The role of IL-6, IL-8 and TNF-α in COVID-19 patients in Karbala Governorate

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Abstract. There are several viruses considered to be ecologically problematic, including COVID-19. The samples were collected from persons with middle and severe cases of patients with COVID-19 as well as other samples from patients who were appeared symptoms or recovered from infection of coronavirus, where the number of cases 100 and measured levels of cytokines in the serum of patients as IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α), estimated by immune assay called enzyme-linked immunosorbent assay (ELISA). Where serum level of TNF-α in persons not illustrated a significant difference in all groups, while the interleukin 6 in severe and middle case groups of COVID-19 was high significantly compared with normal and recovered cases groups of COVID-19. Also, the concentration of interleukin 8 was low in the serum of patients infected by a coronavirus in the cases of severe and middle respectively compared with normal cases and recovered cases that healing from coronavirus infection which maybe gives a prediction for disease COVID-19, also showing different concentrations of cytokines in cases of the COVID-19 group than the normal group that may belong to several mechanisms of immunoregulatory detected in cases of COVID-19 pathogenesis.

1 Introduction

COVID-19 is a new beta coronavirus at causes infection caused by the novel virus first appeared in China in south Wuhan in December 2019 [1] where the samples been limited in the market of Wuhan and infection may be transmitted from one person to another and the presence of virus that infected the animals was not proved detection, on the other hand, the animal species infection has not been definitively proved at the time of writing [2]. Another study suggested, considering the snakes compared with other animals more likely than others as bearers [3], while subsequent research referred to the bats may be in charge of its...
transfer, which belongs to that same in the genome sequences and caused close to similarity as reached 96.2 % of gene sequence occur among SARS-CoV-2 in the humans [4,5,6,7]. Infection by viruses leads to the over-controlling of cytokines, one of which is Tumor Necrosis Factor-alpha (TNF-α), which plays important role in inflammation as a mediator [3,4]. Several cytokines caused decreased inflammation [8], and the number of T cells decreased in the stages of infection in the first periods of viral infection, essentially in the cases of severe due to altering in the organized function of immunity [9]. Thus, it is considered a benefit to know the levels of cytokine aggregated in the serum of persons infected with the virus compared to the cases normal and recovered to understand the mechanism of the cellular after infection by a coronavirus. So, the study showed the concentrations of cytokines TNF-α, IL-6, and IL-8, were detected by ELISA via measured levels in the serum of persons as those cases of normal and cases of severe and middle infected by a coronavirus and in the also patients regain normal health after infected with the causative agent.

2 Materials and Methods

The study occurred in Al-Hussain hospital in Karbala city in Iraq where collected of 100 samples were from persons distributed in four groups, normal, severe, middle, and cases of patients that regain normal health after exposure to the virus between August to November 2022 (Fig.1), then all cases of patients suffering from symptoms of a coronavirus were identified as positive cases for a causative agent initially by rapid test and laboratories of Al-Hussain hospital, then after the appearance of symptoms in patients suffering from COVID-19 and who developed symptoms were given a drug such as hydroxychloroquine (200 mg/day) and azithromycin (200 mg/day) at three to seven days after the appearance of developed symptoms. In the severe COVID-19 case group whose serum samples were collected, twelve of the patients that suffered from severe cases were dealt with hydroxychloroquine and azithromycin for one week (seven days) before taking a sample, while in a group normal samples were collected from persons who were not suffered from any symptoms, additionally, all samples were collected in sterile conditions. The concentration of interleukins was measured by an immune assay called enzyme-linked immunosorbent assay (ELISA) to estimate the levels of IL-6, IL-8, a TNF-α in all groups of persons by utilizing of Ealbscience kits and 10 pg/mL was less concentration that identification by the kit.

2.1 Statistical analysis

The utilization of a Statistical program called graph pad prism program (GPP) version 5, to analyze the data and compared the results of all cases groups in the current study at a p-value lower than the significance, (p < 0.05) [10, 11].
Fig. 1. The cases of volunteer persons in the current study (n = 80 assay).

3 Results and discussion

The reasons that led to an alteration in the concentrations of cytokines may be caused by triggering of the immune response that takes place in the development of disease [12]. The current study showed levels of TNF-α in serum in all groups of persons appeared variance, where the levels of interleukins reached 78.3 ± 4.2, 60.3 ± 5.2, 70.7 ± 4.2, 49.9 ± 2.8 in the cases of normal, severe, middle, and cases of recovered from COVID-19 respectively that can be showed in Table 1, thus there was not significantly of variance at (P>0.05), therefore can be seen that the concentration of TNF-α in the serum of some patients increased when infected by coronavirus especially when infection occurred in the respiratory system of patients [13] and in the cases of patients with severe and middle infection with coronavirus appeared significant increased that might be demonstrated by the newest study that referred to the severity of disease related with the syndrome of the severe acute respiratory, syndrome of middle east respiratory, and lung injury [14, 15].

Table 1. The cytokines concentrations in serum of the volunteer persons in the current study (n=80).

<table>
<thead>
<tr>
<th>Cases of Group</th>
<th>Cases No</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal case (control)</td>
<td>10</td>
<td>78.3 ± 4.2</td>
<td>49.8 ± 6.2</td>
<td>264 ± 58</td>
</tr>
<tr>
<td>Infection case (Sever)</td>
<td>30</td>
<td>60.3 ± 5.2</td>
<td>480.3 ± 410*</td>
<td>141 ± 18*</td>
</tr>
<tr>
<td>Infection case (Middle)</td>
<td>20</td>
<td>70.7 ± 4.2</td>
<td>187.4 ± 83*</td>
<td>188 ± 15*</td>
</tr>
<tr>
<td>Recovered case (healthy)</td>
<td>20</td>
<td>49.9 ± 2.8</td>
<td>72.4 ± 38.7</td>
<td>239 ± 111</td>
</tr>
</tbody>
</table>

Note. (*) referred to the presence of a difference significantly at (P>0.05).

On the other hand, the level of IL-6 in the serum of patients suffering from symptoms of coronavirus infection was high level, where the concentrations of IL-6 cases in persons of all groups were 49.8 ± 6.2, 480.3 ± 410, 187.4 ± 83, and 72.4 ± 38.7 pg/ml and in the cases of normal, severe, middle, and recovered patient group, respectively. Thus, these levels
were significantly higher principally in two cases of patients in severe and middle groups that appeared symptoms of coronavirus compared with the normal cases group, while no significant difference appeared that identified among the normal cases and recovered cases group from the infection by a coronavirus, therefore the intensity or severity of IL-6 was connected to the intensity of coronavirus infection.

During the period of an early increase in the epidemic of coronavirus infection, the concentration of IL-6 was referred to as an indicator of disease severity and gave a prediction of the ventilator immune system [17,18,19]. Pedersen researcher who suggests that the increase in the concentration of IL-6 has a relationship significant to intensive care unit entry and also recovering from the disease [20]. On the other hand, another researcher as Prompetchara presented that the patients were not placed in the intensive care unit and the concentration of IL-6 was highly increased and reached fifty-two percent [21], but another study referred to especially the cases of symptoms of severe of patients an increase in the indicators other in addition to IL-6, as well as other studies showed the height express of , IL-6 in the patients suffering from symptoms of coronavirus disease were accelerated process of inflammation, but there was appeared several studies that the levels serum in patients infected by the coronavirus from interleukin 6 increases and thus, considered significant and connected to the severity of infection [22,23,24].

In the current study, the concentration of IL-8 was low in the serum of patients infected by coronavirus where reaching 141±18 and 188 ± 15 in the cases of severe and middle respectively compared with normal cases and recovered cases that healing from coronavirus infection, thus the levels of IL-8 in the cases groups of patients suffering from coronavirus appeared decreased significantly in compared with 264 ± 58 and 239 ± 111 belong to the normal and recovered cases groups respectively. In addition to that, there was no significant difference between the normal group and the recovered from coronavirus disease group, Merza and his colleagues suggested that the lower concentration of IL-8 belongs to suppression of the immune response that relies on the T-lymphocyte and depletion of these cells in the serum of patients with coronavirus, that considered the reasons to decline levels of IL-8 in the current study [25]. Our current study might be related closely to other studies introduced by Chen and colleagues who showed that the concentration of interleukin 6 increased in the serum of patients while the remaining concentration of IL-8 and TNF-α unchanged in the serum of patients with coronavirus [26].

Table 2 showed treatment influenced the levels of cytokines in the serum of patients with COVID-19, the current study showed the analysis of statistics no significant differences in the cases of middle and severe in the cases of patients who received treatment after patients division and appeared severity symptoms of coronavirus disease, one group treated and another group untreated of patients then estimated the levels of cytokines and noticed that the short time for treatment that not appeared effect on the concentration of cytokines in the serum of patients with coronavirus, thus it's required should be measured the concentration of cytokines in the fluid of broncho alveolar tissues of the patients that may assist in the detected of cytokines involved in the tissue of lung injury or in pathogenesis with coronavirus [25, 26].

<table>
<thead>
<tr>
<th>Cases of Group</th>
<th>Cases No</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal case (control)</td>
<td>8</td>
<td>43.2 ± 4.25</td>
<td>74.2 ± 8.3</td>
<td>19.7 ± 52</td>
</tr>
<tr>
<td>Infection case (Sever) with treatment</td>
<td>12</td>
<td>50.3 ± 10.2</td>
<td>200.6 ± 60*</td>
<td>188.7 ± 28*</td>
</tr>
<tr>
<td>Infection case (Sever) without treatment</td>
<td>12</td>
<td>46.4 ± 5.7</td>
<td>270.6± 230*</td>
<td>147.3 ± 35*</td>
</tr>
</tbody>
</table>

*: Referred to the presence of a difference significantly at (P>0.05).
4 Conclusion

The alteration in the concentrations of cytokines in the serum of patients that suffer from coronavirus infection referred to the statement of immunity to these patients different from other infections by another virus, so the concentrations of cytokines in the serum of patients in the current study may be reflection degree of signs the severity of disease by a coronavirus and these signs gave vary of events to organize of the immunity through and after infected by a coronavirus and also facilitated to known of disease pathogenesis.

References

1. Y.R. Guo, Q.D. Cao, Z.S. Hong et al, Military medical research 7(1), 1-10 (2020)
13. X. Chen, B. Zhao, Y. Qu et al, Clinical infectious diseases (2020)