Original Article

SARS-CoV-2 IgG and IgM Analysis in Patients Who Attended a University Hospital During the COVID-19 Epidemic in Iran

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Abstract

Background: The world is facing a new coronavirus that causes a respiratory infection called COVID-19. Therefore, there is an increasing request for antibody tests in recovered individuals since they want to evaluate their immunity against SARS-CoV-2 reinfection.

Materials and Methods: In our study, we had 1000 blood samples from patients admitted to the Ghiassi Hospital, Tehran, Iran, or were asked to perform serological SARS-COV-2 IgM and IgG tests by their physicians were collected. The antibody levels were assessed via the ELISA assay method using S and N antigens during various waves of the COVID-19 epidemic in Iran.

Results: The highest IgG level (2.77) compared to the average (with 95% confidence) is observed in patients infected in the third wave, which is confirmed by the ANOVA test. The mean IgM concentration in the second wave was equal to 0.77 and more than the IgM level in the third wave and the beginning of the fourth wave, which was confirmed by the ANOVA test.

Conclusion: Detection of SARS-COV-2 IgG, IgM has significant potential for evaluating the severity and prognosis of COVID-19. In addition, all seroepidemiology data in each community can help Health Commissions for controlling this pandemic. These data also can be used for epidemiological modeling and assessing the prevalence of COVID-19 immunity in society.

Keywords: SARS-CoV-2, Antibody measurement, COVID-19, ELISA assay

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Introduction

Since December 2019, when the SARS-CoV-2 was detected in Wuhan, China, lots of challenges caused by this virus occurred, and the COVID-19 pandemic is still present[1]. The mortality of infected patients is increasing every day. Extensive development and the high speed of this pandemic, along with numerous waves in various countries, have broken the traditional standards of physicians and laboratorians for diagnosing and treating diseases and forced them to utilize faster and more accurate screening and diagnostic tests[2,3]. The global COVID-19 pandemic has already caused the establishment of specific laws such as social distancing and curfews worldwide. Some strict rules, like quarantine conditions in cities, and business

closures, all indicate the problematic situation due to the pandemic[4,5]. The demand for laboratory tests for screening and diagnosis increased; thus, rules and protocols changed[6]. Utilizing various diagnostic kits, employing innovation, and applying novel methods with high sensitivity and specificity are laboratory technicians' primary and permanent goals for screening infected individuals.

In some cases, it has been observed that tests (for example, RT-PCR in SARS-CoV-2) cannot accurately meet all the requirements for detecting the prevalence of SARS-CoV-2. Therefore, serological tests and epidemiological studies play a significant role in assessing the prevalence and length of the immunization caused by the COVID-19 infection or vaccination[4,7]. Evaluating the prevalence of SARS-CoV-2 in the community, assessing the resistance to and the possibility of re-infection, measuring immunity against SARS-CoV-2 after vaccination or COVID-19 infection, and monitoring the immune responses of infected individuals after recovery is some of the items that make the serological and seroepidemiological tests essential[4,8]. The study has revealed that seroconversion occurs in all infected individuals. All recovered COVID-19 patients have synthesized the antibodies, and changes in their SARS-CoV-2 IgG were detectable after 10-18 days, particularly if they do not have an immune system defect[9, 10]. As shown in Figure 1, changes in IgG and IgM antibodies peaked in all patients between the third and fourth weeks from the onset of symptoms and can be monitored. IgM usually decreases in patients after the fifth week and disappears approximately in the seventh week despite IgG remaining high for seven weeks or more[1].

We must remember that "wave" and "peak" have two different meanings. Incremental fluctuations and disease outbreaks are called "peaks", but "wave" is defined as the period when a disease is still epidemic and has not reached the stage of extinction or complete inhibition[5, 11]. Because the word "wave" is used in all COVID-19 reports, we also call the increase in infected cases a wave, and we call the highest fluctuation of each wave a peak in the following of this article. The official time of COVID-19 onset in Iran was announced on February 20, 2020. So far, five waves of COVID-19 have been reported in Iran. The first wave was from late April 2020 to late June 2020; the second wave occurred from late July 2020 to late August 2020; the third wave happened from early October 2016 to late December 2016[5], while the fourth wave was from mid-February 2021 to mid-May.

This study aimed to investigate the concentration of IgG and IgM antibodies against SARS-CoV-2 for nine months during three seasons and four waves (which included three disease peaks). The antibody evaluation

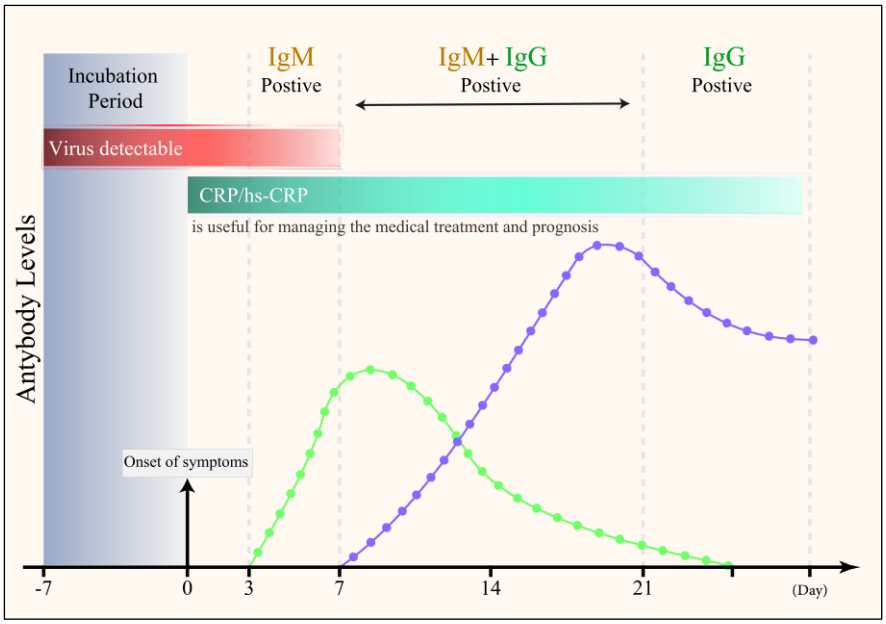

Figure 1. Synthesis and changes in antibodies levels in SARS-CoV-2 infection over time.

was conducted in COVID-19-suspected individuals who visited the Ghiasi hospital. The Ghiasi hospital is one of Iran's oldest (established in 1952) hospitals. It is currently one of the best-equipped hospitals in the Valiasr town of Tehran.

Methods

The ethics committee approved this study of Shahid Beheshti University of Medical Sciences with the ethics code of IR.SBMU.RETECH.REC.1399.117.

Sample collection: In this study, 1000 blood samples of clients were used to determine SARS-CoV-2 antibodies concentration. All of the clients were residents of Valiasr town in Tehran. They attended the laboratory of Ghiasi Hospital located in this town. The sera of these individuals were stored in a refrigerator after isolation and were tested in less than 48 hours.

Materials and Methods: This study used SARS-CoV-2 IgM and IgG kits of Atieh Ideal Diagnosis Company for measuring Igs concentration. These kits are designed to detect specific IgM and IgG antibodies against SARS-CoV-2. The SARS-CoV-2 IgM diagnostic kit was developed based on the Sandwich ELISA assay and the capture antibody type, which exploited human anti-IgM antibodies coated in wells of the ELISA plate. The enzymatic conjugate of this kit contains the nucleocapsid (N) and spike (S) antigens of the SARS-CoV-2 bound to the HRP enzyme. Finally, by forming an immune complex after adding sera samples and then rinsing the ELISA plates, the specific IgM antibody in the patient sample against N and S antigens of the virus could be detected. The 96A-MR Mindray ELISA reader was used to analyze the test responses. The outcomes of the reading device are evaluated by considering the cut-off value resulting from the negative control. As a result, values higher than 1.1 were deemed positive, and those lower than 0.9 were admitted as negative. A negative result indicated the absence of detectable amounts of IgG and IgM antibodies against SARS-CoV-2, and a positive result exhibited the presence of these antibodies.

Sensitivity, accuracy, and specificity of the measuring kits: IgG kit sensitivity was announced at 81.82%, and its specificity was declared at 94.83% by the manufacturer. In the case of the SARS-CoV-2 IgM kit, the sensitivity value was reported to be 95%, and specificity was estimated to be 99.4%, with 95% confidence (announced by the manufacturer). If results need to be interpreted to diagnose or rule out the disease, false-negative and false-positive outcomes should be considered in SARS-CoV-2 serological tests. For this reason, interpreting the results to diagnose the current disease in clients has been avoided in the present study, and these tests have not been used as diagnostic tests. It should also be stated that kits with the same Lot Number were used for all subjects to make the kits' sensitivity, accuracy, and specificity equal and the same for all tests.

Results

Since the primary purpose of this study is to evaluate the antibodies produced in patients infected in the

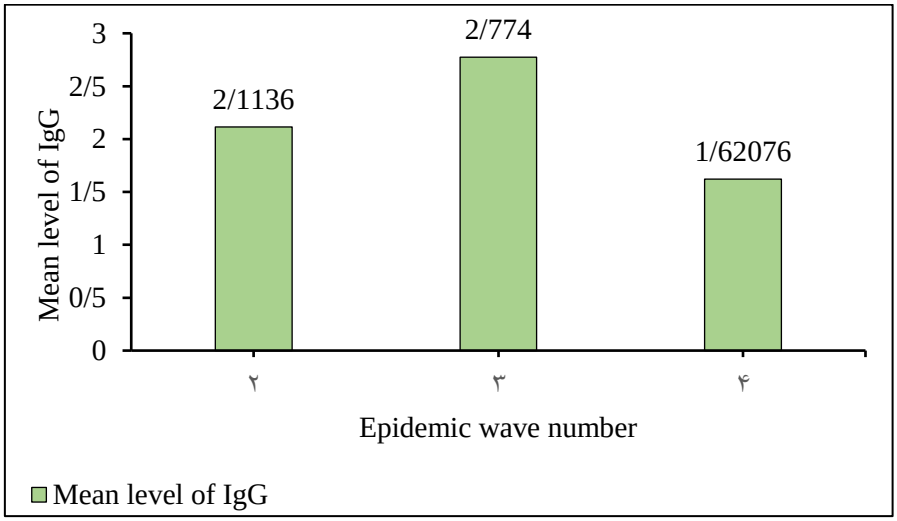

Figure 2. Histogram graph of average level of IgG in SARS-CoV-2 patients in different waves of COVID-19 epidemic in Iran.

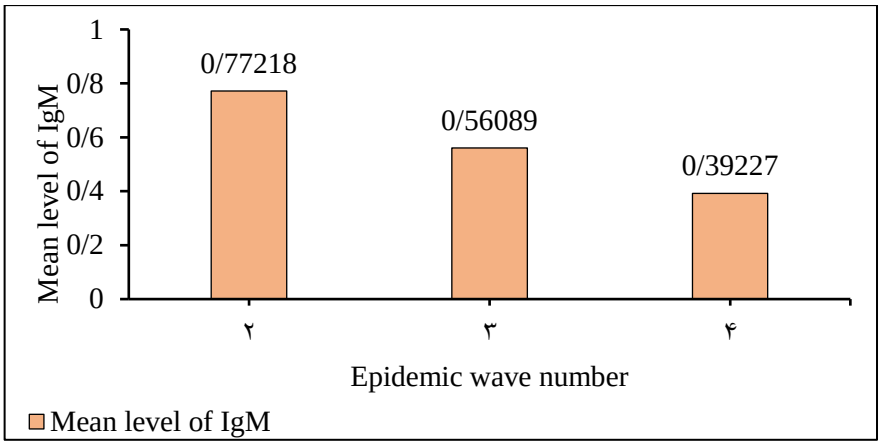

Figure 3. Histogram graph of average level of IgM in SARS-CoV-2 patients in different waves of COVID-19 epidemic in Iran.

Table 1: Average of IgG and IgM concentration in studied SARS-CoV-2 patients.

Descriptive Statics						
	N.	**Minimum**	**Maximum**	**Sum**	**Mean**	**Std. Deviation**
IgG	999	0.005	19.100	2468.425	2.47090	4.665901
IgM	998	0.003	9.400	585.686	0.58686	1.379336
Valid N (list wise)	998					

Table 2: Mean levels IgG in the second, third, and beginning of the fourth wave of COVD-19 epidemic in Iran.

Descriptive IgG								
					95% confidence interval for mean			
	N.	**Mean**	**Std. Deviation**	**Std. Error**	**Lower bound**	**Higher bound**	**Minimum**	**Maximum**
2	228	2.11360	4.432338	0.293539	1.53519	2.69201	0.010	17.430
3	639	2.77400	4.937111	0.195309	2.39047	3.15752	0.005	19.100
4	132	1.62076	3.420891	0.297750	1.03174	2.20978	0.040	14.600
Total	999	2.47090	4.665901	0.147623	2.18121	2.76058	0.005	19.100

Table 3: Significant differences in mean SARS-CoV-2 IgG in the second, third, and beginning of the fourth wave based on the ANOVA test.

ANOVA (IgG)					
	Sum of squares	**df**	**Mean of squares**	**F**	**Sig.**
Between Groups	183.214	2	91.607	4.235	0.015
Within Groups	21543.875	996	21.630	---	---
Total	21727.089	998	---	---	---

diverse wave of the COVID-19 pandemic in Iran, statistical tests were performed to reach precise results. Table 1 shows the mean of SARS-CoV-2 IgG and IgM (0.58 for IgM and 2.47 for IgG, respectively). Table 2 also shows the average IgG concentration in the second, third, and fourth waves. The highest IgG level (2.77) compared to the average (with 95% confidence) is observed in patients infected in the third wave, which

Table 4: Mean levels IgM in the second, third, and beginning of the fourth wave of COVD-19 epidemic in Iran.

Descriptive IgM								
					95% confidence interval for mean			
	N.	Mean	Std. Deviation	Std. Error	Lower bound	Higher bound	Minimum	Maximum
2	228	0.77218	1.590971	0.105365	0.56457	0.97980	0.007	8.600
3	639	0.56089	1.323087	0.052382	0.66375	0.66375	0.003	9.400
4	132	0.39227	1.216660	0.105897	0.60175	0.60175	0.009	7.900
Total	999	0.58686	1.379336	0.043662	0.67254	0.67254	0.003	9.400

Table 5: Significant differences in mean SARS-CoV-2 IgM in the second, third, and beginning of the fourth wave based on the ANOVA test.

ANOVA (IgM)					
	Sum of squares	df	Mean of squares	F	Sig.
Between Groups	13.259	2	6.630	3.502	0.031
Within Groups	1883.600	995	1.893	---	---
Total	18.96.859	997	---	---	---

is confirmed by the ANOVA test shown in Table 3. The same result is illustrated graphically in Figure 2. The mean IgM concentration in the second wave was equal to 0.77 and more than the IgM level in the third wave and the beginning of the fourth wave, which was confirmed by the ANOVA test (Table 5). Furthermore, Figure 3 shows the highest level of IgM in the second wave. In general, in the research period, the maximum amount of IgG was 19.1, and the maximum level of IgM was 9.4, which can be seen in Table 1. The fourth wave was from the winter of 2020 and continues in the spring of 2021. The present study included patients from July 2020 to the end of March 2021. Therefore, only the beginning of the fourth wave is included.

Discussion

The SARS-CoV-2 IgG and IgM levels in 1000 patients who attended the Ghiasi Hospital were statistically analyzed and presented in the results section. Considering that the time of requesting and performing serological tests is critical and should be used for discussing results[10], we will discuss the study results. With a significant difference and 95% confidence, IgG showed its highest value in the third wave compared to the average. At the same time, IgM demonstrated its most significant value compared to the second wave's average. These outcomes could mean that in the second wave, which was from late July 2020 to late August 2020, COVID-19 suspected cases showed clinical signs more quickly and were referred to the hospital faster than patients in other waves. The COVID-19 patients in the second wave became aware of their disease. They followed it by performing an IgM test and being positive. In the third wave, from early September 2020 to the end of December 2020, the disease may have occurred with milder symptoms in the study population, or people were asymptomatic or with fewer symptoms. Therefore, they have passed the disease's initial period and then performed a serological test to ensure their condition or on the physician's order[12].

For this reason, we face high IgG and low IgM during the third wave. Another interpretation of these results is based on the prevalence of experimental equipment for COVID-19 diagnosis. In the summer of 2020, PCR and antibody detection kits (which used ELISA assay) entered the market in the second wave. The boom of domestic ELISA kits began in the same season.

Therefore, physicians admitted more suspects at the onset of the disease or sent them to laboratories. It could cause a higher concentration of IgM in the second wave than in other waves. The significant difference in mean IgG level (which is 2.7) in the third wave compared to the second wave (that is about 2.1) and the fourth wave[1,6] can be the approach of patients to perform PCR test in the early stages of the disease and then perform antibody tests after recovery and passing the acute phase of the disease[8, 9]. Certainly, serological tests for detecting SARS-CoV-2 antibodies are highly applicable in epidemiological studies during a pandemic. All data from different studies and various populations can help researchers in their future epidemiological studies by providing sufficient data about the production, amount, and persistence of antibodies against SARS-CoV-2 before and after vaccination. The results of our research can supply small population statistics as a model for more extensive studies in the future.

Conclusion

As we know, various factors such as age, underlying diseases, sex, and BMI have essential roles in the immune system's response to pathogens and the amount of synthesized antibodies. However, our study testified that in COVID-19, in addition to stated factors that may affect the concentration of Igs, the period of disease and wave of epidemics may also influence the amount of Igs. However, more studies are required to evaluate this issue accurately; this study can remarkably impact epidemiological modeling and data interpretation.

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