Serum levels of α1-antitrypsin, interleukin-1β and interleukin-6 in Iraqi COVID-19 patients: A cross-sectional study [version 1; peer review: awaiting peer review]

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Abstract

Background: More than half of the individuals diagnosed with coronavirus disease 2019 (COVID-19) have been found to have high levels of interleukin (IL)-6. A recent report showed that more elevated serum IL-6 level predicts COVID-19 disease severity and patients’ clinical outcomes. Therefore, this study aimed to compare the serum levels of α1-antitrypsin (AAT), IL-1β, and IL-6 between COVID-19 patients and healthy individuals.

Methods: During the data collection phase, 90 individuals were enrolled, 45 healthy controls, and 45 patients confirmed with COVID-19 using reverse transcription-quantitative PCR (RT-qPCR) at a specialized isolation hospital in Baghdad between November 2021 and March 2022. In this cross-sectional research, venous blood samples were taken, and serum was isolated and stored for quantitative ELISA measurements of AAT, IL-1β, and IL-6 (ELISA). IBM SPSS version 24 was used to analyze the data.

Results: This study revealed a significant increase in the serum levels of AAT, IL-1β, and IL-6 in the COVID-19 patients' group compared to the healthy control group with p-values < 0.001 for each of these markers.

Conclusions: AAT concentrations were higher during COVID-19; this elevation is essential during infection. IL-1β and IL-6 levels were also elevated during the infection period; however, dysregulated high levels may lead to cytokine release syndrome. Therefore, these three biomarkers can be regarded as diagnostically crucial parameters.

Keywords

COVID-19, SARS-CoV-2, α1-antitrypsin, interleukin-1β, interleukin-6
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Introduction

A cluster of pneumonia cases with unknown origins emerged in Wuhan, Hubei Province, China, in December 2019. On January 7, 2020, the causal microorganism was identified as a novel coronavirus (CoV) known as 2019-nCoV.1–4 The virus was later termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).5–7 The World Health Organization (WHO) termed the illness coronavirus disease 2019 (COVID-19). The illness has spread internationally, and WHO designated it a pandemic on March 11, 2020.8–10 COVID-19, like the other coronavirus infections, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), have been associated with increased cytokine production. The cytokine release syndrome, often known as a “cytokine storm”, has been broadly acknowledged as the principal cause of morbidity in these coronavirus outbreaks.11,12 In addition, cytokine storm has been linked to the development of acute respiratory distress syndrome and multiorgan dysfunction syndrome after COVID-19 infection.13 SARS-CoV-2 activates the NLR family pyrin domain containing 3 (NLRP3) inflammasome, resulting in increased synthesis and release of two cytokines, interleukin (IL)-1β and IL-18.14,15 In turn, IL-1β stimulates IL-6 production,16 while IL-18 stimulates interferon (IFN)-γ production.17 Commonly, the IL-1β pathway is more prevalent in COVID-19 patients than the IL-18 pathway.18

Moreover, half of those diagnosed with COVID-19 were reported to have high levels of IL-6.19 According to a recent meta-analysis, a more significant blood IL-6 level is a predictor of COVID-19 disease severity and patient clinical consequences.20 Another meta-analysis concluded that increasing the blood IL-6 level, the more severe the COVID-19 condition and that considerably higher serum levels are observed in patients who did not recover than in people who survived. In addition, they appear more frequently in severely and critically sick COVID-19 sufferers than in moderately unwell COVID-19 patients, and they occur more frequently in individuals who died from the condition than in those who live.21 The Food and Drug Administration (FDA) licensed two types of IL-6 inhibitors, anti-IL-6 receptor monoclonal antibodies (mAbs) and anti-IL-6 mAbs, to modify IL-6 levels or effects to minimize the severity of COVID-19 in patients with systemic inflammation.22 Serum IL-1β concentrations were found to be much higher in COVID-19 patients, and many trials looked at the use of anakinra, an IL-1β receptor blocker, in managing COVID-19 cases of acute severe respiratory distress, with mixed outcomes.23–26 Landi et al., showed that giving canakinumab, an anti-IL-1β mAb, to COVID-19 patients with acute severe respiratory failure results in clinical improvement.27

α1-antitrypsin (AAT) is an acute phase reactant primarily synthesized in the liver and released into circulation serving a range of physiological effects. Monocytes/macrophages, activated neutrophils, and epithelial cells express it less.28 In addition, several cytokines, including IL-1β, IL-6, tumor necrosis factor (TNF)-α, and bacterial lipopolysaccharide, induce hepatocytic expression.29 AAT is the prototypical member of the superfamily of serine protease inhibitors (serpins). The primary role of AAT is not to block proteases but to modulate their proteolytic activity, such as encouraging effective host resistance to infections while preserving healthy tissue from proteolytic harm.30 In addition to protecting against proteolytic injury, AAT has an essential role in reducing acute pulmonary damage by interfering with inflammation, coagulation, and induction of apoptosis.31–33

Both in chronic obstructive pulmonary disease (COPD) and emphysema, AAT deficiency has been associated with alveolar cell destruction.34 AAT from healthy human donors may be used to boost alpha-1 levels in the blood and lungs of individuals with AAT deficiency who have been diagnosed with emphysema.35 Yang et al., suggested that people with AAT deficiency are more likely to get COVID-19 and also have worse prognosis compared to healthy people and that for COVID-19 patients with AAT deficiency who are receiving augmentation therapy with human plasma purified AAT, a dose more significant than just the standard maintenance dosages must be considered.36 In addition, several investigations have shown that human AAT has anti-inflammatory and anti-COVID-19 properties.37–39

This research aimed to compare the blood levels of AAT, IL-1β, and IL-6 in COVID-19 patients to those in seemingly healthy people and to look for any possible links between these inflammatory markers.

Methods

Ethical approval

This study was carried out in compliance with the declaration of Helsinki.39 and was approved by the University of Baghdad’s Faculty of Pharmacy’s Ethics Committee (ethics board approval code: 193221) on 10th April 2021. Before signing their involvement agreements and written consent, all participants were informed of the study’s purpose and potential benefits.

Study design

This study was designed as a cross-sectional study. Blood samples were taken from patients admitted to isolation hospitals in Baghdad for clinical investigation during the period from November 2021 to March 2022.
**Subjects**
This research included 90 individuals of both sexes. A total of 45 of them were diagnosed clinically with COVID-19 and proven with a positive result of nucleic acid amplification testing by reverse transcription-quantitative (RT-q) PCR of respiratory specimens, which were nasal/oropharyngeal swabs, to fulfill the COVID-19 patient group, with 45 healthy adults of an equivalent age and sex representing as the control group.

**Variables**
The main objective of the present research was to assess the levels of AAT, IL-1β, and IL-6.

**Sample size**
The software G*Power (RRID: SCR_013726) version 3.1.9.7 was used to calculate the number of participants. The sample size has to be at least 80 individuals with a 95% confidence interval, 90% power, a two-tailed alpha of 0.05, and an effect size of 0.80 (f). This research included 45 patients in the diseased group and 45 participants in the healthy controls.

**Inclusion criteria**
The inclusion criteria for the COVID-19 patient group have been limited to non-vaccinated adults who were clinically diagnosed by a specialist, patients who showed a positive result for COVID-19 by RT-qPCR, had a fever and pulmonary symptoms (cough, shortness of breath, chest tightness, and pain), and patients with radiological findings of consolidation on either a chest X-ray or computed tomography (CT) scan, oxygen saturation was 70–85%. Additionally, the control group consisted of healthy, non-vaccinated people.

**Exclusion criteria**
Exclusion criteria include individuals who have recently had surgery, probable bacterial pneumonia, renal illness, pregnancy, immunological disorders, trauma, liver disease, respiratory diseases, and vaccine recipients who had a negative RT-qPCR result for COVID-19.

**Bias**
The desirable research population is well-defined, easily available, reliable, and has a high possibility of achieving the desired conclusion. To eliminate bias, participants were selected in a way that did not favor those with unusually high or low amounts of COVID-19 exposure. To detect volunteer bias in a sample, we asked control participants whether they thought they were infected.

**Study procedure**
Blood samples (3 ml) were obtained from each subject and put in a gel tube for 30 minutes to coagulate. The samples were centrifuged at 5,000 RPM for 5 minutes to collect serum for AAT, IL-6, and IL-1β analysis using the enzyme-linked immunosorbent assay (ELISA). All ELISA kits and instruments used in this investigation are listed in Table 1.

**Statistical analysis**
For statistical analysis, IBM SPSS Statistics (RRID: SCR_016479) version 27 software for Microsoft Windows was used throughout the statistical analysis process. According to the Shapiro-Wilk test, the data were not regularly distributed. To

<table>
<thead>
<tr>
<th>ELISA kits (96 wells)</th>
<th>Supplier</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human ELISA (AAT) kit</td>
<td>Bioassay Technology Laboratory-China</td>
<td>E0753Hu</td>
</tr>
<tr>
<td>Human ELISA (IL-1β) kit</td>
<td>Bioassay Technology Laboratory-China</td>
<td>E0143Hu</td>
</tr>
<tr>
<td>Human ELISA (IL-6) kit</td>
<td>Bioassay Technology Laboratory-China</td>
<td>E0090Hu</td>
</tr>
</tbody>
</table>

**Instruments**

<table>
<thead>
<tr>
<th>Origin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge</td>
<td>Kokusan-Japan</td>
</tr>
<tr>
<td>Gel tube</td>
<td>Xinle-China</td>
</tr>
<tr>
<td>Deep freezer (-20°C)</td>
<td>Vestel-Turkey</td>
</tr>
<tr>
<td>Micropipettes (different volumes)</td>
<td>Capp-Denmark</td>
</tr>
<tr>
<td>Microtiter plate reader</td>
<td>Human reader HS-Germany</td>
</tr>
</tbody>
</table>

Where: ELISA= enzyme-linked immunosorbent assay; AAT= α1-antitrypsin; IL-1β= interleukin-1β; IL-6= interleukin-6.
compare the COVID-19 patients group with the control group, the non-parametric Mann-Whitney U test was performed. Each participant’s median and interquartile range (IQR) value were calculated. Spearman’s Rank correlation coefficient was also used to analyze the relationship between AAT levels and the other indicators tested, IL-6 and IL-1β. A chi-squared test was used to examine categorical variables.

**Results**

The participants in this research varied in age from 39–65 years old in the illness group to 37–67 years old in the healthy control group. There was no difference in mean age between the patient and control groups (P=0.215). Regarding sex, 23 (51%) of the 45 patients were men, while 22 (49%) were women. A total of 20 (44%) of the 45 people in the control group were men, whereas 25 (56%) were women. Table 2 shows no significant difference (P=0.527) between the sexes of the participants in this research.

According to the results of the study, there was a significant difference in the measured value of AAT between the two groups. The median (IQR) levels of AAT in the patient and control groups were 5.55 (1.86) mg/ml and 4.11 (2.82) mg/ml, respectively, with a P=0.001 (Figure 1). In terms of IL-1β, this research discovered a substantial difference between the analyzed groups, with the median (IQR) of IL-1β in the COVID-19 patient group being 1,672 (475) pg/L and 923 (178) pg/L in the control group, P=0.001 (Figure 2). Also, there is a significant difference in the measured levels of IL-6 between the comparison groups, as indicated in Table 3 and Figure 3. IL-6 levels in the COVID-19 patient and control groups were 105.8 (39.24) ng/L and 51.15 (12.76) ng/L, respectively, P=0.001.

Correlation studies between AAT, IL-6, and IL-1β of the participants (n=90) are shown in Table 4. Serum AAT has a significant positive correlation with IL-1β and IL-6. Also, serum IL-6 has a significant positive correlation with IL-1β (P<0.01).

### Table 2. Assessment of sociodemographic variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>COVID-19 patient group (n=45)</th>
<th>Control group (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, year</strong></td>
<td>Median IQR</td>
<td>Median IQR</td>
<td>P=0.215 (Mann-Whitney U test)</td>
</tr>
<tr>
<td></td>
<td>50 (12)</td>
<td>47 (11)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Male, n (%) Female, n (%)</td>
<td>Male, n (%) Female, n (%)</td>
<td>P=0.527 (Chi-squared)</td>
</tr>
<tr>
<td></td>
<td>23 (51) 22 (49)</td>
<td>20 (44) 25 (56)</td>
<td></td>
</tr>
</tbody>
</table>

Where: COVID-19= coronavirus disease 2019; IQR= Interquartile range; n= number.

![Figure 1. Serum AAT level in studied groups. AAT= α1-antitrypsin.](image)
Figure 2. Serum IL-1β level in studied groups. IL-1β = interleukin-1β.

Table 3. AAT, IL-1β and IL-6 levels measured in this study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>COVID-19 patient group (n=45)</th>
<th>Control group (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
</tr>
<tr>
<td>AAT (mg/ml)</td>
<td>5.55</td>
<td>1.86</td>
<td>4.11</td>
</tr>
<tr>
<td>IL-1β (pg/L)</td>
<td>1672</td>
<td>475</td>
<td>923</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>105.8</td>
<td>39.24</td>
<td>51.15</td>
</tr>
</tbody>
</table>

Where p-value was for Mann-Whitney U test; AAT = α1-antitrypsin; IL-1β = interleukin-1β; IL-6 = interleukin-6; COVID-19 = coronavirus disease 2019; IQR = interquartile range; n = number.

*statistically significant when P<0.05.

Figure 3. Serum IL-6 level in studied groups. IL-6 = interleukin-6.
Discussion
Because of its anti-inflammatory and anti-viral effects, AAT is a crucial protease inhibitor in the pathophysiology of SARS-CoV-2 infection. Similarly to the current study, the findings of a prior study conducted by Ercin et al. (2021), which included 86 participants, 44 patients diagnosed with COVID-19 and 42 as the control group, revealed that patients with COVID-19 infection demonstrated a significant increase in AAT threshold, which is a great predictor for disease termination. IL-6 is a crucial modulator of the inflammatory and immunological response to infection or trauma. Excessive IL-6 levels are associated with cytokine storm, which may result in multiorgan dysfunction and respiratory failure. Controlling systemic IL-6 levels in SARS-CoV-2 infected individuals is thus critical. The present research found that patients in the COVID-19 group had substantially higher IL-6 levels (P=0.001) than participants in the healthy control group, with a median (IQR) of IL-6 for patients being 105.8 (39.24) ng/L and the healthy control group being 51.15 (12.76) ng/L. Tang et al., (2021) performed a cross-sectional study with 100 COVID-19 patients categorized into three subgroups: common (n=56), severe (n=28), and critical (n=16); the mean ± SD for each group being 23.93 ± 9.64, 69.22 ± 22.98, and 160.34 ± 26.15, respectively, P=0.05, indicating a significant difference between the three groups. IL-1β is a cytokine that plays a crucial role in immune system activity. Because high levels are linked to cytokine storm syndrome, keeping IL-1β levels under control following COVID-19 infection is critical. The present research found a substantial increase in IL-1β levels in the COVID-19 patient group compared to the healthy control group, with a median (IQR) of 1.672 (475) pg/L for the patient group and 923 (178) pg/L for the control group (P=0.001). Furthermore, Lu et al., (2021) discovered that the levels of IL-1β, along with other examined biomarkers, were considerably higher in COVID-19 patients compared to healthy subjects. As shown in Table 4, there is a significant positive correlation between IL-1β and IL-6 (P<0.001), this is because IL-1β stimulates IL-6 production. Also, there is a significant positive correlation between AAT, IL-6, and IL-1β (P<0.001) because the hepatocytic expression of AAT is stimulated by several cytokines, two of them are IL-1β and IL-6.

Limitations
Many exclusion criteria were used in the study to limit the confounding effect. However, because the number of patients in our study was small, we recommend increasing the number of patients in future studies.

Conclusions
Because high levels of AAT are crucial during COVID-19 infection, the physician may request a serum AAT level test to prescribe AAT supplements if levels are low. It may also be regarded as a favorable prognostic factor throughout the illness phase, and similar results apply if COVID-19 patients do not have AAT deficiency before being infected with the SARS-CoV-2 virus. IL-1β and IL-6 are significant proinflammatory indicators, and excessive levels of either may cause cytokine release syndrome. In addition, they are a strong predictor of COVID-19 infection.

Data availability
Underlying data
Zenodo: Demographic data along with a comparison of α1-Antitrypsin, Interleukin-1β and Interleukin-6 levels measured in this study. https://doi.org/10.5281/zenodo.6895591.
This project contains the following underlying data:

- Serum levels of α1-Antitrypsin, Interleukin-1β, and Interleukin-6.xlsx (demographic data and laboratory results)

Data are available under the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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References


44. Shareef LG: Demographic data along with a comparison of α1-Antitrypsin, Interleukin-1β and Interleukin -6 levels measured in this study. [Dataset]. Zenodo. 2022. [Publisher Full Text]
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