Evaluation of IL-6 and IL-10 among Patients with the Covid 19 virus in Wasit Governorate

Rasha Amer Hassoon

University of Ilam, Faculty of sciences, Department of Biology, Ilam, Iran.
Corresponding author, Email: a.rostamzad@ilam.ac.ir.

Abstract. Coronavirus disease 2019 (COVID-19) first occurred in China in December 2019 and has since expanded worldwide. High levels of Interleukin-6 (IL-6) and Interleukin-10 (IL-10) in COVID-19 patients imply cytokine storm can play a significant role in the pathogenesis and are regarded a useful criterion in indicating the most acute course of illness. The aim of this study was to evaluate IL-6 and IL-10 levels in ill COVID-19 patients who were confirmed by q-PCR and to evaluate their relationship with patient severity and number of days to elicit an immune response. We conducted a study of patients admitted to the intensive care unit with diagnosis of COVID-19 between March 2021 to May 2021, and demographic, clinical and laboratory data were collected upon admission. On the day of measuring the concentrations of IL-6 and IL-10 in the serum, then we fixed the number of days after the onset of symptoms and confirmation of infection. A total of 90 patients were conducted in this research, out of 45 patients were infected with COVID-19 that, confirmed by q-PCR, and 45 healthy people were used as control. The results of this study showed that there was a significant increase in the rate of the levels of IL-6 and IL-10 (p =0.0 < 0.05). The study results also indicated that the concentration of IL-6 and IL-10 increases with the development of infection also the IL-6 and IL-10 levels in patients with Covid-19 has strong association with contracting this disease ( OR=79.75., P= 0.0001 and OR=219.07., P= 0.0002) respectively.

Keywords: IL-6,IL-10,Covid-19.

INTRODUCTION

Since December 2019, a virus known as “acute respiratory distress syndrome coronavirus 2” (SARS-CoV-2) has been circulating in Wuhan, China, and has expanded swiftly throughout the world 1. The World Health Organization (WHO) has designated SARS-CoV-2-caused illness as “coronavirus disease 2019” (COVID-19). The majority of COVID-19 patients have mild to severe symptoms and recover quickly; however, patients who acquire severe manifestations have a significant mortality rate, which can reach up to 60% in patients hospitalized to the Intensive Care Unit (ICU) and treatment with ventilators 2. According to certain research, lymphopenia and cytokine release syndrome (CRS) are related to the severity of the illness 3. CRS is an inflammatory process response that can be induced by a number of causes, including infections, poisons, or an idiopathic reaction to medicines, and is defined by an elevation in pro-inflammatory cytokines, such as IL-6 and IL-10. This seeming "cytokine storm" can induce several organ damage in a matter of minutes and has been thought to define not only the intensity but also the prognosis of COVID-19 4. Elevated levels of IL-6 and IL-10 also are linked to several clinical characteristics, such as maximal body temperature and the development of bilateral lung involvement on chest radiography 5, 6. Multiple clinical investigations have found increased levels of both pro-inflammatory and anti-inflammatory cytokines in COVID-19 7. A recent thorough meta-analysis highlighted elevated levels of IL-2, IL-2R, IL-4, IL-6, IL-8, and IL-10 in a severe group of patients, whereas no significant rise in IL-1β and IL-17 levels was identified 8. Infections with SARS-CoV-2 involves two overlapping phases: first one, marked by the virus’s strong replicative activity, is followed by a counteractive host immunological response 9. Several studies have suggested a group of cytokines that may represent selective therapeutic targets, as IL-6 and IL-10 has been considered important in the pathogenesis of COVID-19 disease and may also be antagonistic from existing drugs. IL-6 and IL-10 are inflammatory substance that produces primarily by macrophages and T lymphocytes in response to pathogens and is pivotal for the control of many viral infections 10,11. In our study, we characterized serum IL-6 and IL-10 levels during the entire infectious process. Where we showed increased levels of interleukin-6 according to the stage of the disease and it’s associated with the development of the disease that may lead to respiratory failure. Quantitative determination of IL-
6 and IL-10 levels may allow early diagnosis of patients, which may help in taking necessary measures to reduce the cytokine storm, which can be fatal in most cases.

**Methods**

The study was conducted from March 2021 to May 2021 in Al-karama teaching hospital in Wasit province in Iraq. This study included two groups. The first group included 45 patients with covid-19 and 45 papules healthy as control. Venous blood samples were taken by 5 ml from each patient and control as well, all blood samples were placed in a cool-Box under aseptic condition and this tube was stored in the freezer at (-20°C). Serum was isolated by centrifugation at 3000 rpm for 10 min and the serum was divided into aliquots in eppendorf tubes until estimation of IL-6 and IL-10. We analyzed the following variables: total IL-6 and IL-10 by using ELISA method and special kits from Elabscience Company. Statistical analyses was done using Microsoft SPSS version 24 which include the following (mean +SD), P-value of less than 0.05 was considered a significant.

**Results**

The results of IL-6 are presented in Table (1). These results revealed a significant increase in IL-6 in serum of patients with covid-19 in comparison with that of the control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IL-6 Mean + SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with covid-19</td>
<td>A 49.0±44.59</td>
</tr>
<tr>
<td>Controls</td>
<td>B 11.3±4.17</td>
</tr>
</tbody>
</table>

As shown in Figure (1): the concentration of IL-6 increased with the development of infection, as there is a clear and direct correlation between the concentration of IL-6 and increase in the number of days after the onset of symptoms and infection.
Figure 1: Chart showing the relationship between IL-6 level and the number of days after symptoms appeared in patients with Covid-19, where there is no direct correlation between the increase in the number of days and the increase in IL-6 concentration where $r = 0.7$, $p = 2.1$. The IL-6 in patients with Covid-19 has strong association with contracting this disease OR=79.75. The difference is significant $P=0.0001$ as shown in the table (2).

Table 2: Odd ratio of Covid-19 patients and control by IL-6.

<table>
<thead>
<tr>
<th>IL-6</th>
<th>Control</th>
<th>Covid-19 patients</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSTIVE</td>
<td>1(2.2)</td>
<td>29(64.4)</td>
<td>0.0001</td>
<td>79.75(10.0231 to 634.5380)</td>
</tr>
</tbody>
</table>

The results of IL-10 are presented in Table (3). These results revealed a significant increase in concentration of IL-10 in serum of patients with covid-19 in comparison with the control group.

Table 3: Concentration of IL-10 in studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>IL-10</th>
<th>P-value</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with covid-19</td>
<td>No.</td>
<td>A</td>
<td>76.9 ± 54</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>B</td>
<td>8.04±3.7</td>
<td>yes</td>
</tr>
</tbody>
</table>

As shown in Figure (2) the concentration of IL-10 increased with the development of infection, as there is a clear and direct correlation between the concentration of IL-10 and the increases in the number of days after the onset of symptoms and infection.
Figure 2: Chart showing the relationship between IL-10 level and the number of days after symptoms appeared in patients with Covid-19, where there is no direct correlation between the increase in the number of days and the increases in IL-10 concentration where $r = 0.22, \ p = 0.1$. The IL-10 in patients with Covid-19 has strong association with contracting this disease OR=219.07. The difference is significant $P= 0.0002$ As shown in the table (4).

Table (4): Odd ratio of Covid-19 patients and control by IL-10.

<table>
<thead>
<tr>
<th>IL-10</th>
<th>Control</th>
<th>Covid-19 patients</th>
<th>P-value</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSTIVE</td>
<td>13(0.28)</td>
<td>45(100)</td>
<td>0.0002</td>
<td>219.07 (12.5663 to 3819.2162)</td>
</tr>
</tbody>
</table>

**Discussion**

There is a considerable degree of heterogeneity between studies in terms of the features and outcomes of COVID-19 patients, since these results are impacted by the nations’ demographics, clinical settings, and health-system resources. In terms of previously published research12. Our research focused on IL-6 because its documented unique function in the cytokine storm that occurs in COVID-19 patients, its strong connection to disease severity, the likelihood of needing mechanical ventilation, or mortality, and, most significantly, because it may be exploited as a pharmaceutical target. According to our findings, IL-6 is an essential marker of inflammation that can help doctors identify individuals with severe COVID-19 early in the disease's course. The results of our study agree with Huang C et al. 2020, where the results of the study showed that there is a significant increase in the levels of IL-6 in patients with COVID-19 13. Our study showed that 29 (64.4 %) patients infected with Covid-19 out of a total of 45 suffer from an increases and varying in the concentration of the IL-6 (Positive). Our results showed that the positive rate (51.1 %) was higher on day 5–9 of the disease onset such as showed in figure (3).

Figure 3: IL-6 concentration changes in 29 patients. Within 5-9 after the onset of symptoms, the IL-6 value is higher.
Elevated IL-6 levels in COVID-19 patients have been regarded as the most goal exhibition of the ongoing "cytokine storm," as they have been noted in patients suffering from septic shock, undergoing cardiac surgery 14. Our findings show that IL-6 levels in critically sick COVID-19 patients were greater than normal, but much lower than in other severe acute inflammatory syndromes with organ failure. Additional research is needed to determine if the relationship of high IL-6 with bad outcome is just a measure of severity or a possible target for particular IL-6 blocking treatments. It has been observed that the degree of pulmonary immune damage corresponds with significant neutrophil and macrophage infiltration in lung tissues 15. The majority of prior research found a link between high IL-6 levels and the development of COVID-19 severity and bad prognosis. Our result agree with a research conducted by Grifoni et al.,2020 16. Where their study stated that high levels of IL-6 may enhance the risk of Covid-19 and may lead to death due to the so-called cytokine storm. IL-10 is a multifunctional cytokine with the main role of suppressing the inflammatory response. However, IL-10 is also known to trigger T-cell energy or non-responsiveness in anti-tumor cell responses as well as viral infection 17. Blocking IL-10 using antibodies against IL-10 or its receptor, or genetically removing IL-10, resulted in viral infection eradication 18. Although cytokine levels are high in acute COVID-19 patients, the implications in terms of therapy are unknown. On COVID-19 patients, corticosteroids that might potentially decrease cytokines by blocking the NF-B transcription factor were administered. Many of studies including SARS-CoV, SARS-253 CoV-2, and MERS-CoV infection found an elevated death risk ratio (RR 2.11, 95 percent CI: 1.13 – 254 3.94) for individuals treated with corticosteroids (Yang Z et al.,2020). Despite the unclarity on therapeutic potential of IL-10, their observed levels of elevation in severe COVID-19 patients have prompted clinical researchers to explore use of them as prognosticators. In an earlier study with pneumonia-affected children, IL-10 on admission was found to be an indicator of severe disease 19. Perhaps the results of the study agree with Wu H et al. Where the results of their study showed a significant increase in the levels of IL-10 in most of the affected children, and this may indicate the high and continuous activity of this IL-10 even at advanced ages 20. Whereas other studies suggest the opposite, as Qin C et al indicated that They show that IL-10 is the only cytokine related with child COVID-19, although rises in all six are found in adult patients 21. In a other study of 71 adults, IL-10 was the only immune mediator among a panel of 34 that was increased as early as the first week after the onset of clinical COVID-19 symptoms in those who formed disease (18 patients) well beyond second week compared to those who continued to have medium disease (53 patients) 22.

The results of our study IL-10 also agree with several Chinese studies from the beginning of the epidemic that indicated that blood levels of IL-10 correlated with the severity of COVID-19 23, 24, 25, serum levels of IL-10 were observed to gradually drop over the course of 2–3 weeks or when symptoms resolved 26. Nevertheless, no precise data on COVID-19-convalescent blood has been recorded. IL-10 has been shown to play an important role in the stimulation and production of cytokines in and on mast cells so Mast cell activation syndrome, according to some experts, leads to hyper inflammation in severe COVID-19 and could be a therapeutic target. However, in the lack of concrete evidence for this pro-inflammatory mechanism in COVID-19, we believe IL-10's proven anti-inflammatory properties to be important for its hypothesized function in both lung defense and viral clearing 27. It is difficult to anticipate the overall impact of treatment IL-10 signaling inhibition in COVID-19, whether enhanced lung inflammatory vulnerability or desirable antiviral immunity. Antibody-mediated IL-6 receptor inhibition, for example, originally appeared to reduce hyper inflammation in COVID-19 patients 28. We believe that the rise in IL-10 represents a specific COVID-19 disease endotype that helps to lung protective but also interacts with viral clearance very quickly after infection, up to major illness.

Conclusion
For patients with COVID-19 IL-6 and IL-10 levels can be acceptable as a good indicator of endpoint progression to severe illness or in-hospital mortality or it may be the best predictor of negative outcomes.

Limitations
Our research has several limitations. First It is challenging to identify risk variables for increased IL-6 levels using a multifactorial adjusted analysis due to the small number of patients. Second The absence of association between IL-6 and other biological factors in samples collected after many days in the ICU might be impacted by additional factors unrelated to COVID-19. Third IL-6 levels were not monitored at regular intervals, and individuals with mild COVID-19 forms were not included, which may have affected IL-6's ability to predict severity and results. Finally only a minority of patients presented very high IL-6 levels during COVID-19.
Recommendations

Repeated measurements of IL-6 can help the clinicians in identifying critically COVID-19 patients with the highest risk of poor prognosis. The elevated of IL-6 has an association with COVID-19 and increase the probability of risk contracting the disease (OR=79.75). In our COVID-19 group, IL-6 levels appear to be a good predictor of endpoint joint progression to severe disease or mortality and appear to be the best predictor of adverse outcomes. Our study supports the hypothesis that targeting the cytokine storm induced by COVID-19 with anti-IL-6 drugs could be a valid therapeutic option to improve outcomes in COVID-19 patients.

REFERENCES


