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Evaluation of changes in IgG level in patients diagnosed with COVID-19; a single center study in Isfahan, Iran

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Abstract

Introduction: With the advent of the COVID-19 pandemic, extensive efforts have begun worldwide to identify the factors that reduce the incidence of the disease. Various methods have been launched to combat the disease. Changes in serum immunoglobulin levels are among the factors that have been considered in COVID-19.
Objectives: The aim of this study was to determine the changes in IgG levels in patients diagnosed with COVID-19 in 2020 at Al-Zahra hospital in Isfahan.
Patients and Methods: In this prospective study, 65 patients with COVID-19 were evaluated. Their serum IgG levels were assessed before and at three and six months after the start of treatment, and changes in the levels of this immunoglobulin before and after treatment were compared.
Results: The mean IgG level at the time of referral was 1214.2±672.1 mg/dL and increased to 1490.8 ±621.5 mg/dL at the three months after treatment. At six months after treatment, IgG levels decreased compared to three months later and reached 1375.1±609.1 mg/dL. There was a significant difference in changes in IgG levels during the six months after the onset of the disease compared to repeated measures analysis of variance (P=0.001).
Conclusion: The present study's findings showed that IgG levels increased significantly in three months after COVID-19 and decreased significantly in 6 months. Since IgG levels begin to rise at least four weeks after exposure to the virus, this immunoglobulin cannot be conducted as a diagnostic tool to detect COVID-19.

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Introduction

COVID-19 was identified following some viral pneumonia cases in late 2019 in China. COVID-19's pathogenic agent was named severe acute respiratory syndrome (SARS) for short and spread rapidly in three months worldwide and turned into a pandemic (1-3). Following human-to-human transmission, the disease quickly spread to more than 200 countries, and the number of infected people and deaths due to this disease is still increasing (4,5).

Most patients with COVID-19 have had symptoms such as a dry cough, a sore throat, and fever. The disease gets better automatically in most cases. However, in some cases, the patient may suffer from mortal symptoms, including organ failure, septic shock, pulmonary edema, severe pneumonia, and severe acute respiratory syndrome (6-8). Patients who are old and have one or more background illnesses, including cardiovascular, neurological, endocrine, digestive, and respiratory problems, usually require intensive care units (ICU) (6).

Key point

This study aimed to analyze changes in IgG levels in COVID-19 patients at Al-Zahra hospital in Isfahan. They studied 65 patients and found that IgG levels increased significantly after three months of treatment but decreased at six months. However, the delayed IgG response to the virus means it is unsuitable as a diagnostic tool for COVID-19.

As COVID-19 symptoms, including fever and dry cough, are similar to other bacterial and viral infections (7-9), diagnosing this disease is difficult, especially during the influenza season (10).

Despite all efforts, there need to be more studies on this virus, and most of the available information is based on similar coronaviruses. However, according to the studies so far, this virus mainly transforms from human to human via respiratory droplets. Respiratory viruses are usually

transmittable during the symptomatic period of the disease. However, the increasing evidence about this virus shows that human-to-human transmission may happen in its incubation period (between 2-10 days) (11,12).

Currently, the preliminary tool for diagnosing this disease is reverse transcription polymerase chain reaction (RT-PCR) to assess the virus's nucleic acid, computerized tomography (CT) scan, and hematology tests (13). Using RT-PCR kits has many limitations, including needing trained operators, equipped laboratories, high expenses, and delays in announcing the results. Using RT-PCR also has a pseudo-negative for detecting COVID-19 (14).

Using the specific antibodies of SARS-CoV-2 in the patient's blood sample is a simple high-sensitive fast method for diagnosing COVID-19. Before producing immunoglobulin G (IgG), which is essential for long-term safety and immunological system memory, the first line in confronting viral infections is producing immunoglobulin M (IgM). It is reported that after SARS infection, IgM and IgG antibodies could be detected in the patient's blood sample 3-6 days and 1-3 weeks after disease onset, respectively (15, 16).

As the agent who develops COVID-19 disease is a member of a large family of viruses that can cause SARS and Middle East respiratory syndrome (MERS), it is speculated that producing antibodies in them is similar, and detecting IgM and IgG antibodies in patients demonstrates the infection. While the IgM antibody shows a recent confrontation with the SARS-CoV-2 virus, the IgG antibody indicates an older confrontation with the given virus (17). For two years have passed since the pandemic of this disease, there have been no comprehensive seroepidemiologic studies on the production of immunoglobulins for COVID-19.

Objectives

This study was conducted to determine the changes in IgG levels in patients with COVID-19 diagnosis.

Materials and Methods

Study design

This study was prospective research that was performed in 2020 at Al-Zahra hospital of Isfahan. The study's target population was patients infected with COVID-19 who had visited the center, as mentioned above.

The criteria for entering the study included the definite diagnosis of COVID-19 based on PCR results, not having immunity system failure diseases, not having autoimmune diseases, not using the immunity system's weakening drugs, not being previously infected with COVID-19, and the patient's consent for participation in the study. In addition, the other criteria for exiting the study were the patient's death before finishing the study and not visiting the next time to measure IgG level.

The sample volume required for the study was estimated using the formula for determining sample volume to compare the averages and with a 95% confidence level, 80% test power, a standard deviation of 0.15 mg/dL IgG level, and a minimum significant difference of 0.1 before and after infection with COVID-19. Based on the criteria above, 60 patients were estimated for the study.

The research method was in the following way; after the necessary coordination, the patients visiting the hospital who were diagnosed with COVID-19 and had other criteria entered the study. The patient's demographic data, including their age and gender, was at first determined and recorded in the data collection form. Before doing any therapeutic measures and after gaining the patient's consent, an amount of 10 cc of venous blood was taken from the patient and sent to the hospital's laboratory to determine the IgG serum level. All patients were called to the hospital three and six months after discharge and referred to the laboratory to determine the IgG level. Then the mentioned results were recorded in the data collection form. The 700 to 1400 mg/dL were considered the normal IgG level.

Statistical analysis

The data were analyzed using SPSS software version 26. A variance analysis test with repeated observations analyzed the changes in IgG levels before and after treatment. Moreover *t* test and variance analysis with repeated observations were conducted to compare IgG level changes in different age and gender groups. The significant level was considered less than 0.05.

Results

In this study, 65 patients with COVID-19 were studied in this research. The average age of patients was 53.45 ± 14 years, with a range of 28-83 years. Twenty-six patients (40 %) were below 50, and 39 (60 %) were 50 years old and over. Forty-four patients (67.7 %) were male, and 21 patients (32.3 %) were female (Table 1).

The average IgG level at the time of referral was 1214.2 ± 672.1 mg/dL. It increased three months later and reached 1490.8 ± 621.5 mg/dL. Compared with three months after treatment, the IgG level decreased six months later and was 1375.1 ± 609.1 mg/dL. According to the variance analysis test with repeated observations, the IgG level changes had a significant difference six months after the onset of the disease ($P \leq 0.001$). The changes in IgG level during the study are shown in Figure 1.

Compared to the referral time, the average changes in IgG level three months after the disease increased by 229.2 ± 291.5 mg/dL. However, six months later, it had

Table 1. Distribution of demographic variables of studied patients

	Variable	Number	Percent
Age group (y)	<50	26	40
	≥50	39	60
Gender	Male	44	67.7
	Female	21	32.3

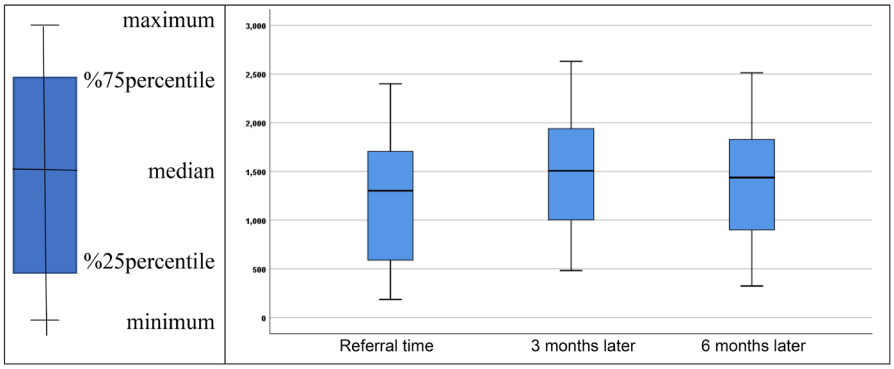

Figure 1. Median, range, and 25-75 % percentile of IgG level from the disease onset time to 6 months later.

increased by 193.3 ± 173.2 mg/dL. A comparison of the IgG level between six and three months after the disease showed that the antibody level decreased by 123.9 ± 110 mg/dL. Additionally, T-paired tests on the mentioned data indicated that the IgG level changes three and six months after the onset of the disease had a significant change ($P <$ 0.001; Table 2).

The average and standard deviation of IgG level from the disease onset time to 6 months later based on patients' age and gender are shown in Table 3. According to the results, the average IgG level at the referral time, three and six months later, had no significant difference between males and females. Based on the variance analysis test with repeated observations, the procedure of changes in IgG level had a significant change in both males and females ($P<0.001$). However, the changes in IgG level had no significant difference between males and females (0.66). The assessment of IgG level based on age group showed that the IgG level at the referral time was higher in patients 50 years or over ($P=0.036$).

Nevertheless, three and six months later, the mentioned antibody level had no significant difference between the two groups. In the intragroup evaluation, the IgG level changes during the entire study period significantly differed between the two age groups below 50 and 50 years over ($P<0.001$). However, in the intergroup comparison, the procedure of changes in IgG level had no significant difference between the two age groups ($P=0.061$). The IgG level changes based on age and gender are shown in Figures 2 and 3.

The study findings show a significant statistical relation between IgG serum level and age. We found a direct and significant correlation of 0.34 between age and IgG level at the referral time ($P=0.006$). The correlation between age and IgG level was 0.26 ($P=0.042$) and 0.27 ($P=0.035$) three and six months later.

Discussion

This study was conducted to determine the changes in IgG levels in patients with COVID-19. Sixty-five

Table 2. Average changes in IgG level (mg/dL) 3 and 6 months after treatment

Comparison criterion	Average difference	95% CI	*P*
IgG0-IgG3	229.2 ± 291.5	234.3–348.8	<0.001
IgG0-IgG6	193.3 ± 173.2	123.7–222.7	<0.001
IgG3-IgG6	-110 ± 123.9	78.3–141.7	< 0.001

Table 3. The average and standard deviation of IgG level at the referral time and three and six months later based on patients' age and gender

Variable	Time	IgG level (mg/dL)			*P***	*P****
		Referral time	3 months later	6 months later		
Gender	Male	1203 ± 705.8	1483.4 ±618.6	1348.2 ± 631.5	<0.001	0.66
	Female	1237.8 ± 611.2	1507.1 ± 643.7	1434.5 ± 568.2	<0.001	
	*P**	0.85	0.89	0.61		
Age group	Below 50	1001.5 ± 742.5	1344.8 ± 707.9	1201.3 ± 699.5	<0.001	0.061
	50 years and over	1356 ± 588.6	1590.7 ± 542.1	1480.2 ± 529.7	<0.001	
	*P**	0.036	0.12	0.083		

* The Significance level difference between the two age and gender groups at each time point based on the T-test

** The significance level of IgG level changes in age and gender groups based on variance analysis test with repeated observations

*** The significance level of IgG level changes between age and gender groups based on variance analysis test with repeated observations

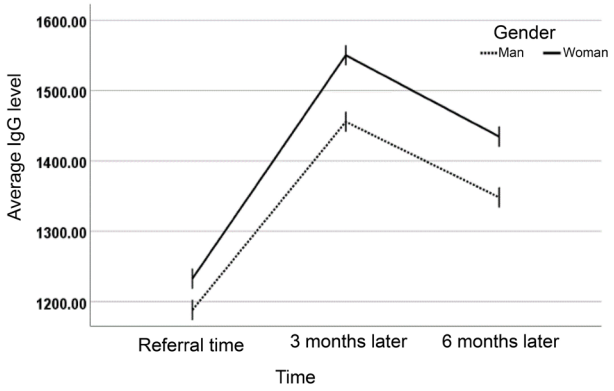

Figure 2. The average changes in IgG level (mg/dL) at the diagnosis time to 6 months after the disease based on gender ($P = 0.66$).

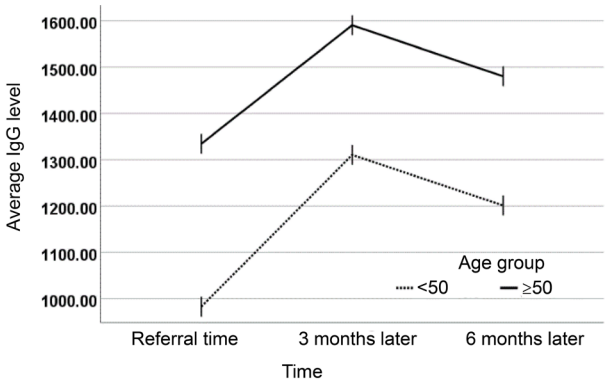

Figure 3. The average changes in IgG level(mg/dL) at the diagnosis time to 6 months after the disease based on age group ($P = 0.61$).

patients infected with COVID-19 were studied in this research, and their immunoglobulin G (IgG) levels were evaluated at the referral time and 3 and 6 months later. The patients had an average age of 53.45 ± 14 years, and 67.7 % of them were male. Regarding the IgG level, the changes in this immunoglobulin significantly differed from the disease diagnosis time to 6 months later. Still, the highest IgG was three months after the disease, and then it gradually decreased. Ma et al studied the IgM and IgG immunoglobulin levels in 87 patients with COVID-19 and found that the IgM serum level had a significant increase two weeks after the disease diagnosis, however compared with the base time, the IgG levels had a significant increase eight weeks after treatment. These findings are consistent with the results of our study (18). In another study by Van et al to determine the diagnostic value of IgG in infection to COVID-19, the IgG levels were measured 14-25 days after the onset of the clinical symptoms, and they had 89.5 sensitivity for the enzyme-linked immunosorbent assay (ELISA) method (19).

In a controlled case study, Andrey et al investigated 45 patients with COVID-19 and 45 healthy people to assess their IgG levels. According to the research findings, the immunoglobulin levels in the patients were higher than in the control group. Based on the results of this study, the IgG levels measurement 14 days after infection with COVID-19 had 88 % sensitivity (20). Thus, regarding the mentioned findings, it seems that measuring immunoglobulin G levels has a high value in diagnosing COVID-19 disease however; the accuracy of the test depends on the time of IgG level measurement. As the IgG level is not increased during the first days of the disease and gradually reaches a high level in a couple of weeks, it does not seem that measuring IgG is a suitable way for early diagnosis of the disease. In addition, the IgG test results, presence of clinical symptoms, the time interval between the onset of symptoms and gaining the sample, and other diagnostic tests, including molecular and imaging tests, must be considered for interpreting the test results, too (21). The results should be interpreted based on other mentioned variables. In case of negative test results and doubts about the disease, it is recommended that sampling is repeated and test repetition conducted 1-2 weeks later (22). On the other hand, as a high percentage of people are infected with mild and asymptomatic disease types, the IgG test's positive result may be due to cross-reaction with pre-produced antibodies and other factors (23). Therefore, before any decision-making, the positive test results must be conformed to the patient's clinical findings (24).

Additionally, people who are carrying the virus may have a positive antibody answer during the infection period, measuring IgG level is probably valuable in seroepidemiological studies and determining the safety level of society against COVID-19.

Conclusion

The study's findings showed that the IgG level increased significantly three months after infection with COVID-19. However, the IgG level decreased significantly six months after the infection with COVID-19. Since IgG levels begin going up at least four weeks after confronting the virus, and due to the previous confrontations with the virus, the patients may have a high IgG level at the disease onset time; hence, this immunoglobulin could not be exploited as a diagnostic tool to detect COVID-19. Regarding the present conditions of the disease.

Limitations of the study

The main limitations of the present study were the small sample size.

Authors' contribution

Conceptualization: Mohammadreza Yazdani, Morteza Pourahmad.
Data curation: Mohammadreza Yazdani, Morteza Pourahmad.
Formal analysis: Abolfazl Habibi.
Investigation: Abolfazl Habibi.
Methodology: Mohammadreza Yazdani, Morteza Pourahmad.
Project administration: Mohammadreza Yazdani, Morteza Pourahmad.
Resources: Abolfazl Habibi.
Validation: Morteza Pourahmad.

Visualization: Mahnaz Momenzadeh, Morteza Pourahmad.
Supervision: Morteza Pourahmad.
Writing–original draft: Mahnaz Momenzadeh.
Writing–review and editing: Mahnaz Momenzadeh.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Isfahan University of Medical Sciences (Ethical code #IR.MUI.MED.REC.1400.302). Written informed consent was obtained from all participants prior to intervention. The study was extracted from Abolfazl Habibi's MSc thesis at the university (Thesis #54483). The authors have fully observed ethical issues, such as plagiarism, data fabrication, and double publication.

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