Evaluation of Immunoglobulins, CD markers , CD4: CD8 ratio and Interleukin-6 in COVID-19 patients in Iraq

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Abstract

Background: In late 2019, the outbreak of novel coronavirus (COVID-19) infection began in China, which spread rapidly and cases were recorded worldwide.

Objective: In this study, we sought clarification of the clinical characteristics and importance of changes in the lymphocyte group, antibodies, CD markers, and interleukin-6 in the serum of COVID-19 patients, in order to help the clarification, the pathogen and develop new biomarkers.

Methods: Venous blood samples were taken from patients before using any medications. Sera had been separated and saved at (-20? C) until analysis. In plasma samples, SARS-CoV-2 serum immunoglobulins (IgG, IgA, and IgM) were also determined using enzyme-linked immunosorbent assays (ELISA), and Serum IL-6. The indices of the CD markers were measured using ELISA technique.

Results: Significant difference in laboratory findings were noticed between healthy control group and COVID-19 patients' group, including median IgM (P=0.0213), IgG (P?0.0008) and IgA (P?0.0017), and they were decreased in patients comparing with control group. There is a significant decrease in CD3 and CD4 compared to healthy individuals in patients infected with COVID-19 (P < 0.0001). Also, CD19 fall in patients compared to the control group (P <0.0001). After calculating CD4 / CD8 ratio, a significant drop in patients (P < 0.0001) was observed. The only parameter that was increased in patients was CD56 (P <0.0001), a significant decrease of IL-6 was noticed in COVID-19 patients (58.3) in comparing with control values (37.787) (P<0.0001).

Conclusions: As conclusion of our study we noticed some significant changes in COVID-19 patients compared to normal people which were lower IgM, IgG and IgA levels, higher CD8 levels, lower CD3 and CD4 levels, higher CD56 levels, lower CD19, and CD4 / CD8 levels, and interleukin 6 levels.

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We affirm that the submission represents work that has not been published previously and is not currently being considered by another journal. Also, I confirm that each author has seen and approved the contents of the submitted manuscript.

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Keywords: IL-6, COVID-19, IgG, IgA, IgM, CD markers

Introduction:

On 30 January 2020, the Global Health Organization looked at the latest SARS-Cov-2 epidemic in China and its accelerated global spread in 2019;[26] issued a Statement of International Concern on Emergency in Public Health, which was caused by coronavirus (COVID-19) [1]. As of 16 February 2020, China (primarily in Wuhan) had reported A total of 58,182 laboratory-confirmed instances including 1,696 fatal incidents according to official figures from the Chinese Government. [2]. COVID-19 has been confirmed to present a higher risk of occurrence in older men with comorbidities [3]. What is a poorly functioning immune system? The pathophysiology for COVID-19 of exceptionally high pathogenicity has not yet been fully understood as a new form of highly

infectious disease in humans Several studies have shown that SARS associates increased serum proinflammatory cytokine concentrations with pulmonary inflammation and severe damage to the lung [4]. We are living in extraordinary times Extreme Viral pneumonia COVID-19 that induces extreme respiratory failure syndrome in adults (ARDS), aggravated globally by intensive care units [5]. Without A vaccination or effective anti-viral treatment but without herd protection, anti-cytokine therapy; more specifically anti-IL-6 therapy and others, including IL-1, indicated relief of hyperinflammation. [6]. Lymphocytes and subsets are CD4, CD8, B cells, and natural killer cells to keep the immune system active; they play a major role. Changes in the overall number of lymphocytes and subsets following viral infection vary with various forms of the virus, suggesting potential association Modification of lymphocyte subsets and viral pathogens [7]. Recent studies in COVID-19 patients showed a clear decline in peripheral lymphocytes but any changes in the subsets were still unknown [8]. This infects all other age groups equally among the more than 1,000 patients surveyed in Wuhan and rarely among children and adolescents. Approximately 15% of confirmed cases are progressing to the moderate phase, while patients over 65 are more likely to enter the serious phase [9]. We sought clarification of the clinical characteristics in this study and the importance of changing the group of lymphocytes, antibodies, CD markers, and interleukin-6 in the serum of COVID-19 patients, which may help to clarify the pathogen and develop new biomarkers.

2. Materials & methods:

2.1. Population in study

30 patients (15 mals and 15 females) diagnosed with COVID-19 positive in Central Lab-Baghdad were included in this work. Initially, all the patients were fully diagnosed with clinical symptoms and then confirmed with RT-PCR (qRT-PCR) quantitative analysis of swab throat samples. The control group had also selected 30 healthy people of similar age and gender. In contrast, people with a history of prior chronic illnesses were diagnosed with immunosuppressive medications earlier than the initiation of infection with COVID-19 and those who suffered from the disease; We were robbed of this report. All subjects were acquainted with the goals of the research and were following the consent documents. Both samples were collected and approved for alleged human cases of novel coronavirus (nCoV) infection protocols, in compliance with the laboratory review. [10]

2.2. Laboratory procedures

Venous blood samples had been accumulated from patients before taking any medications. Sera had

been separated and saved at (-20℃) until analysis. Serum immunoglobulins (IgG, IgA, and IgM) The use of enzyme-linked immunosorbent assays (ELISA) using (Biokit, Spain) as directed by the manufacturer was determined against SARS-CoV-2 in plasma samples. Serum IL-6 was determined by Human IL-6 PicoKineTM ELISA Kit, Boster, USA. The ELISA technique (lab science company, USA) was used to measure CD markers indices.

2.3. Analysis statistics

Statistical analysis was conducted in seventeen variants of SPSS (SPSS, Inc., Chicago, IL, USA) and adopted as a mean ± standard deviation (SD) T-test was used to evaluate the critical class differences. In the past the statistically significant P value of<0.05 was found.

2.4. Ethical considerations

All subjects consented to be included in the study. The research was reviewed and approved through the local Committee of Study.

Results:

1- Evaluation of Immunoglobulin in COVID-19 infections.

A significant difference in laboratory findings was noticed between healthy (control) group and COVID-19 patients' group (Table 1) (Fig1.B), including median IgM (P=0.0213), IgG (P□0.0008) and IgA (P□0.0017), were decreased in patients comparing with a control group.

2- Evaluation of CD markers and CD4: CD8 ratio in COVID-19 infections.

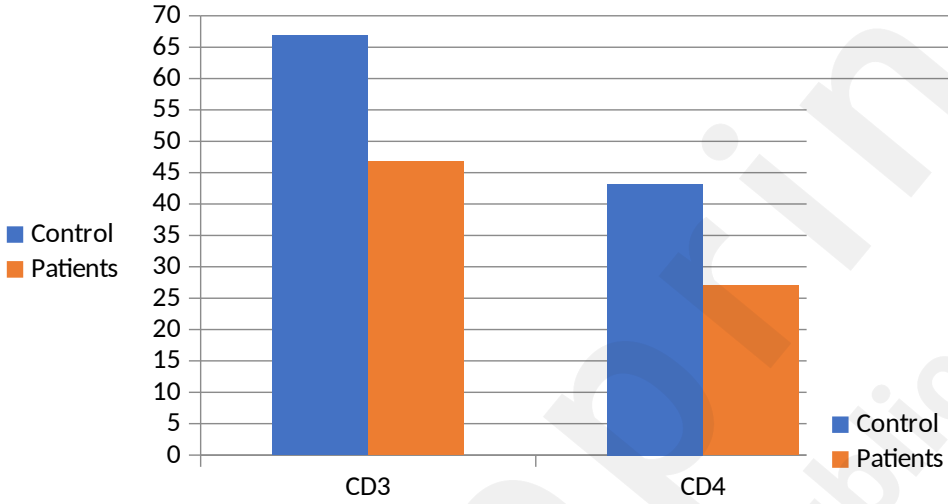
Among COVID-19 infected patients, there is a substantial decrease among CD3 and CD4 relative to healthy individuals (P < 0.0001) (Fig1.A); But the difference in CD8 (Fig1.A) was not significant. Decline of CD19 inpatient vs control group (P < 0.0001). After the measurement of the ratio CD4 / CD8, a significant drop in patients was observed (P <0.0001) (Fig1.C). The only parameter that increased in patients was CD56 (P <0.0001).

Table1: Evaluation of Immunoglobulin, CD markers, and CD4: CD8 ratio in COVID-19 infections.

Tests	Groups		*P*-value
	Control	Patients	
IgM	319.80 ± 5.84* A	291.90 ± 10.23 B	0.0213
IgG	1916.93 ± 51.10 A	1617.87 ± 67.76 B	0.0008
IgA	467.67 ± 25.09 A	337.00 ± 30.79 B	0.0017
CD3	66.853 ± 0.868 A	46.839 ± 1.704 B	<0.0001
CD4	43.192 ± 0.905 A	27.020 ± 0.983 B	<0.0001
CD8	24.8750 ± 0.711	25.3467 ± 0.587	N.S.**

CD19	11.2963 ± 0.355 A	7.6933 ± 0.389 B	<0.0001
CD4/CD8 ratio	1.78246 ± 0.0512 A	1.07702 ± 0.0414 B	<0.0001
CD56	10.7790 ± 0.605 B	17.8867 ± 0.414 A	<0.0001
IL-6	37.787±0.456 B	58.3±1.795 A	<0.0001

* Means ± Standard Error.
** N.S.: Non-Significant
a, b, c: means in the same Row with different superscripts differ significantly at probability value (P≤0.05).



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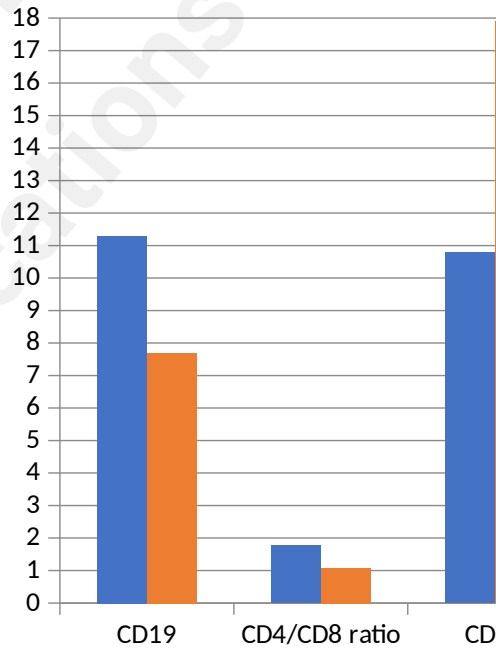
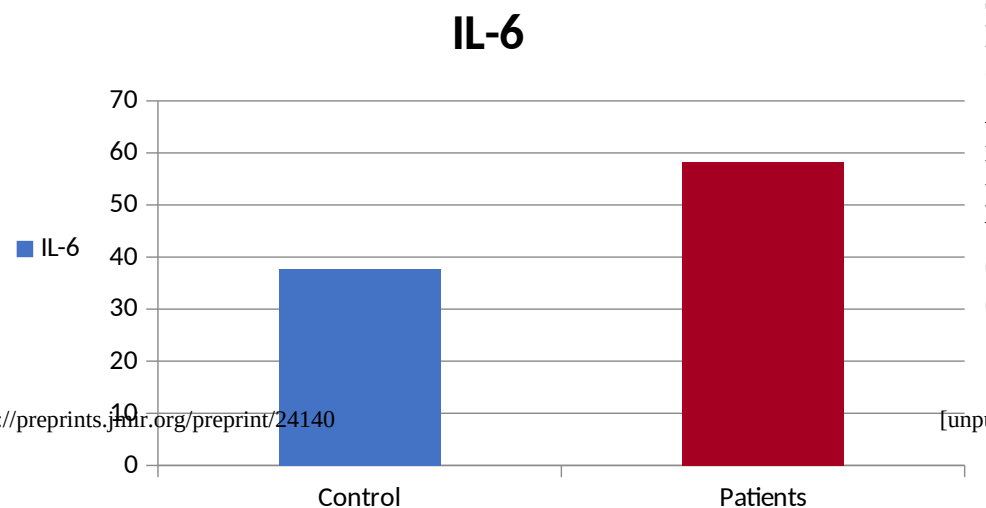


B **C**

Figure1:
A- **distribution CD3, CD4 and CD8 among COVID-19 infection.**
B- **distribution IgM, IgG, and IgA among COVID-19 infection.**
C- **distribution CD19, CD4/CD8 ratio CD56 among COVID-19 infection.**



3- Evaluation of Interleukin-6 in COVID-19 infections.
A significant decreased noticed in COVID-19 patients (58.3) comparing with control values (37.787) (P<0.0001) (Table 1).

Figure 2: distribution Interleukin-6 among COVID-19 infection

Discussion:

1- Evaluation of Immunoglobulin in COVID-19 infections.

This research aims to find the immune changes of Immunoglobulin in Covid-19 patients, the current results showed a decrease in the level of IgM, IgG and IgA when compared to healthy people as shown in (Figure1.B) Therefore, this study is consistent with administering immunoglobulin to infected patients These immune IgG antibodies against COVID-19 in newly infected patients will be specific in improving the immune response. Different methods may be employed to eliminate or deactivate possible resistant pathogens from plasma IgG coronavirus antigen [11]. IgG antibodies comprise two specific parts: the antigen identification fragment F(ab′)2 and the crystal fragment (Fc) This is critical when communicating with IgG antibodies to trigger the immune response. B-cells and other inflammatory cells inborn Fcγ receptors,[12] A recent study of three Chinese COVID-19 cases found that seroconversions occurred between 7 and 12 days after the symptoms began. [14]. More detailed studies on antibody response kinetics (e.g. IgA, IgM, IgG, neutralizing anticorps) are now urgently needed to better understand immune system dynamics. COVID-19 Reply. Our study results indicated a decline in IgM, IgA, and IgG. The effect of early or late serological transformation is unknown and should be investigated on the severity of the condition. What is interesting is that there was no positive IgM, no positive IgG. In general, IgM is first produced and then the production of IgG is shifted [14], But SARS-CoV studies often suggest simultaneous development of IgM and IgG [15].

2- Evaluation of CD markers and CD4: CD8 ratio in COVID-19 infections.

The current results showed a rise in the level of CD8 and a decrease in the level of CD3 and CD4 when compared to healthy people as shown in (figure1.A), The natural and acquired immune system activation is consistent with the reaction to the viral infections. Activation of cellular immune response is the most powerful solution to several viral infections; T cell activation in particular. [16] As for the high level of CD8 in patients, this result corresponds to one of the studies and differs with the same study regarding the level of CD4 where it decreased when compared to healthy people and the decrease was noticeable in which this result says despite the absence of The level of expression of CD8 was significantly In COVID-19 patients, a significant difference in the ratio of CD4 to CD8

between Two groups was greater than in normal individuals. This finding demonstrates the production of Cellular immune response following infection with COVID-19 by excessive CD8 expression and lymphocyte T cytotoxic hyperactivity. [17]. As for CD3, it was low in patients, but it is present in plasma and This result is compatible with the findings from one of the recent studies that reported positive CD3, CD4, and CD8 lymphocyte at entry. [18] In the (figure 1.C) the results showed a rise in the level of CD56 and a decrease in the level of CD19 and CD4 / CD8 ratio This result is consistent with the result. [19] Lymphocytes containing CD45 and naturally occurring killer cells (CD16, CD56) increased dramatically. However, no significant effects were observed on CD3 T, T-suppressor (CD3, CD8), T-helper (CD3, CD4) and CD19 B lymphocytes. Our study is in line with a study that dramatically reduced in COVID-19 patients, white blood cells, lymphocytes, and platelets ($P < 0.05$) The contrast to the CD4 is: ratio CD8, frequency CD4 T-cell. [17]

3- Evaluation of Interleukin-6 in COVID-19 infections.

In our study results, we found a rise in the level of interleukin-6 for people with Covid-19 when compared with healthy people. Cytokines are vital for the regulation of Inflammatory and immune reactions. Due to its pleiotropically effects, IL-6 is of paramount importance among them. [21-20] Results of our study correspond to a recently published study a possible joint mechanism for cytokine-induced Lung damage from COVID-9 has been previously seen in patients with respiratory dysfunction. [22] It also corresponds with the study that the 'cytokine storm' states. In Particular, interferon-gamma, alpha interleukin 17 (IL-17), the significant increase in serum cytokines; interleukin 8 (IL-8), and interleukin 6 (IL-6) tumor necrosis factor are hallmark characteristics of the deep inflammatory condition in COVID-19 patients suffering from pneumonia and hypoxia. [23] Coronaviruses can activate immune responses in the dysregulated host. As suggested by exploratory studies, In complicated COVID-19 cases interleukin-6 (IL-6) levels are high, and biological tocilizumab anti-IL-6 may be beneficial [24] Our findings show that serial measurement of circulating IL-6 rates could be essential to detect disease development among COVID-19 infected patients.. Consequently, as we have noted, it is reasonable to carry out an immediate initial IL-6 level assessment of COVID-19 patients hospital admission due to its potential benefits in assessing worsening clinical characteristics and progression of disease in COVID-19.

Conclusions: Decrease in the level of IgM, IgG and IgA and give immunoglobulin to infected patients Increasing the immune response in newly infected patients will make these immune antibodies COVID-19 specific, increasing CD8 levels and lowering CD3 and CD4 levels and increasing CD56 levels and lowering CD19 and CD4 / CD8 levels; Increase in interleukin-6 levels for patients with Covid-19 In line with our findings, it is therefore appropriate the prompt initial

evaluation of IL-6 rates will be carried out after referral to the hospital in patients with COVID-19 owing to the possible effects of the measurement of deteriorating clinical features and illness.

Conflict of Interest: No declare
Funding : N\A

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