

Evaluation of Interleukin-8, Tumor Necrosis Factor-Alpha, and Interferon-Gamma in COVID-19 Patients with and without Chronic Diseases

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) is a highly infectious respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since its emergence in Wuhan, China, in December 2019, COVID-19 has rapidly spread worldwide, leading to a global pandemic declared by the World Health Organization (WHO) on March 11, 2020. The severity of COVID-19 symptoms varies, with individuals having chronic diseases experiencing a heightened inflammatory response, which may contribute to worse clinical outcomes. Pro-inflammatory cytokines, such as interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ), play a critical role in the immune response to SARS-CoV-2 infection. **Objective:** This study aimed to evaluate serum levels of IL-8, TNF- α , and IFN- γ in COVID-19 patients with and without chronic diseases and compare them with control groups of healthy individuals with and without chronic diseases. **Methods:** A case-control study was conducted, measuring IL-8, TNF- α , and IFN- γ levels in COVID-19 patients using enzyme-linked immunosorbent assay (ELISA). The study included COVID-19 patients with chronic diseases, COVID-19 patients without chronic diseases, and two control groups consisting of healthy individuals with and without chronic diseases. **Results:** IFN- γ levels were significantly higher in COVID-19 patients with chronic diseases compared to the control group with chronic diseases ($p = 0.0005$) and were also significantly elevated in COVID-19 patients with chronic diseases compared to those without chronic diseases ($p = 0.036$). IL-8 levels were significantly higher in COVID-19 patients with chronic diseases than in those without chronic diseases ($p < 0.001$) and were also elevated in COVID-19 patients with chronic diseases compared to the control group with chronic diseases ($p < 0.01$). Additionally, TNF- α levels were significantly increased in COVID-19 patients with chronic diseases compared to the control group with chronic diseases ($p = 0.029$). **Conclusion:** The findings indicate that COVID-19 patients with chronic diseases exhibit a heightened inflammatory response, characterized by elevated levels of IL-8, TNF- α , and IFN- γ . These cytokines may contribute to disease severity and poor outcomes in patients with underlying health conditions. Further studies are recommended to explore the potential of these inflammatory markers in predicting COVID-19 progression and guiding clinical management strategies.

Keywords: IL-8, TNF- α , IFN- γ , ELISA, Chronic Diseases.

INTRODUCTION

Acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the COVID-19 disease, appeared in December 2019 in China [1], and rapidly spread around the world, being declared by the World Health Organization as a global pandemic in March 2020 [2, 3]. SARS-CoV-2 is an enveloped, positive-sense, and single-stranded Ribonucleic acid (RNA) beta-coronavirus, capable of infecting animals and humans. Regarding its biochemical constitution, the SARS-CoV-2 genome contains around 30,000 nucleotides, encoding structured proteins such as spike (S), membrane (M), envelope (E), and hemagglutinin esterase (HE), and nucleocapsid (N) [4]. The two SARS viruses' S proteins were shown to have similar and low nanomolar binding affinities to human angiotensin-converting enzyme 2 (ACE2), the host surface receptor that the viruses exploit to enter cells [5]. Symptoms of COVID-19 include fever, cough, shortness of breath, tissue damage, multiorgan failure, and death. The outcomes of COVID-19 vary from mild symptoms to acute resolved or fatal pneumonia. Most COVID-19 patients were classified as mild (81%), 14% as severe, and 5% as critical. The overall case-fatality rate (CFR) was 2.3% and all the deaths were from critical patients who represented

about almost half of critical cases [6]. Elderly patients and those with preexisting co-morbid conditions have higher risks to develop critical illness and die from the COVID-19 disease [7]. SARS-CoV-2 infection induces exuberant inflammatory responses by secretion of many of cytokines such as IL-8, TNF- α and IFN- γ . Interleukin-8 is a member of the chemokine family. Among the first chemokines discovered, IL-8 was identified as a chemotactic factor secreted by activated monocytes and macrophages that promotes the directional migration of neutrophils, basophils, and T lymphocytes [8]. IL-8 was found to play an important role in autoimmune, inflammatory, and infectious diseases, because of its potent pro-inflammatory properties, IL-8 is tightly regulated, and its expression is low or undetectable in normal tissues [9]. It exerts its effects on neutrophils via two different cell surface receptors known as CXCR1 and CXCR2, respectively. CXCR1 and CXCR2 were the first chemokine receptors to be identified and are the major chemokine receptors on neutrophils [10]. TNF- α is one of the most critical pro-inflammatory cytokines of the innate immune response and mediates pleiotropic effects, which implies action on the production of inflammatory mediators, cell proliferation, and cell

death. TNF is produced by macrophages, T-cells, B-cells, natural killer(NK) cells, dendritic cells, and fibroblasts [11]. TNF- α exists in a soluble and transmembrane form. The processed sTNF- α then facilitates various biological activities through type 1 receptors (TNFR1) and type 2 receptors (TNFR2). tmTNF- α also acts on both TNFR1 and TNFR2, but its biological activities are expected to be mediated mainly through TNFR2. In general, TNF- α binds to its receptors, mainly TNFR1 and TNFR2, which initiate signal transduction pathways. These pathways lead to various cellular responses, including cell survival, differentiation, and proliferation [12]. INF- γ is a type II interferon that is produced by various cells, such as CD4+ T cells [13]. The production of IFN- γ is mainly regulated by natural killer (NK) and natural killer T (NKT) cells in innate immunity while CD8+ and CD4+ T-cells are major paracrine sources of IFN- γ during adaptive immune response [14]. Functionally, IFN- γ can bind to the IFN γ R1/2 to activate the Januskinase(JAK)signal transducer and the transcription protein(STAT)pathway to coordinate different cell functions, such as immune regulation, leukocyte transportation, cell proliferation, apoptosis, anti-microbial, antitumor, and pro-tumor effects [15].

MATERIAL AND METHODS

Study Design

This study included Sixty sample of patients of COVID-19, 30 sample with chronic diseases (16 male and 14 female) and age range between (28-89) years, and 30 sample without chronic diseases (16 male and 14 female) with age range between (17-80) years. All samples of patients were collected from Al-hayat Center in Al-Zahraa Teaching Hospital, and from external laboratories after confirming their infection using nose swab PCR test, in Wasit Province, Iraq. While, samples of control groups involved 20 sample, 10 control samples with chronic diseases (4 male and 6 female) and age range between (30-60) years, and 10 control samples without chronic diseases (2 male and 8 female) with age range between (16-51) years. Control samples were collected from people not infected with covid-19 and was used covid-19 rapid test to prove it.

Methods

A-Samples of collection

Venous blood sample of 5 ml was drawn using a sterile syringe, which can be disposed of after use. Then, we put 5 ml of blood in a gel tube for the purpose of the immunological study (ELISA) enzyme-linked immune-sorbent assay and left the sample for 15 minutes at room temperature (20-25 °C) after that we put the samples in a centrifuge approximately 2500-3000 cycles per minute and for 5 minutes to obtain the serum, We distribute the serum into three eppendorff tubes, after which the tubes were placed in the freezer at a freezing degree of -20°C.

B- Serological Test (ELISA)

This test was used to evaluate the presence of Human Interleukin8(IL-8), Tumor Necrosis Factor-alpha(TNF- α) and Interferon gamma(IFN- γ) using enzyme-linked immunosorbent assay technique (ELISA). ELISA kits (YLA1210Hu, Human IL-8), (YLA1337Hu, Human TNF- α) and (YLA1525Hu, Human IFN- γ) for (Shanghai YL Biotech. Co., Ltd./ China) according to the instructions of the manufacturer.

Statistical Analysis

The data were analyzed using the following software, Microsoft excel, IBM SPSSV26. The results reported in this study were expressed as mean $\pm$ SD.

Results & Discussion

Comparative analysis of IFN-gamma Levels in patients with and without chronic diseases and the control group.

The presented data showed that there was significance difference ($P=0.036$) in serum level of IFN- γ between patients with and without chronic diseases, and also significance difference between patients with chronic diseases and control group ($P=0.00005$). The means were (153.335) mg/dL in patients of chronic disease, (127.301) mg/dL in patients of non- chronic disease, and (98.824) mg/dL in control group of chronic disease shown in table (1).

Table 1: Analysis of IFN-gamma Levels measured in mg/dL by ELIZA method in covid-19 patients with and without chronic diseases and control group.

IFN- γ	Mean	$\pm$ SD	SE	P-value
Patients of Chronic Disease	153.335	$\pm$ 37.324	6.814	0.036
Patients of Non- Chronic Disease	127.301	$\pm$ 55.085	10.057	
Patients of Chronic Disease	153.335	$\pm$ 37.324	6.814	0.00005
Control of Chronic Disease	98.824	$\pm$ 3.677	1.163	

The mean IFN- γ level in group of patients with chronic diseases is notably high at 153.335, suggesting a pronounced presence or activation of this cytokine among these individuals. The standard deviation (SD) is 37.324, indicating a considerable spread in the IFN- γ levels within this group, reflective of individual variations in disease state or immune response. Results shows strikingly low p-value of 0.00005 strongly suggests that the observed differences in IFN- γ levels are statistically significant, possibly reflecting an altered immune status in this group. This analysis reveals a significant difference in IFN- γ levels between patients actively suffering from chronic diseases in coincide with covid-19 infection in compare with control group.

The elevated IFN- γ levels in the former group could be indicative of a strong immune response, potentially linked to the covid viral

infection leads to exacerbation by coincidence with chronic diseases. In COVID-19 patients with chronic diseases, as in study by Zheng *et al*, [16], indicate that serum level INF- γ were higher in COVID-2019 patients with diabetes mellitus(DM)than patients with non-diabetes mellitus (NDM) ($P < 0.05$). His study showed the immunological changes in COVID-19 patients with DM, who presented a higher percentage of CD4+, but a lower percentage of CD8+ than those in nondiabetic patients. Most of the patients [52 (73.24%)] presented no increased CD4+ percentage, and 19 (26.76%) of patients presented increased abnormal CD4+ percentage, especially in the patients with DM, which was not completely identical to the results of other studies. Qin *et al*, [17], reported that the decrease of CD4+ Tcells and no significant change in the number of CD8+ cells were common among patients with COVID-19, especially in severe cases. Chen *et al*, [18],

reported that lower CD4⁺ T cell count was an indicator of immunosuppression and was significantly associated with ICU admission.

Therefore, COVID-19 might damage T lymphocytes, and the immune disorders occurred during the period of disease, especially in patients with DM. Patients with SARS-CoV-2 infection had high levels of various cytokines, specifically IFN- γ was identified as a potent marker of disease severity that can be identified as an indicator of disease progression and a therapeutic goal [19].

Table 2: Analysis of IL-8 Levels measured in mg/dL by ELIZA method in covid-19 patients with and without chronic diseases and control group.

IL-8	Mean	$\pm$ SD	SE	P-value
Patients of Chronic Disease	112.938	43.019	7.854	< 0.001
Patients of Non- Chronic Disease	48.394	13.746	2.510	
Patients of Chronic Disease	112.938	43.019	7.854	< 0.01
Control of Chronic Disease	50.682	6.904	2.183	

Here in this study IL-8 level been measured in different groups of study. The results shows clearly that COVID-10 patients who had chronic diseases coincide with show much high IL-8 level, indicating a significantly higher IL-8 level in patients with chronic diseases compared to the ones with no chronic diseases (p -value < 0.001), also its shows significant increase in IL-8 level in patients with chronic diseases compared to the control group of chronic disease (p -value < 0.01), shown in table (2). Thus, IL-8 is clearly a pro-inflammatory cytokine that may recruit neutrophils to the infected areas and has been associated with tissue damage. IL-8 can be used as a biomarker for the disease prognosis of COVID-19 patients and as a potential therapeutic target to treat COVID-19 patients. Plasma levels of IL-8 were elevated in both mild and severe COVID-19 patients and increased with disease progression [20, 21]. In study by Kesmez Can *et al.*, [22], revealed that IL-8 level was the highest in the severe patient group (140.40 ± 65.03) and also higher in the mild-moderate group (76.79 ± 25.78) as compared with the healthy volunteers (22.54 ± 6.49) with Statistically significant difference ($P = 0.001$). Also, in study by Li *et al.*, [20], found that IL-8 levels in the sera of COVID-19 patients were significant higher compared to healthy people. In COVID-19 patients with chronic diseases, as in study by Iskandar *et al.*, [23], found that level of IL-8 was significantly higher ($p < 0.05$) in COVID-19 patients with diabetes mellitus (DM) comorbidity compared to those without

Comparative analysis of IL-8 Levels in patients with and without chronic diseases and the control group.

The findings in this study showed serum level of IL-8 in patients with chronic diseases was significantly higher than patients without chronic diseases ($P < 0.001$), and also there was significance difference ($P < 0.01$) in level of IL-8 between patients with chronic diseases and control group. The means were (112.938) mg/dL in patients of chronic disease, (48.394) mg/dL in patients of non- chronic disease, and (50.682) mg/dL in control group of chronic disease shown in table (2).

DM, this due to decreased immune function, endothelial and alveolar dysfunction, hyperglycemia and also hypercoagulable state.

Comparative Analysis of TNF- α Levels in Patients with Chronic Diseases and the Control Group.

The current study found significance difference in serum level of TNF- α in patients of chronic diseases compared with control group of chronic diseases ($P = 0.029$). The means were (71.509) mg/dL in patients of chronic disease, (43.474) mg/dL in control group of chronic diseases, shown in table (3). The results below found significance difference in serum level of TNF- α in patients of chronic diseases compared with control group of chronic diseases ($P = 0.029$). In patients with certain chronic diseases, like rheumatoid arthritis or inflammatory bowel disease, often have elevated baseline levels of TNF- α due to the inflammatory nature of their conditions.

On the other hand, level of TNF- α has been consistently higher in severe COVID-19 patients and those who suffer from comorbidities such as obesity, hypertension, and chronic heart failure [24]. This provides robust evidence for adopting TNF- α as a more promising predictor for the prognosis of severe and life-threatening forms of COVID-19 [25].

Table 3: Analysis of TNF- α Levels Measured in mg/dL by ELIZA Method in Covid-19 Patients with Chronic Diseases and Control Group.

TNF-alpha	Mean	$\pm$ SD	$\pm$ SE	P-value
Patients of Chronic Disease	71.509	58.02	10.593	0.029
Control of Chronic Disease	43.474	20.17	6.379	

Conclusion

Level of IFN- γ in patients with and without chronic diseases higher than control groups. This indicator that IFN- γ is a key cytokine in the immune system and has been the subject of extensive research in the context of COVID-19, especially concerning patients with chronic diseases.

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