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Evaluation of Immunoglobulins, CD4/CD8 T Lymphocyte Ratio and Interleukin-6 in COVID-19 Patients

COVID-19 Hastalarında İmmünoglobülinler, $CD4^+/CD8^+$ T Lenfosit Oranları ve Interlökin-6 Düzeyinin Değerlendirilmesi

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Abstract

Introduction: The outbreak of novel coronavirus COVID-19 infections that started in China late 2019 has spread rapidly and cases have been recorded worldwide. So, in this study, we sought clarification of the clinical characteristics and importance of changing the lymphocyte group, antibodies, CD markers, and interleukin-6 in the serum of COVID-19 patients, which may help to clarify the pathogen and develop new biomarkers.

Material and Methods: Venous blood samples had been accumulated from patients before taking any medications. Sera had been separated and saved at (-20°C) until analysis. Serum anti-SARS-CoV-2 immunoglobulins (IgG, IgA, and IgM) were determined in plasma samples using enzyme-linked immunosorbent assays (ELISA) and Serum IL-6 was assessed.

Results: Median IgM (p=0.001), IgG (p<0.0001), and IgA (p<0.001), were decreased in patients comparing with control the control group. There is a significant decrease in $CD3^+$ and $CD4^+$ cells compared to healthy individuals in patients infected with COVID-19 (p<0.0001). $CD19^+$ cell count decreased in COVID-19 patients compared to that of the control group (p<0.0001). After calculating $CD4^+/CD8^+$ cell ratio decreased in COVID-19 patients (p<0.0001). However, $CD56^+$ cells were found to be increased (p<0.0001).

Conclusions: IgM, IgG, IgA levels and $CD19^+$, $CD4^+$ cells, $CD4^+/CD8^+$ cell ratio were found to be decreased whereas $CD8^+$, $CD3^+$, $CD4^+$ cells were detected to be increased in COVID-19 patients compared to those of healthy controls.

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

Öz

Giriş: Çin'de 2019 yılı sonlarına doğru başlayan Yeni Koronavirüs Hastalığı (COVID-19) hızla tüm dünyaya yayıldı. Bu çalışmada, COVID-19 olan hastaların serumlarındaki interlökin-6 (IL-6) düzeyleri, değişen lenfosit alt-grupları ve antikorlar araştırılarak bu patojenin daha net anlaşılması ve yeni biyobelirteçlerin geliştirilşmesi amaçlandı.

Gereç ve Yöntemler: Hastalardan herhangi bir ilaç kullanmadan önce venöz kan örnekleri toplandı. Bu kanlardan serumlar elde edildi ve bu serumların irdelenmesine kadar (-20°C'de) saklandı. Serumdaki SARS-Cov-2'ye karşı gelişmiş immünoglobulinler (IgG, IgA ve IgM) ve IL-6 düzeyi ELISA testi ile ölçüldü.

Bulgular: Kontrol grubu ile karşılaştırıldığında, COVID-19 hastalarında IgM (p=0.001), IgG (p<0.0001) ve IgA (p<0.001) seviyeleri anlamlı derecede azaldı. Benzer şekilde $CD3^+$ ve $CD4^+$ T hücreleri kontrollere göre anlamlı dercede düşük saptandı (p<0.0001). $CD19^+$ B hücreleri ve $CD4^+/CD8^+$ hücre oaranları kontrol grubuna göre daha düşük (p<0.0001), buna karşılık $CD56^+$ hücrelerde artış saptandı.

Sonuçlar: COVID-19 hastalarında IgM, IgG ve IgA düzeyleri ile $CD19^+$ ve $CD4^+/CD8^+$ hücrelerin oranı sağlıklı kontrollere göre daha düşük, $CD8^+$, $CD3^+$, $CD4^+$ ve IL-6 düzeyleri daha yüksek olarak saptanmıştır.

Anahtar Sözcükler: IL-6, COVID-19, IgG, IgA, IgM, CD belirteçleri

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Introduction

On 30th of January 2020, World Health Organization (WHO) regarding the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) in China On 30th of January 2020, World Health Organization (WHO) regarding the severe acute respiratory syndrome coronavirus 2(SARS-Cov-2) in China; issued a Statement of International Concern on Public Health Emergency regarding the latest epidemic of SARS.[1] As of 16 February

2020, China (primarily in Wuhan) had reported A total of 58.182 laboratory-confirmed cases including 1.696 fatalities 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] Several studies have shown that SARS associates increased serum pro-inflammatory cytokine concentrations with pulmonary inflammation and severe damage to the lung.[4] We are living in extraordinary times in which COVID-19 induces extreme respiratory failure syndrome in adults (ARDS), aggravated globally.[5] Without a vaccination or an efficient anti-viral treatment anti-IL-6 and anti-IL-1 treatment may provide relief of hyper-inflammation occurring together with viral-induced ARDS.[6] $CD4^+$, $CD8^+$ T lylmphocytes, B cells and natural killer cells.[6] Changes in the overall number of lymphocytes and subsets following viral infection vary due to various forms of the virus, suggesting a potential association 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] Approximately 15% of confirmed cases are progressing to the moderate phase, while patients over 65 are more likely to have severe disease status.[9] We sought clarification of the clinical characteristics in this study and the importance of changing antibodies, lymphocyte subgroups and IL-6 in the serum of COVID-19 patients, which may help to develop new biomarkers.

Materials and Methods

Study Population

Thirty patients (15 males and 15 females) diagnosed with COVID-19 in Central Lab-Baghdad were included in this work. Initially, all patients were diagnosed based entirely on clinical symptoms and then confirmed by quantitative analysis of swab throat samples by RT-PCR (qRT-PCR). The control group consisted of 30 healthy people of similar age and gender. Patients with a history of prior chronic illnesses, cases who were administed with immunosuppressive medications earlier than the initiation of infection with COVID-19, and those who suffered from the disease; were excluded from the study. 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]

Laboratory Procedures

Venous blood samples were collected from patients before taking any medications. Sera were separated and saved at -20°C until analysis. Serum immunoglobulins (IgG, IgA, and IgM) The use of enzyme-linked immunosorbent assays (ELISA) using (Biokit, Spain) as described by the manufacturer was used for the determination of SARS-CoV-2 antigen in plasma samples. Serum interleukin-6 (IL-6) was determined by Human IL-6 PicoKineTM ELISA Kit, Boster, USA. CD markers indices were measured by the ELISA technique (lab science company, USA). The wells were filled with 100 µl a standard work solution incubated for 90 minutes at 37°C. Then 100 µl of biotinylated detection Ab working solution was added to each well, the wells had been mixed and incubated at 37°C for 1 hour and were covered by a platform sealer. The solution was sucked from each well, cleared by adding 350 µl of wash buffer to each well, wash three times for 1-2 min. Then each well has been filled with an HRP conjugate work solution (100 µl). Then incubated for 30 min at 37°C, covered with the sealer. The substrate reagent (90 µl) was added to each well, coated with a fresh plate sealer. It has been incubated at 37°C for 15 minutes. The stop solution (50 µl) was added to each well. With the micro-plate reader set at 450 nm, the optical density (OD) of each well was determined at one time.

Statistical Analysis

Statistical analysis was carried out in seventeen Statistical Package Social Science (SPSS, Inc., Chicago, IL, USA) versions and introduced as a mean ± standard deviation (SD). One-way ANOVA is used to show the mean differences between all samples.

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.

Median IgM ($p=0.001$), IgG ($p<0.0001$) and IgA ($p<0.001$) were decreased in patients with COVID-19 compared to those of control group (Figure 1b).

2. Evaluation of lymphocyte populations and CD4: CD8 ratio

Among COVID-19 infected patients, $CD3^+$ and $CD4^+$cells decreased compared to control group ($p<0.0001$) (Figure 1a); But the difference in $CD8^+$ cells (Figure 1a) was not statistically significant. Decline of CD19 inpatient vs control group ($p<0.0001$). $CD4^+/CD8^+$ cell ratio decreased in patients ($p<0.0001$) (Figure 1c). $CD56^+$ cells were statistically significantly increased ($p<0.0001$).

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

Serum IL-6 levels were decreased in COVID-19 patients compared to those of healthy controls ($p<0.0001$) (Table 1).

Discussion

Serum IL-6 levels were decreased in COVID-19 patients compared to those of healthy controls ($p<0.0001$) decreases in the level of IgM, IgG and IgA when compared to healthy people. Therefore, this study is consistent with the idea of administering immunoglobulin to infected patients. These immune IgG antibodies against COVID-19 in newly infected patients might improve the immune response against COVID-19. Different methods may be employed to eliminate or deactivate possible resistant pathogens from plasma.[11] IgG antibodies comprise two specific parts: the antigen identification fragment F (ab′) 2 and the crystal fragment (Fc). Fc binds to Fcγ receptor to trigger B cells.[12] A recent study of three cases of Chinese COVID-19 patients indicated that there were sero-conversions 7 to 12 days after symptoms started.[13] More detailed studies on the kinetics of antibody response (e.g. IgA, IgM, IgG) are now urgently needed to better understand the dynamics of the immune system. Our study results indicated a decline in IgM, IgA, and IgG levels. The effect of early or late serological transformation is unknown and should be investigated in terms of the severity of the condition. In general, IgM is first produced and then the production of IgG is shifted[14], but SARS-CoV studies suggest that IgM and IgG often develop simultaneously.[15] The current results showed a rise in the level of $CD8^+$ cells and a decrease $CD3^+$ and $CD4^+$ cells when compared to healthy people. The natural and acquired immune system activation occurs to viral agents. Activation of cellular immune response is the most powerful solution to several viral infections; T cell activation in particular.[16] $CD4^+/CD8^+$ cell ratio was found to be decreased in patients with COVID-19. This finding could be due to higher number of $CD8^+$ cytotoxic T cells in COVID-19 infection.[17] Decreased $CD3^+$ cells was reported before.[18] Higher

Table 1. Evaluation of immunoglobulin, lymphocyte populations, and $CD4^+CD8^+$ cell ratio in patients with COVID-19 infections and control group.

Tests	Groups		*P*-value
	Control	Patients	
IgM	319.8±32.0* A	291.9±56.0 B	0.001
IgG	1917±202 A	1618±307 B	<0.0001
IgA	468±137 A	337±168 B	<0.001
$CD3^+$ cells	66.9±4.7 A	46.8±9.3 B	<0.0001
$CD4^+$ cells	43.2±4.9 A	27±5.3 B	<0.0001
$CD8^+$ cells	24.9±3.9	25.3±3.0	N. S. **
$CD19^+$ cells	11.3±1.9 A	7.7±2.1 B	<0.0001
CD4/CD8 ratio	1.8±0.3 A	1.1±0.2 B	<0.0001
$CD56^+$ cells	10.8±3.3 B	17.9±2.3 A	<0.0001
IL-6 pg/ml	37.8±1.8	58.3±9.8	<0.0001

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

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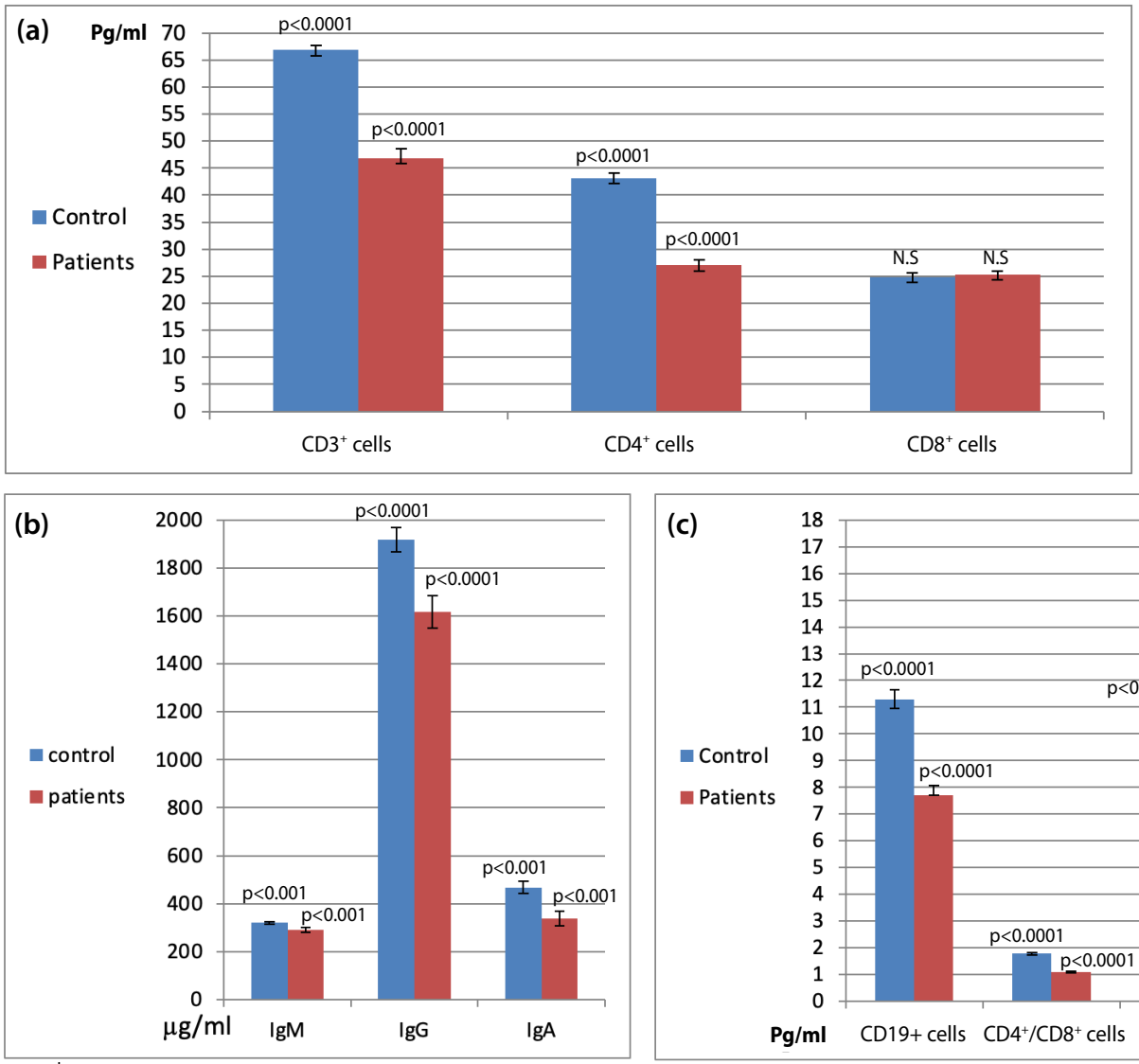

Figure 1. Distribution CD3, CD4 and CD8 among COVID-19 infection **(a)**. Distribution IgM, IgG, and IgA among COVID-19 infection **(b)**. Distribution CD19, CD4/CD8 ratio CD56 among COVID-19 infection **(c)**.

$CD56^+$ cells and lower $CD19^+$ cells adn $CD4^+/CD8^+$ cell ratio were consistent with the results of others.[19] In our study, we found that, $CD56^+$ cells increased whereas $CD19^+$ cells did not change significantly. The lowered $CD4^+/+/CD8^+$ ratio was reported before.[20] In our study Il-6 level was found to be increased in COVID-19 patients. Cytokines are vital for the regulation of inflammatory and immune reactions.[21] Due to its pleiotropically effects, IL-6 is of paramount importance among them.[21] Higher IL-6 levels in COVID-19 patients.[22] IL-6, IL-7, IL-8 and IL-17 were increased in COVID-19 patients with pneumonia and hypoxia.[23] Coronaviruses can activate immune responses in the dysregulated host.[23] In complicated COVID-19 cases IL-6 levels are high, and anti-IL-6 therapy may be beneficial.[24] So our findings indicate that serial measurement of circulating IL-6 rates could be essential in screening the development of disease in patients with COVID-19.

Consequently, as we have noted, it is reasonable to carry out an initial IL-6 level assessment of COVID-19 patients at hospital admission suggesting its potential benefits in assessing the worsening clinical characteristics and progression of disease in COVID-19.

Ultimately, Type I interferons, lymphocyte function and viability were not evaluated in our study.

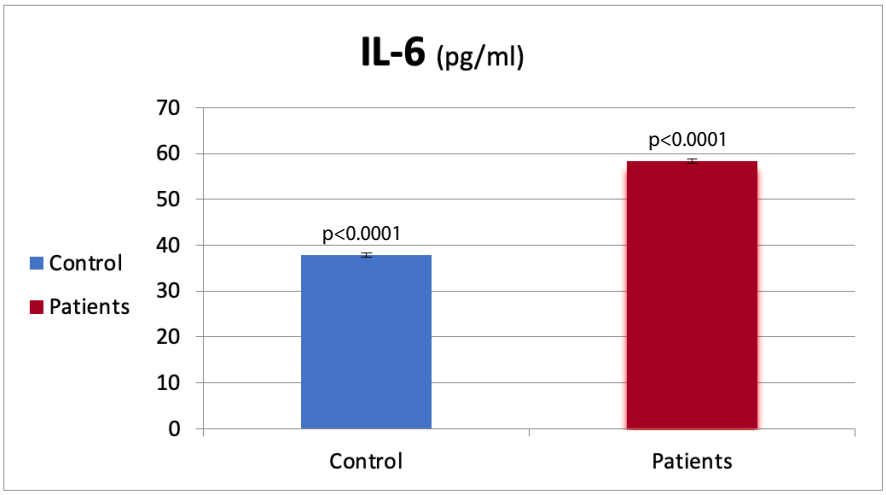

Figure 2. Distribution interleukin-6 among COVID-19 infection.

Conclusions

IgM, IgA and IgG levels decreased in COVID-19 patients. It has been also found that, $CD8^+$ cells, $CD56^+$ cells increased wheras $CD3^+$, $CD4^+$ cells and $CD19^+$ cells and serum IL-6 levels decreased. The prompt initial evaluation of IL-6 level is recommended to evaluate the clinical situation of the patients.

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Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declared that no conflicts of interest existed.

Contribution of Authors: Concept: MMS, AAS, RAA; Design: MMS, AAS; Data Collection or Processing: RAA, MMS, ATJ; Analysis or Interpretation: MMS, ATJ, AAS; Literature Search: MMS, ATJ; Writing: MMS, ATJ; Critical Review: AAS, RAA.

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