The Trend of IgG and IgM Antibodies During 6-Month Period After the Disease Episode in COVID-19 Patients

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Abstract
SARS-CoV-2 is a newly emerged coronavirus that has been widely transmitted since late 2019. It has caused a pandemic and infected roughly 450 million people globally. Hitherto, there is no approved anti-COVID-19 treatment, and vaccination is the only experienced preventive strategy. It mainly promotes the immune system, which is vital as a barrier against COVID-19. Humoral immunity (antibody-mediated immunity), among the various functions of the immune system against the coronavirus, plays an outstanding role in preventing infection. Consequently, we intended to assess IgG and IgM antibodies, three and 6 months after infection, to trend their titer and see how long COVID-19 antibodies remained in the human body. According to the research-designed criteria, only 98 patients out of 4500 suspected cases of SARS-CoV-2 infection remained for analysis. Blood samples were taken in three time periods (Day Zero (T₀), three and 6 months post-infection) and examined for COVID-19’s IgG and IgM antibodies titration using the ELISA platform. Though both IgG and IgM were still detectable for some subjects at the end of the period, the decline in their levels (from 14.45 ± 5.88 to 2.52 ± 2.33 for IgG [85% decline of antibody titer] and 8.3 ± 0.99 to 0.37 ± 0.14 for IgM [95.5% decline of antibody titer]) was statistically significant (P value 0.0001). There was no correlation between gender and IgG and IgM levels. Although the levels of both antibodies were overall higher in the senior group (> 60 years old), statistical analysis showed a significantly higher level just for IgM in this group (P value: 0.005). Following the results, although anti-SARS-CoV-2 IgM and IgG antibodies can persist in the blood for 6 months post-infection, their levels steeply declined over time. Therefore, relying on humoral immunity as a trustworthy barrier against SARS-CoV-2 infection calls for more extensive research.

Keywords SARS-CoV-2 · COVID-19 · IgG · IgM · ELISA

1 Introduction
COVID-19 is a horrible pandemic spread worldwide, resulting in hundreds of millions of infected cases and millions of fatalities. The disease initially appeared in Wuhan, China, spread quickly, and eventually became a pandemic (Lu et al. 2020; Zhou et al. 2020). Fever, dry cough, and exhaustion are the most common symptoms of this disease. A large number of infected individuals get severe pneumonia, and some develop acute respiratory distress syndrome (ARDS) (Huang et al. 2020; Liu et al. 2020a, b). A real-time reverse transcription-polymerase chain reaction (qRT-PCR) test utilizing nasopharyngeal and oropharyngeal swabs is currently the standard diagnosis technique for COVID-19. Although qRT-PCR plays an essential role in detecting this condition, it does have certain drawbacks. For example, in a research conducted in Wuhan, China, on 4880 cases with COVID-19 symptoms or who had close contact with patients, the positive qRT-PCR rate for COVID-19 was 38%, with a high false-negative response (Liu et al. 2020a, b).
There is a significant false-negative rate due to the challenges of obtaining good-quality swab samples at different stages of the disease and the qRT-PCR test’s troubleshooting. Regarding these milestones, identifying and quarantining infected individuals and breaking the...
dissemination cycle of this highly contagious disease will be difficult. Antibodies, on the other hand, are among the most critical elements in diagnosing and treating diseases.

Antibody assays have a significant benefit over qRT-PCR so that they can identify people who had SARS-CoV-2 infection even if they have never undergone testing while acutely ill (Sidiq et al. 2020). Serological tests can be completed more quickly, cheaply, and with fewer steps than molecular approaches (Lee et al. 2020; Wolff et al. 2020).

Nucleic acid amplification tests (NAATs) can be performed in conjunction with serological diagnostics when the viral load of patients is below the detection limit of qRT-PCR assays (Wolff et al. 2020; To et al. 2020).

Furthermore, serological tests can be practical in the following situations: (1) diagnosing patients with negative qRT-PCR results and strong clinical evidence suggesting infection, (2) diagnosis of patients more than 1 week after the onset of symptoms, and (3) figuring out the potential immunity and the likelihood of protection against a reinfection (Rastawicki and Rokosz-Chudziak 2020; GeurtsvanKessel et al. 2020; Lassaunière et al. 2020; Wang et al. 2020).

IgM is the earliest immunoglobulin raised against viral invasion. Then IgG levels begin to grow, with higher specificity and viral neutralizing activity (Dispinseri et al. 2021). The antibody assay is a confirmed test to detect the presence and trend of COVID-19 disease. Li et al. (2020) developed a 15-min point-of-care lateral flow immunoassay to detect IgM and IgG in human blood. According to their primary research, the total testing sensitivity and specificity were 88.66, and 90.63%, respectively (Li et al. 2020).

Some studies have revealed that IgM and IgG antibodies against SARS-CoV-2 rise nearly four days after the appearance of the COVID-19 symptoms and peak one month later (Song et al. 2021; Xie et al. 2020). However, there is a lack of concepts regarding how long these antibodies are sustainable. This study intended to evaluate serum levels’ trend of anti-SARS-CoV-2 IgM/IgG over 6 months.

2 Methods

2.1 Patient Enrollment

Between May 1, 2020, and June 1, 2021, all referring cases to the laboratory for COVID-19 were asked to sign the consent form for study and publication. Patients have the right to refuse to sign the consent form; refusal to sign this form will not affect their care in any way.

Passing the exclusion/inclusion criteria, all individuals with qRT-PCR-confirmed COVID-19 disease participated in this longitudinal observational study. Blood samples were taken from patients as part of the routine protocol. The baseline of IgM and IgG immunoglobulins against COVID-19-specific proteins was tested for all included patients for the first time (Day Zero (T₀)), and IgM and IgG levels were again tested three and 6 months later.

Regarding three steps of exclusion with reasons (vaccinated individuals, pregnant patients, those with reinfection, patients with comorbidities, consumption of medications impacting the immune system, such as cancer and chemotherapy, etc.), the data of 98 individuals remained and qualified to be analyzed (Fig. 1. Flowchart of patients’ enrolment). The Biomedical Research Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran, approved the study under the code IR.BMSU.REC.1399.068 (https://ethics.research.ac.ir).

2.2 Analysis and Instrumentation

Samples were evaluated using the ELISA platform to explore the anti-SARS-CoV-2 IgG (indirect ELISA) and IgM (capture antibody) antibodies following the manufacturer manual (PT-SARS-CoV-2, Pishtaz Teb Co.) (Fig. 2). Reported sensitivity and specificity of this kit claimed to be 94.1% and 98.3% for IgG and 85.4% and 99.4% for IgM, respectively. According to the kit manual, index values higher than 1.1 were considered positive, and below 0.9 were considered negative cases for both IgG and IgM. Cases whose index values were between 0.9 and 1.1 were considered suspicious.

Additionally, the CRP latex test was created to quickly and accurately detect and measure C-reactive protein (CRP). It is a fast slide agglutination technique based on a serum modification of the latex fixing method. Accordingly, the increase in the CRP levels above the upper limit of the reference was indicated by the presence of an apparent agglutination in the samples and patients’ CRP levels were shown to be a marker of COVID-19 severity (Fig. 3).

2.3 Statistical Analysis

SPSS Statistics V22.0 was used to analyze the collected data. The difference between IgM and IgG positive levels was assessed using the Chi-square test. The data were presented using the mean and standard deviation. For normally distributed data, the t-test was employed, and for data with a non-normal distribution, the Mann–Whitney U test was utilized. To compare qualitative variables, a Chi-square test was used. The statistical significance criterion was chosen at a P value of less than 0.05 (statistical abbreviations; Df, degrees of freedom; F, F value; P: P value).
3 Results

3.1 Demographic Data and CRP Levels

Over the study time, 4500 patients referred to the laboratory for COVID-19 testing. After 3 months, 1254 cases remained meeting the inclusion criteria. At the end of the follow-up, the research comprised a total of 98 individuals. In this study, more than 1000 cases got vaccinated and were ruled out of the study (Fig. 1). The patients varied in age from 4 to 83 years old, with an average of 49.67 ± 14.6 years. Males accounted for 61.2% of the cases, while females accounted for 38.8%. The CRP testing was negative in 27.55% while strongly positive in 13.27% of the subjects (Fig. 3).

3.2 Comparison of IgG Levels at Different Time Intervals

According to the findings of repeated observational analysis, the mean IgG level over time was significantly different \((Df \ 2.96, \ F \ 544.59, \ P = 0.001)\). So, after data correction for patients’ age and gender, the difference in IgG levels at different time intervals was statistically significant. The positive rate dropped down to 99% at 3 and 66.3% at 6 months post-infection \((P = 0.001)\). (Tables 1, 2; Fig. 4). Furthermore, no significant association between gender and IgG levels was seen at various time intervals. Additionally, there was no significant association between age and average IgG levels throughout the time intervals \((Df \ 1, \ F \ 2.208, \ P = 0.141)\). In other words, there was no noticeable impact of patient age on IgG levels at different time intervals, as seen in Fig. 5.
3.3 Comparison of IgM Levels at Different Time Intervals

The average level of IgM over the study time was significantly different \((Df = 2.96, F = 84.34, P = 0.001)\). In light of the results, after data correction for the patients of different ages and genders, the diversity in IgM levels at different time intervals was statistically significant. So that the positive rate dropped down to 1% at 3 and 6 months post-infection \((P = 0.001)\) (Tables 3, 4; Fig. 6). Meanwhile, at different periods, a significant association between age and IgM levels was found \((Df = 1, F = 8.18, P = 0.005)\). In other words, there was a noticeable impact of patient age on IgM levels at different time intervals. According to the graph, patients aged over 60 had a higher starting IgM level, whereas those under 60 had a higher average IgM level in the second and third periods (Fig. 7).
4 Discussion

Antibody responses to SARS-CoV-2 in 1–3 months have been extensively investigated, while long-term (6 months or more) are still little studied. The durability of IgG and IgM antibody levels against SARS-CoV-2 at a 6-month interval was investigated in this study. Likewise, the manner of age (over and less than 60) was then evaluated. In this regard, tough exclusion criteria such as reinfection, immunologic comorbidities, and vaccination were considered reaching precise results. Moreover, suspicious cases (IgG ≤ 1.1), follow-up failing, and pregnancy were considered exclusion criteria.

The outstanding highlight of the present research is that such a study will be difficult to undertake in the future due to the near-impossibility of finding the target population since the target population should be infected for the first time, have not been vaccinated, and should not be reinfected throughout the study, in addition to fulfilling general admission criteria. As a result, given the worldwide scope of the COVID-19 pandemic and the wide range of available vaccines, locating a qualified population will be difficult.

During this study, in addition to measuring the IgG/IgM level, the CRP level was also determined. CRP levels may predict the severity of the disease and lung damage at the beginning of COVID-19. According to Liu et al. (2020a, b), patients with COVID-19 had a 65% increase in CRP. According to the studies with COVID-19 axis, CRP levels are significantly different between the deceased

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| Variable          | First       | Month 3    | Month 6    | $F$ (df) | $P$ value |
|-------------------|-------------|------------|------------|----------|-----------|
| IgG level (mean ± SD) | 14.45 ± 5.88 | 7.49 ± 4.88 | 2.52 ± 2.33 | 544.59(2.96) | 0.0001    |
| Age (year)      |             |            |            |          |           |
| < 60            | 14.01 ± 5.29 | 7.32 ± 4.91 | 2.52 ± 2.39 | 2.208(1)  | 0.141     |
| ≥ 60            | 16.97 ± 6.90 | 10.21 ± 4.06 | 3.53 ± 1.80 |          |           |

Table 1 Comparison of the mean of IgG levels at different time period

| IgG statusa | Negative N (%) | Suspected N (%) | Positive N (%) | $P$ value |
|-------------|----------------|-----------------|--------------|-----------|
| Day zero ($T_0$) | 0 (0%) | 0 (0%) | 98 (100%) | 0.001 |
| Month 3      | 0 (0%) | 1 (1%)  | 97 (99%)  | 0.001    |
| Month 6      | 26 (26.5%)| 6 (6.1%) | 65 (66.3%) | 0.001    |

Table 2 Status of individuals based on IgG antibody titers at Day Zero ($T_0$), three and 6 months after infection

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Fig. 4 Comparison of IgG levels at different time intervals. The overall trend was significantly descending. At the initial phase (day zero ($T_0$)), the highest amount of IgG was recorded (14.455). After 3 months, this amount dropped to 7.49, and eventually, after 6 months, it decreased to 2.52. *Significant value in comparison with third period. #Significant value in comparison with other two periods

Fig. 5 The effect of age on the mean IgG levels at different time intervals. $T_0$ stands for the Day Zero of testing. < 60 refers to individuals under 60 years old. ≥ 60 relates to individuals 60 or over 60 years old. The total mean and trend didn’t demonstrate any significant interaction between age and IgG levels

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aThe index values higher than 1.1 were considered as positive and below 0.9 were considered as negative cases for IgG titer. Cases whose index values were between 0.9 and 1.1 considered suspicious
party and the recovered population, which could serve as a predictive indicator of disease severity (Ruan et al. 2020; Agache et al. 2021). CRP is an exemplary assay for evaluating patient conditions in conjunction with other clinical findings because it indicates a strong correlation between patient severity and COVID-19 patient symptoms. In the present study, 72.45% of patients showed positive CRP, which is nearly consistent with other reports.

Our findings showed that the IgG levels for 66.3% of patients were positive even after 6 months but eventually decreased to one-sixth of their beginning levels (from 14.45 ± 5.88 to 2.52 ± 2.33 for IgG [85% decline in antibody titer]). These are consistent with Luo’s research, which looked at average IgG/IgM antibody titers against SARS-CoV-2 antibodies from symptom onset to 210 days. They discovered that typical titers peaked within 30 days of symptom onset and then steadily decreased to one-fifth at 180 and 210 days (Luo et al. 2021).

In the present study, the positive rate for IgG dropped to 99% at 3 and 66.3% at 6 months post-infection. It was more dramatic for IgM, so its positive rate dropped down to 1% at 3 and 6 months post-infection. Maine et al. (2020) found that after 3 months of the onset of symptoms, the positive rate of IgM drops to 30.8%. On the other hand, IgG levels maintained positive in 92.3% of patients 3–6 months after the beginning of symptoms (Maine et al. 2020), which is consistent with the findings of the current investigation.

Another study conducted on 54 SARS-CoV-2-positive people reported that after reaching a peak in IgG titers on

### Table 3
Comparison of the mean IgM levels at different time period

| Variable                | First    | Month 3  | Month 6  | F (df)     | P value |
|-------------------------|----------|----------|----------|------------|---------|
| IgM level (mean ± SD)   | 8.30 ± 0.99 | 0.43 ± 0.23 | 0.37 ± 0.14 | 84.34(2.96) | 0.0001  |
| Age (year)              | 8.18(1)  | 0.005    |          |            |         |
| < 60                    | 5.63 ± 5.88 | 0.496 ± 1.51 | 0.44 ± 0.9  |          |         |
| ≥ 60                    | 10.83 ± 6.11 | 0.36 ± 0.19 | 0.3 ± 0.22  |          |         |

### Table 4
Status of individuals based on IgM antibody titers at Day Zero (T₀), three and 6 months after infection

| IgM statusa | Negative N (%) | Suspected N (%) | Positive N (%) | P value |
|-------------|----------------|-----------------|---------------|---------|
| Day zero (T₀) | 21 (21.4%) | 1 (1%) | 76 (77.6%) | 0.001 |
| Month 3     | 96 (98%)     | 1 (1%)         | 1 (1%)        | 0.001  |
| Month 6     | 97 (99%)     | 0 (0%)         | 1 (1%)        | 0.001  |

aThe index values higher than 1.1 were considered as positive and below 0.9 were considered as negative cases for IgG titer. Cases whose index values were between 0.9 and 1.1 considered suspicious
day 60, they steadily declined to 50% on day 180. There was no correlation between IgG titer and age, sex, or comorbidities in their research, although patients with acute and severe symptoms had much higher IgG levels (De Donno et al. 2021), while we observed a steeper decline in IgG titer and discovered a significant correlation between age and IgM but not with IgG antibody levels. Since antibody titers and plasma neutralization activity have been shown to correlate with disease severity, the rapid decline in antibody rate in our cohort may be related to the mild to moderate symptoms verbally reported by individuals. On the other side, Luo et al. (2021) showed a significant correlation between IgG and IgM antibody levels with age and illness severity but not with patient sex. One of the elements influencing the humoral immune response is antigen persistence in the patients’ bodies (Rezaie et al. 2019). The median length of viral persistence is antigen persistence in the patients’ bodies with age and illness severity but not with patient sex.

Higher levels of IgM levels at the beginning of infection and lower levels 6 months after infection.

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**Declarations**

**Conflict of interest** The authors declare no conflict of interest.

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