Study of Human Interleukin 12 (IL-12), Human Interleukin 3 (IL-3) and Interferon gamma (IFN-γ) in patients with COVID-19 in Wasit Province, Iraq

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Abstract

In late December 2019, a small number of patients with mysterious fever and signs of lower respiratory tract infections were discovered in Wuhan, China's Hubei province's largest metropolitan city. It was originally identified as pneumonia of unclear etiology because the etiology of this unknown respiratory infection could not be determined. The causative pathogen was identified and designated 2019 novel coronavirus after further investigation by the local authority of the Chinese Center for Disease Control and Prevention. (2019- nCoV). COVID-19, the acronym for "coronavirus disease2019," was announced on February11, 2020 as the disease caused by this new coronavirus. Coronavirus 2 causes severe acute respiratory syndrome (SARS-CoV-2, SARS2, 2019-nCoV or COVID-19 virus), This pathogen causes a syndrome that can lead to a critical care respiratory condition, which requires specialized treatment of certain intensive care units (ICUs).

A study was conducted to study some immunological markers in patients with COVID-19 between patient and control in Wasit province. The results showed a highly significant increase in the IL-12 (62.03%) , IL-3(93.80%) and IFN-γ(13.16%) for patient that have COVID-19 and this results was Compared with controls and this study was divided according to age groups for patients and results showed the highest value for patients that age lowest 40 IL-12 (12.72), IL-3 (94.77) and IFN-γ (64.35) compared with patients that age highest than 40.

Keyword: *COVID-19 virus, Interleukin 12 (IL-12) , Interferon gamma (IFN-γ), Human Interleukin 3 (IL-3).*

INTRODUCTION

Coronaviruses have one of the largest genome sizes of RNA viruses, ranging from 26 to 32 kilobases. They have distinctive club-shaped spikes that protrude from their surface and, in electron micrographs, resemble the solar corona, from which they get their name.

Coronaviruses, which have been isolated from a variety of species, are a group of large, enveloped, single plus stranded RNA viruses that have previously been associated to acute rhinitis and diarrhea in humans (1,2).

The SARS epidemic in 2002–2003 was associated to a human coronavirus known as SARS-CoV (severe acute respiratory syndrome coronavirus). (3,4)

accompanied by viral amplification, host immune responses become activated, which is

supposed to clear the virus and cure the patients. But why a portion of patients had more severe disease development like MODS is still unknown. We hypothesized cytokine storm plays important role in the pathogenesis of severe cases of COVID-19 (5,6).

Cytokine storms can be triggered by various infectious or non-infectious diseases (7), and cause severe damages to multiple organs. Pathogen infections are recognized by the immune system, which consists of two types of responses: an innate immune response that recognizes pathogen-associated molecular patterns (PAMPs) and an antigen-specific adaptive immune response. In both responses, there are several activated cells of the immune system, which play a key role in establishing the environment of cytokines (8.9). However, exaggerated, excessive synthesized cytokines lead to an acute, severe systemic inflammatory response known as “cytokine storm”.

IL-12 important cytokine secreted mainly by macrophages and dendritic cells is interleukin-12, which has two important subunits, including IL-12p35 and IL-12p40. Interleukin-12 can activate IFN-γ secretion in the body through CD T cells (10) IL-12 has been shown to inhibit the replication of viruses by increasing and inducing IFN-γ activity, and can increase the quality of the CD8 + T cell response (11).This type of interleukin acts on its receptor (IL-12R) after being secreted against stimuli such as microbial or viral derivatives. One thing to keep in mind is that it has been shown that these receptors are usually expressed by certain cells, including T and NK cells, and it has also been shown to increase the serum concentration of this interleukin in patients with high COVID-19 infection (11,12).

IFN-γ is another important cytokine that can be made and secreted by NK cells and T lymphocytes and plays an important role in the body's immunity(13). The cytokine IFN-γ is one of the important cytokines that is important and vital for the body's defense against viruses. It has been shown that this cytokine, when the virus enters the body, inhibits the replication of the virus on the one hand and increases the cytotoxic T lymphocyte killing activity in the body on the other hand (14). Various studies have shown that T and NK cells reduce IFN-γ expression in the body when the patient has immunodeficiency. (15).

IL-3 a hematopoietic growth factor produced by T cells and in a lesser extent by mast cells, eosinophils, and innate response activator B cells, was described to play a key role during inflammatory diseases(16).

Material and Methods

Study design

This study was approved by the AL-karama Teaching hospital and External laboratories after fixing their infection, in Wasit provinces/Iraq , All patients (n=40) with COVID-19 enrolled in this study were diagnosed with SARS-CoV-2 infection between December 2021 to May 2022.

the patients age range was from 14 to 70 years , there were 18 male and 22 female, the whole blood collected after diagnosed with SARS-Cov-2(positive nasopharyngeal swab for SARS-Cov-2), and C-reactive protein and CBC reporter and 40 control without COVID -19, there were 27 male and 13 female, the patients age range was from 9 to 55 years Control samples were for people who did not suffer from blood pressure or other diseases and used covid-19 rapid test to prove not infection COVID -19.

Laboratory examination of blood samples

Approximately 3-5 ml of peripheral blood was obtained with collection tube from the subjects in each group, serum samples were separated by 2000 rpm /20 min centrifugation.

IL-3 , IL-12 and IFN-γ tested using Enzyme-Linked Immunosorbent Assay (ELISA) kit the step of work was including Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature, Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C, Add 50ul standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody, Add 40ul sample to sample wells and then add 10ul Human (IL-3, IL-12 and IFN- γ) antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C, Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material, Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark, Add 50ul Stop Solution to each well, the blue color will change into yellow immediately, Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Statistical analysis

SPSS 22.0 statistical software was used for statistical analysis. Count data were analyzed by the $\chi 2$ test. A P value < 0.05 indicates statistical significance.

Results and Discussion

This study was designed to search for some immunological markers in patients with COVID-19 in 80 samples as a case group compared with control ,used 40 patient 40 control for immunological study by enzyme-linked immunosorbent assay technique (ELISA).

As illustrated in Table (1) that presented the serum level of IL-3 in patients and control groups, the statistical analysis of data observed that there is a significant increasing ($p<0.001$) in IL-3 levels in SARS-Cov-2 patients in comparison to control group (healthy individuals). The means were (62.93±3.44) and (93.80±7.29) pg/mL in control and patients groups respectively.

Table (1): The mean of IL-3 in patients and controls.

Parameter	Groups	Mean±S.E (pg/mL)	P value
IL-3	Control	62.93±3.44	<0.001**
	Patients	93.80±7.29	

The results of the current study showed that the percentage of IL-3 in patients was higher than that of controls this is due to immunity system was activation IL-3 by B and T cells after personls infection with covid-19. This result was disagreed with (17) suggesting that plasma IL-3 does not appear to feed the cytokine storm, and plasma IL-3 levels were related with disease severity in SARS-CoV-2 infections. from this vantage point. And agreed with (18) In our COVID-19 case patient, levels of interleukin 3 (IL-3), which is secreted by activated T cells, were high. Since inflammatory monocytes and neutrophils are produced more readily as a result of IL-3,

sepsis from various etiologies is associated with a cytokine storm.

In addition, IL-12 serum level also significantly elevated (p<0.001) in SARS-Cov-2 patients as compared to control group, where the mean of IL-12 was (13.16±1.10) pg/mL in patients and (7.98±0.42) pg/mL in control group (Table 3-2).

Table (2): The mean of IL-12 in patients and controls.

Parameter	Groups	Mean±S.E (pg/mL)	P value
IL-12	Control	7.98±0.42	<0.001**
	Patients	13.16±1.10	

The results of the current study showed that the percentage of IL-12 in patients was higher than that of controls and values of cytokines IL-12 significantly higher in patients with COVID-19 than in healthy controls (P<0.001) , IL-12 production, and secretion is associated with virus entry into the cell and it rapidly induces the gene expression of IL-12. In addition, the next important point, this interleukin has the ability to establish links between innate and adaptive immune responses(19)

Studies in patients with COVID-19 have shown that serum titters of interleukin-12 are increased and in other infections similar to the coronavirus, such as SARS-CoV, this increase in serum interleukin-12 has been observed (20). demonstrated the serum level of IFN-γ in SARS-Cov-2 patients, and the statistical analysis of this study detected a significant elevation (p=0.01) in the level of IFN-γ in the patients in comparison to the healthy individuals, as the means were (51.60±2.03) and (62.03±3.37) pg/mL in control and patients groups respectively.

Table (3): The mean of IFN- γ in patients and controls.

Parameter	Groups	Mean±S.E (pg/mL)	P value
IFN-γ	Control	51.60±2.03	0.01*
	Patients	62.03±3.37	

The results of the current study showed that the percentage of IFN-γ in patients was higher than that of controls and values of cytokines IFN- γ significantly higher in patients with COVID-19 than in healthy controls (P<0.01). During SARSCoV-2 infection-related cytokine storms, IFN-γ irregularities are visible and cell transcripts are seen with over expression of the COVID-19-related gene.

Studies in patients have shown that the level of IFN-γ has increased in children with COVID-19, which has not been high compared to adults with COVID- 19, this indicates that COVID-19 infection is not severe in children with the disease (21).

Conclusions

- Patients with SARS-CoV-2 infection have high levels of various cytokines that can be identified as an indicator of disease progression and a therapeutic goal

- Specific immune profiles of SARS-CoV-2 infection can lead to secondary infections and dysfunction of various organs in the body.
- In the field of drug and treatment, more activities should be done to find a solution to control and replicate the COVID-19 virus and ultimately reduce its side effects.

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