JJBS

Volume 15, Number 3,September 2022
ISSN 1995-6673
Pages 423 – 429
https://doi.org/10.54319/jjbs/150311

Jordan Journal of Biological Sciences

Role of IFN-γ, TNF-α, IL-6 and C-Reactive Protein in Newly Diagnosed Iraqi Corona Patients

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Received: June 24, 2021; Revised: October 2, 2021; Accepted: October 16, 2021

Abstract

The exceptional conditions sweeping the world due to the Corona virus epidemic have prompted researchers to race to study each of the symptoms, phenomena and relevant clinical biochemical parameters to provide science and scientists with valuable information to achieve victory over the virus. The aim of this investigation is to study the early inflammatory features caused by the immune system before a cell storm occurs in Iraqi Corona patients. The investigation was conducted at Yarmouk Teaching Hospital, Baghdad, Iraq, during the period from January 2021 until the end of March 2021. Our team obtained five milliliters of venous blood from 50 participants newly diagnosed with the Coronavirus (24 males and 26 females). Their ages ranged between (25-55) years compared to 38 individuals (18 males and 20 females). Corona virus patients had statistically significant higher ($P<0.01$) with Low density lipoproteins-cholesterol (LDL-C), urea, C-Reactive Protein (CRP), and ($P<0.001$) with D-dimer when they were compared with control group. There was a significant increase in the value of Interleukin-6(IL-6) in people infected with the virus compared to the reviewers whose swab results showed that they were not infected with the virus. For both interferon-ɣ (IFN) and Tumor necrosis factor -α (TNF- α), the data showed a significant decrease in morale of reviewers diagnosed with acute respiratory syndrome (COVID-19) against their non-infected peers. These data indicate that early intervention for IFN antiviral infection could be fundamental in inhibiting fibrosis to improve functional recovery. Any source of cytokine control, such as interferon-ɣ and Tumor necrosis factor -α combined with combination therapies for clinical treatment, will be important in the future for COVID-19 infection.

Keywords: Covid-19, Interleukins, Cytokine storm, C-reactive protein, Lung fibrosis.

1. Introduction

The emergence of a new strain of the respiratory syndrome SARS-Cov-2 in 2019 has been announced globally (Johnson *et al*.,2020). The World Health Organization has launched the term pandemic for this disease, as viruses have widely and rapidly moved from the source of the virus to the rest of the world. The disease has also been called Corona virus 2019 (Covid-19). This disease unknown origin, and it is believed that this virus was transmitted by eating animals that are believed to provide a suitable medium for the development of this strain (Elmasry *et al*., 2020). Infection with this disease occurs when the virus penetrates the human respiratory system and multiplies in the lung. Its speed of spread is due to its ability to remain effective on contaminated surfaces or to transmit through sneezing or coughing and direct contact with infected people (Mohadeseh *et al*.,2021). Coronavirus infection stimulates the body's immune system, at which point the cytokine storm begins, which is the most dangerous cause of acute respiratory distress syndrome (ARDS) and lung fibrosis. Many researchers have provided evidence confirming the association of the inflammatory effect with signs of lung fibrosis in people with coronavirus 2019 (Zhong-Jie *et al*.,2020). The virus has been described as a very fine nanostructured structure surrounded by the lipid sheath of the nucleocapsid, and inside it there is the nucleoprotein (N) and the positive-sense single-stranded RNA ((+) RNA ss) that allows a virus to force human cells to produce virus particles. It binds to the N protein to (+) SSRNA from its N terminus and leads to viral transcription and repetition. Externally, the membrane protein (M) surrounds the surface of the virus more than once, as it works to stack its wallSARS-CoV-2. It also appears under the electron microscope of a spike-like glycoprotein (S). It is the last transmembrane protein that comes outside the surface and regulates its binding to the host cell receptor, leading to the entry of the virus in the host cell (Kirchdoerfer *et al*.,2016). (E) the envelope consists of tiny amino acids united parts .The minor part of the virus plays a critical role in synthesizing the virus. Fig. 1. shows a hypothetical drawing of the SARS-CoV-2 structure (Mohadeseh *et al*.,2021). The human immune system identifies the virus as soon as it enters the body, and then it summons immune factors such as immune cells or first-line receptors (Woo *et al*.,2010; Zhong *et al*.,2003; Drosten *et al*.,2003). Generally, most of prognostic factors related to the varieties of interleukins. Many of severe disease cases which led to death, was due to (pro-inflammatory innate immunity, anti-inflammatory system)

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(Víctor *et al*.,2020), where the virus is identified through pathogen recognition receptors (PRRs) as well as by stimulating cells to interferon (IFN). The mechanisms of action of such viruses include reducing the activity of innate human immune cells by inducing variables in cytokine secretion so that the virus produces IgM and IgG. High IFN had a positive effect on patients' resistance to viral infections; but at low levels of this cytokine, symptoms occur and the complications of SARS-CoV-2 infection and death increase (Miriam and Jerome 2020). T-lymphocytes produce a cytokine that is necessary in cases of an immune response. IFN reduces the proliferation of viruses and acts as a wall of counterattacks. The production of cytokine by T cells increases the toxicity of these cells and thus increases the incidence of lymphocyte mortality (Dalia *et al*.,2021). The continuous rise in IFN results in a doubling of systemic inflammation, increased tissue injury, and organ failure (Lindsay 2016). Recent studies reported severe cases of Covid-19 as a result of pulmonary and systemic thrombosis occurring at low levels of INFs, while moderate levels of IFN lead to a delay in SARS-Cov-2. That is, elevated immunoglobulins are interpreted as a positive therapeutic marker (Mohammed and Asmaa 2019). Besides, a cytokine storm is also present in severely ill patients with elevation of interleukins such as TNF-α and IL-6(Huang *et al*.,2020; Hahn *et al*.,2015). Thus, the host differences are immune responses playing a major role in the severity of COVID-19(Víctor et al.,2020). It is good to understand the effect of interleukins and their role as a diagnostic factor for the severity of Covid 19 patients, which is one of the objectives of this study. The aim of this study is to find the role of IFN-γ, TNF-α, IL-6, C-Reactive Protein and other biochemical parameters in Iraqi Corona Patients, and to demonstrate the early inflammatory features induced by the immune system before the occurrence of a cellular storm.

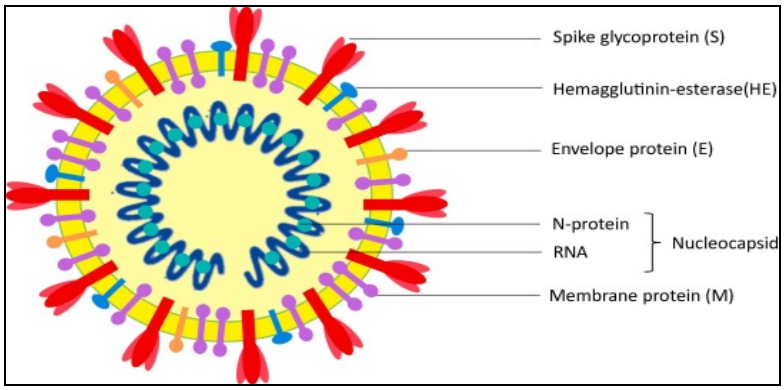

Figure 1. An illustration of the main components of SARS-CoV-2.

2. Materials and Methods

2.1. Patients and control

This investigation was conducted at Yarmouk Teaching Hospital, Baghdad, Iraq, during the period from January 2021 until the end of March 2021. Our team obtained five milliliters of venous blood from 50 participants newly diagnosed with the Coronavirus (24 males, 26 female). Their ages ranged between (25-55) years and compared to 38 people (18 males and 20 females), and it was proved by using a swab that they were not infected with the previously mentioned virus. It should be noted that all patients did not receive any treatment while conducting the research examinations. Patients' data were documented during a private medical interview along with their medical history.

2.2. Methods

2.2.1. Anthropometric Measurement:

Blood pressures were recorded according to the guidelines adopted by (WHO 2019), and body mass index was determined by dividing subjects' weight (Kg) by their height (m^2). BMI= mass (kg)/ (height $(m)^2$) (Simon et al.,2005).

2.2.2. Biochemical Assessment:

Glucose was determined by using the enzymatic colorimetric method (Trinder 1969), serum TC was measured by using an enzymatic method (Richmond1992), serum TG was measured by using method (Fossati and Prencipe 1982), and serum HDL-C was measured by HDL-C kit using an enzymatic method (Lopes-Virella *et al*.,1977). LDL-C was calculated, using (Nauck et al.,2002), the blood urea and serum creatinine were measured, according to an enzymatic method (Helmut and Yvette 1959). Furthermore, C-Reactive Protein (crp) was determined by using automated analyzer (BIOLABO), D – Dimer concentration measured according to minividas kits supplied by Bio Meriux-France., and Serum Biomarkers INF-ᵧ, TNF-α and IL-6 concentrations were measured by the (Biosours) ELISA kit.

2.3. Statistical analysis

All data were expressed as (mean ± SD). Statistical significance was considered at the level of ($P<0.001$),($P<0.01$), and ($P<0.05$). Analysis of variance (ANOVA) for equality of means (testing of coincidence). Statistical analysis used (SPSS programs: Statistical Package for Social Sciences, version 22 (SPSS, Chicago, IL, USA).

3. Results

By tracking (Table 1) which shows the general characteristics and the parametric measurements of the patients who were received in the aforementioned hospital, it was found that the average age was 39.5 for those infected with COVID-19, while the average age of the control group was 29.5, and no significant differences were shown for the ages participating in this research. The number of infected women participating was 52%, while the number of infected men participating was estimated at 48%. The data obtained from the aforementioned table did not record any significant differences between the comparison groups at the level of weight, height and body mass index. At the same time, a significant increase of (P< 0.05) was recorded in the DBP and SBP values, by comparing those infected with the virus and the group of healthy controls. The results obtained are similar to research conducted by researchers from Bangladesh (Syeda *et al*.,2021).

Table 1. Anthropometric Measurements between Patients of Corona Virus& Control

Parameters	COVID-19 Patients Positive Mean ±SD N=50	Control (COVID-19 Patients Negative) Mean ±SD N=38	P-value
Age (Years)	39.5±8.68	29.5± 5.71	0.156 NS
Sex (M/F)	(24/26)	(18/20)	/
Wights	73.3125± 10.77	68±12.11	0.143 NS
Height	168.875±5.20	169±5.73	0.124 NS
BMI (Kg/m^2)	25.68±3.41	23.69±2.94	0.06 NS
SBP (mmHg)	136.875±6.29	123.75±5.18	0.05*
DBP (mmHg)	86.25±5.63	77.5±4.6291	0.05*

NS: Non-Significant, * (P< 0.05) Significant.

As inferred in Table 2, the serum of each of the FBS, TC, TG, HDL & Creatinine level have no significant between the competition groups. However, upon reaching the determination of LDL & Urea values, we found that they bear significance with significantly higher significant differences for the affected patients compared to the healthy ones. Our results are consistent with other studies (Syeda *et al*.,2021; Abdulla *et al*.,2020; Isabella Marcia *et al*.,2020).

Table 2. Mean ±SD Characteristics of some Biochemical Parameters in both Covid-19 & Control Patients′

Parameters	COVID-19 Patients Positive Mean ±SD N=50	Control (COVID-19 Patients Nagative) Mean ±SD N=38	P-value
FBS (mg/dl)	98.438±4.24	90.25±4.77	0.106 NS
TC (mg/dl)	177.625±21.96	152.875±20.82	0.107 NS
TG (mg/dl)	111.9375±19.14	95.625±12.67	.0163 NS
HDL (mg/dl)	47.75±2.77	53.375±4.40	0.136 NS
LDL (mg/dl)	137.25±12.03	83.35±8.44	0.01**
urea(mg/dl)	66.875±5.40	29.375±2.06	0.01**
creatinine(mg/dl)	1.163±0.140	0.7±0.075	0.06 NS

NS: Non-Significant, **(P<0.01) and (P<0.001) high significant.

The biochemical measurements taken from Table 3 show an increase with a highly significant difference of (P<0.01) and (P<0.001) in both D-dimer & CRP for people who tested positive for coronavirus than for non-infected people.

Many studies give support to the phenomena that we obtained, such as the studies of each of (Zhu *et al*.,2020; Zhong-Jie *et al*.,2020; Moutchia *et al*.,2020).

Table 3. Comparative of CRPand D-Dimer concentration Covid-19 &Control Patients

Parameters	COVID-19 Patients Positive Mean ±SD N=50	Control (COVID-19 Patients Negative) Mean ±SD N=38	P-value
CRP (g/dl)	8.5±1.37	2.1875±0.56	0.01**
D-Dimer (ng/ml)	821.875±80.25	144.875±23.012	0.0001**

**(P<0.01) and (P<0.001) high significant

Interleukin values are identical to some extent with other studies that included the same field, as there was a significant increase in the value of IL-6 in persons diagnosed with the virus compared to the reviewers whose negative swab test results showed that they were not infected with the virus in question.

As for each of (IFN -ɤ& TNF- α), it is evident from the data a significant decrease in the morale of the auditors

diagnosed with acute respiratory syndrome (COVID-19) against their uninfected peers. All that has been mentioned previously for the values of interleukin can be tracked by looking at Table 4.

Table 4. Comparative of Interleukins for Covid-19 &Control Patients

Parameters	COVID-19 Patients Positive Mean ±SD N=50	Control (COVID-19 Patients Negative) Mean ±SD N=38	P-Value
IL-6(pg/ml)	46.056± 8.08	15.55±1.87	0.001**
IFN- ɤ (pg/ml)	6.881±1.18	40.375 ± 9.04	0.0001**
TNF- α (pg/ml)	27.544±5.2	44±4.9	0.001**

**(P<0.01) and (P<0.001) high significant

4. Discussion

We are currently facing the largest global health emergency in decades, the devastating outbreak of COVID-19(Wu and McGoogan 2020). Even with a preventive vaccine, it will be critical to determine whether exposed and /or infected people, especially those with mild or asymptomatic forms of the disease who are likely to unintentionally act as major carriers, develop a robust adaptive immunity against SARS-CoV-2(Long *et al*.,2020).

The reports approved by the World Health Organization based on the data collected from different countries, one third of the patients infected with the virus currently spreading have suffered from acute respiratory distress syndrome (ARDS), which is considered the greatest cause of death or may cause later pulmonary fibrosis in survivors (Hui *et al*.,2018). Aging, obesity, diabetes, cardiovascular disease, and high blood pressure are pre-existing factors that increase the risk of severe infection and death (Norbert *et al*.,2021).

Age is an influential factor that plays a very important role in overcoming infection with the emerging coronavirus, as the elderly are more likely to die when infected (Merad and Martin 2020). It has been shown that most of the elderly suffer from chronic diseases, problems such as diabetes, pressure, and other disorders (Norbert *et al*.,2021). An immune aging factor is an additional reason for the increasing of vulnerability of the elderly to complications from the infection (Roel *et al*,2020). The immune system changes dramatically and dramatically over time and behaves inconsistently in elderly people, in particular, because it either treats the infection excessively and thus leads to major infections, or it is too late to react to (Parmigiani *et al*.,2013). The danger is like the virus entering the body, getting worse, and then causing death. It has been revealed that the immune system changes dramatically and dramatically over time and behaves inconsistently in elderly people in particular, because it either treats the infection excessively and thus leads to major infections, or the uncontrol over it, therefore, the virus is intrusion through body. Hence, a fatal health problem could be reported. (Arne and Derek 2020). An irregular blood pressure is appeared as a result of infection, persistent physical complications and nervous tension. Ultimately, blood pressure has a greatly effects on the patient through the performance effective of the internal organs, including the kidney function efficiency, which it leads to an increase of blood urea level (Guyi *et al*.,2020).

A number of manuscripts confirming the fact of a disorder in metabolism of lipid during SARA-CoV-2 infection, wherefore an increase in the level of low-density lipoprotein (Eva *et al*.,2021), hence that a change in lipid levels had been occurring (Shen *et al*.,2020; Boikova *et al*.,2020). The high biological markers of Covid 19 patients have been observed (Shi et al.,2020); the cause is attributed to such a circumstance that the virus may cause direct damage to the organs as in the lungs, kidneys, small intestine, heart, and arteries due to lack of oxygenation and respiratory failure (Moutchia *et al*.,2020). We note in our study, for example, an increase in LDL and urea for patients diagnosed with a positive result of the virus. Or the reason for the increase in each of the inflammatory indicators, such as high D-dimer (a marker of intravascular thrombosis), CRP, and cytokines, the cause of the increase in the systemic hyperinflammation caused by cytokine is the innate immune system syndrome (Merza *et al*.,2021; Wang *et al*.,2020).

An elevated C-reactive protein is an indication of inflammation, and is very useful in that it shows the body's resistance to disease (Shang *et al*.,2020). Several studies have shown an increase in C-reactive protein levels in patients diagnosed with coronavirus (Javanian *et al*., 2020; Ling 2020). This increase plays a role in the incidence of pneumonia and respiratory failure, and so the increase is associated with the development of ARDS (Terpos *et al*.,2020).

Signs of an overexpression of the innate immune system include an increase in a number of indicators, such as the IL-6A end CRP ratio, so that after a viral infection, the innate immune system will be activated early to limit the reproduction of the virus (Hwaiz *et al*.,2021; Luis 2019). D-dimer elevation indicates an increased risk of abnormal blood clotting (Hai-Han *et al*.,2020), as its measurements are used in clinical analyzes to diagnose deep vein thrombosis or pulmonary embolism (Querol-Ribelles *et al*.,2004). Studies have found that its rate is associated with higher deaths from pneumonia resulting from viral infection (Chen N. *et al*.,2020; Tang *et al*.,2020).

IL-6 is one of the main mediators of the inflammatory and immune response resulting from viral infection (Herold *et al*.,2020; Aziz *et al*.,2020). Therefore, many patients develop a fatal immune reaction with persistent damage by the action of cytokines leading to alveolar infiltration by macrophages and monocytes (Abbasifard and Khorramdelazad 2020).

When infection occurs, the affected cells specifically enhance both cytokines and chemokines, and in copious quantities, resulting in a cytostome that can infect endothelial cells and thin blood vessels with a lack of ischemia and oxygen, then the infection increases (Hu *et al*.,2020). Among the results of our study, it was noted that

the IFN and TNF values are lower in patients diagnosed with positive coronavirus test, which is identical to a study in 21 patients with severe COVID-19 compared to the moderate form of patients (Chen G. *et al*., 2020). It has been shown that there is an inverse relationship with results of IFN levels with cases of pulmonary fibrosis in people with COVID-19, so that IFN is produced by specific antigens or by T cells and natural killer cells. The action of IFN lies in the secretion of defensive macrophages in the host against viral infection, as well as activation of the protease maturation protein (POMP), which enhances antigen activity (Kate and Eileen 2014). IFN has been considered a risk factor for developing lung fibrosis in people with COVID-19 (Zhou, 2009). There is a consensus relationship linking a high level of IFN to the risk of developing cell inflammation caused by SARS-COV-2 infection. Inflammatory particles play a role in determining the type of ARDS and pulmonary fibrosis (Torres Acosta and Singer, 2020). The level of this cytokine is inversely related to lung fibrosis (Keane, 2019). The process of securing these molecules and inhibiting pulmonary fibrosis needs future studies.

5. Conclusion

In conclusion, SARS-CoV-2 infection elicited an inflammatory response and led to the formation of fibrosis in COVID-19 patients even after attenuation of clinical symptoms and negative results from an RT-PCR test for RNA extracted from nasopharyngeal swabs. By following the data of the analyzes conducted during the study, it was evident that there was an increase in the values of CRP, IL-6, Urea, LDL and D-dimer, and yet a decrease in the values of INF and TNF was recorded. Baseline IFN-levels were negatively associated with increased fibrosis volume in COVID-19 upon discharge. These data indicate that early intervention of antiviral infection with IFN- could be fundamental in inhibiting fibrosis to improve functional recovery. Any source of cytokine control, such as IFN-and TNF-α combined with combined therapies for clinical treatment will be important in the future for COVID-19 infection. (Main). It is also reasonable to use markers of immune function to predict disease severity, as a comparison of non-severe or severe Covid 19 is associated with increased signs of the immune response such as IL-6, CRP and increased signs of tissue damage and organ damage as in D-dimer and urea, among other signs.

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