plasma levels of pro-inflammatory cytokines [37] , suggesting a correlation between inflammation and the severity of the illness. Patients with severe COVID-19 may also experience significant hyperinflammation, which resembles macrophage activation syndrome, which is characterized by symptoms such diffuse intravascular coagulation, hyperferritinemia, fever, and pancytopenia [38]. However, COVID-19 has significantly raised IL-1Ra levels. This finding would indicate that therapeutic IL-1 inhibition is only partially effective in COVID-19 patients [39] as medications that block IL-1 or IL-1-signaling have been reported to have a rather poor response in patients with high endogenous levels of IL-1Ra [40]. Moreover, increased circulating IL-1Ra levels often represent an IL-1 signature, such as the proper timing of IL-1 blockage in COVID-19, which may be crucial and presumably makes it more difficult to evaluate the data at hand. [41]. Thus,

early treatment of acute hyperinflammatory respiratory failure might have a healing impact. The proinflammatory cytokines and T cell responses are suppressed by the early inhibitory cytokine known as IL-1RA. A cytokine called IL-1RA regulates inflammatory responses at the beginning of immunological activation [42]. IL-1RA, which is produced by monocytes, macrophages, or dendritic cells [18], competitively binds to the IL-1R [43, 44]. The production of Oleksowicz et al. [45], and type I IFN can be modulated byTheofilopoulos et al. [46]. As a result, early IL-1RA synthesis may have an impact on the induction of proinflammatory and antiviral cytokines during this coronavirus infection's early stages. Given the varied detected blood amounts in severe and moderate illnesses, the function of IL-1RA in the immune response may change. In moderate situations, the strong adaptive immune responses to the pathogen may overcome the suppressive impact of increased IL-1RA. However, significantly greater levels of IL-1RA were found in the critical and severe cases compared to mild instances, suggesting an overactive immune response that may have influenced the shift from a regulated and protective immunological milieu to inflammation-induced tissue damage. The possibility of IL-1Ra being a helpful parameter in the diagnosis and follow-up of patients with COVID-19 is suggested by the parameter's positive connection with CRP as well as ferritin and its negative correlation with SpO2.

5. Conclusion

In conclusion, in this study, it was determined that serum IL-1Ra levels were higher in the patients with critical and severe COVID-19 compared with the patients with mild/moderate COVID-19 and related to disease severity. Moreover, correlation analysis identified IL-1Ra as an independent predictor for COVID-19 severity. Therefore, IL-1Ra could be a useful and prognostic biomarker that can be used in the patients with distinguishing critical and severe COVID-19 from the patients with mild/moderate COVID-19.

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Declaration of Interests

The authors declare no conflict of interests

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