Evaluation The Role Of Some Immunological Markers In Coronavirus-19 Infection In A Sample Of Iraqi Patients

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Interleukin, Coronavirus, Enzyme-Linked Immune Sorbent Assay, Angiotensin-Converting Enzyme 2, Viral Transport media

ABSTRACT
Background: cells produce proinflammatory cytokines like il-8, which activates neutrophils and stimulates immunological responses. Il-6 is produced quickly and transiently in response to infections and stimulates hematopoiesis and acute phase responses, aiding host defense. Dysregulated il-6 production can lead to chronic inflammation and autoimmunity despite its transcriptional and posttranscriptional regulation.
Objective: the study aims to identify immunological markers that could serve as diagnostic factors for covid-19 infections, using rt-pcr for diagnosis and estimation of interleukin-6 and interleukin-8 levels and evaluating their association with infection progression, given the importance of covid-19 in various medical fields.
Methods: blood samples collected from al-zafaraniyah general hospital, fatima-alzahra hospital, and al-yarmook teaching hospital in baghdad city identified 40 patients with coronavirus. The study used the enzyme-linked immune sorbent assay (elisa) approach to detect interleukin-6 and interleukin-8 antibodies in the patients ' serum.
Results: the study reveals a strong correlation between immunological markers and corona virus-19 infection, with 40 patients showing higher levels of interleukin-6 and interleukin-8 antibodies, indicating a significant association between these markers.
Conclusions: the study found a significant association between coronavirus-19 infection patients and immunological markers, with high levels of interleukin-6 and interleukin-8 antibodies in serum highly associated with coronavirus -19 incidence.

1. Introduction

Coronavirus is a member of the Nidovirales order and family Coronaviridae. Four genera—α-, β-, γ-, and δ-CoV—are used to categorize coronaviruses. CoVs with α- and β-only infect mammals, whereas those with γ- and δ-mainly infect birds. The human coronavirus (CoV) is made up of four types: Sever acute respiratory syndrome (SARS), OC43 and HKU1, β-CoVs (also found in NL63 and 229E), and MERS-CoV (connected to the Middle East respiratory illness). Corona virus disease-19 (COVID-19) is caused by a β-CoV virus that belongs to the same subgenus as the SARS virus but differs in clade similarity from the SARS CoV, according to genomic and phylogenic studies [1].

The worldwide committee on viral taxonomy has proposed the designation SARSCoV-2 for this particular virus [2]. The virus uses the angiotensin-converting enzyme 2 (ACE2) receptor to enter respiratory cells, and the composition of the receptor-binding gene area is strikingly similar to that of the SARS-CoV [3,19].

In Wuhan, Huanan seafood wholesale market, the initial site to which cases of (COVID-19) was linked to a city in the Hubei Province of China. A novel coronavirus was identified as the cause of a cluster of pneumonia cases of unknown etiology at the end of December 2019 [1].

Utilizing specific biomarkers in COVID-19 patient care may help to mitigate or avoid the disease's consequences [4]. The importance of IL-6 in COVID-19, including its connection to the pathophysiology of cytokine storm and disease severity, is already well-established. Tocilizumab, an anti- interleukin-6 receptor monoclonal antibody, has been repurposed in severe COVID-19 patients as a result of this. Nonetheless, several additional chemokines are strongly correlated with the degree of sickness in critical COVID-19, and other variables, including the existence of organ system failure, have been linked to severe COVID-19 along with IL-6, IL-8, and Tumer necrosis factor-alpha TNF-α [5].

2. Methodology

Samples collection

Samples were collected from the beginning of November 2021 until the end of January 2022. The patients' ages ranged from 20 to 75. 40 patients suffered from COVID-19 (Patient group) and 40 person as healthy control group . The blood sample was collected from the patients who attended Al-Zafaraniyah General Hospital, Fatima-Alzahra Hospital, and Al-Yarmook Teaching Hospital in Baghdad City. Nasopharyngeal and oropharyngeal swabs for each patient were taken at the same time and added immediately into viral transport media (VTM).

Overview of interleukin

An enzyme-linked immune sorbent assay (ELISA) method was used to detect in the serum of coronavirus (COVID-19) patients. Screen kit (Demeditec/China) was used in this assay.

The IL-6, IL-8 and TNF-α human ELISA is a solid phase enzyme amplified sensitivity immunoassay performed on micro titer plate. The assay uses monoclonal antibodies directed against distinct epitopes of IL-6, IL-8 and TNF-α. Calibrators and samples react with the capture monoclonal antibody coated on microtiter well and with a monoclonal antibody labelled with horseradish peroxidase. After an incubation period allowing the formation of a sandwich: coated MAb 1-human - MAb 2, the micro titer plate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the micro titer plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance, which is proportional to the concentration. A calibration curve is plotted and IL-6, IL-8 and TNF-α concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA reader and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration rang

Statistical analysis

The SAS (2012) software was utilized to examine the impact of various variables on the research parameters. In this study, the chi-square test was used to compare percentages significantly, and the least significant difference (LSD) test was employed to compare means significantly [7].

3. Results and discussion

Molecular detection of coronavirus by RT-PCR

Every patient had nasopharyngeal and oropharyngeal samples that were chosen and subjected to an RT-PCR coronavirus assay. Fifty samples had coronavirus detection; the coronavirus was identified via assaying a subset of these samples. One complimentary test that was run was RT-PCR, Fig 1. Results of Some Immunological antibodies in COVID-19 patients have been studied as Biomarker IL-6 antibodies and IL-8 antibodies, Table 1.

The effect of COVID-19 on the respondents regarding the IL6 was shown by statistical analysis, which showed that all respondents who tested positive for SARS scored higher than those who tested negative for all tests. Table (2) shows that the mean of IL6 in the positive is 126.43 with a standard deviation of 63.97, while the mean of the negative cases was 20 with a standard deviation of 4.6, so there were significant differences between the two groups, where Pvalue = 0.000, which is smaller than sig = 0.05. The current study's findings are consistent with previous research, which indicated a significant correlation between elevated IL-6 levels and worse clinical COVID-19 outcomes, such as ICU hospitalization and acute respiratory distress syndrome (ARDS). Furthermore, individuals with such complex COVID-19 variants had higher blood IL-6 levels [8,18]. Additionally, the findings showed that a longer COVID-19 is linked to elevated IL-6 (9). Furthermore, according to Kareem *et al.* [10], serum IL-6 is a crucial predictor of COVID-19 severity in patients residing in Baghdad, Iraq. Additionally, the findings of Rasheed *et al.* [11] revealed that the majority of COVID-19 patients in

Baghdad, Iraq, who were moderately to severely afflicted, had significantly raised levels of IL6.

The statistical analysis also showed the effect of SARS on the respondents' IL8, with an average of 30.33 and SD of 8.16 in the negative instances and 119 in the positive case, according to the data. Furthermore, notable distinctions exist between the two cohorts. The current study's findings are consistent with previous research, which found that elevated levels of IL-8 are biomarkers for the prognosis of coronavirus illness patients in 2019 (10).

Additionally, Li et al.'s findings [12] discovered that patients with severe or serious COVID-19 had longer disease durations when their blood levels of IL-8 were elevated (Fig 2).

Additionally, the current study's findings support the notion of a positive correlation between inflammation and the (SARS-CoV-2) [13]. Furthermore, the current study's findings support previous research, which suggested that IL-8 may be a useful predictive indicator of disease severity in COVID-19 patients [14.20].

The current study's findings are consistent with prior research, demonstrating a strong correlation between elevated TNF levels and COVID-19 illness severity [15]. Furthermore, Mortaz *et al.* [16] revealed that individuals with severe COVID-19 regularly had elevated blood levels of TNF-α. The average and deviation of ages were calculated for males and females; the average age for males was 40.6 years, with a standard deviation of 11.59, while for females, it was 41.48 years, with a standard deviation of 13.96. There are no significant differences at the gender level if the p-value = 0.862, which is more critical than sig = 0.05, Table 3.

Distribution of COVID-19 patients according to the vaccination

A vaccine homogeneous sample was taken from respondents who had previously taken two doses, one dose and had not taken any dose, and the results were as follows: The IL6 test showed that there were significant differences between the unvaccinated and the vaccinated, while there were no significant differences in people who took one dose and two doses. The IL8 test showed that there were substantial differences between the three groups, and after conducting the (LSD) test, the three groups showed that they were different from each other in terms of influence. As for the TNF test, there are no significant differences between the three groups [17], Table 4.

4. Conclusion and future scope

This report exhibits a remarkable association between patients who suffered from COVID-19 infection and immunological markers. The results showed that IL-6 antibodies and IL-8 antibodies in the serum of COVID-19 were highly associated with the incidence of COVID-19. The report explains that the highest IL-6 antibodies and Interleukin-8 antibody concentrations were higher in 50 COVID-19 patients.

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Author contributions

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Datasets/Data Availability Statement

The data supporting the findings of this study are available within the article and its supplementary material.

Ethical Approval

The institute's required approval was achieved according to the Helsinki Declaration.

Conflicts of interest

None.

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