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*Original Article*

# Increasing Levels of Serum Anti-Spike S1-RBD IgG after 120 Days of the Pfizer-BioNTech-mRNA Second Dose Vaccination

**Abdul-Wahab Kadhum, A[1 *], Rushdi Abdullah, A[1], Mujahid Al-Shuwaikh, A[2]**

*1. Medical Microbiology Department, Medical College, AL-Iraqia University, Baghdad, Iraq*
*2. Medical Microbiology Department, Medical College, AL-Nahrain University, Baghdad, Iraq*

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Corresponding Author: ayaat.a.kadhum@students.alairaqia.edu.iq

**Abstract**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines, such as Pfizer-BioNTech, have demonstrated high efficacy; however, there is limited data on the duration of immune responses besides their relationships with age, gender, body mass index (BMI), and the presence of previous coronavirus disease-2019 (COVID-19) infection. This study aimed to evaluate SARS-COVID-19 Anti-Spike IgG levels after 30 days (one month) and 120 days (four months) of the 2nd dose of Pfizer-BioNTech vaccine given to medical students at Al-Iraqi University, Baghdad, Iraq. This study was performed after the obtainment of the acceptance and approval of the Medical College of Al-Iraqi University and the Iraqi Ministry of Health. Two groups of students were randomly picked up from the Medical College of Al-Iraqi University. They were completely vaccinated by administering two doses of Pfizer-BioNTech/0.5 ml for each dose. After taking their permission, 5 ml of their blood (one group after one month and the second group after four months of vaccination) was drawn in the Higher Education lab inside the Medical College of Al-Iraqi University. It took approximately four months to collect the samples (from October 2021 until February 2022). Following that, serological analysis was done for measuring the SARS-CoV-2 spike protein IgG by using Elabscience/SARS-CoV-2 spike protein IgG ELISA Kit (USA) (+ve <0.06) that was performed in the Higher Education lab of Medical College of Al-Iraqi University. Demographic data were also collected from participants, including age, gender, BMI, blood group, and the presence of previous COVID-19 infection. For statistical analysis, SPSS (version 26) and STATISTICA (version 12) were used to input, check, and analyze data. Standard approaches of frequencies and percentages were used for qualitative variables, while for quantitative variables, mean±standard deviation was used. A P-value of <0.05 was considered a significant plasma level of the SARS-COVID-19 Anti-Spike IgG. The study results showed that in group 1 (after one month of the 2nd dose), the male-female ratio was 62.2: 37.8, the mean age of the vaccinated students was 28.2000 years old, and the BMI was 25.5454 kg/m2 with 33.3% previously COVID-19 infected individuals. In group 2 (after four months of the 2nd dose), the male-female ratio was 44.4: 55.6, the mean age of the vaccinated students was 25.8444 years old, and the BMI was 24.7584 kg/m2 with 24.4% previously COVID-19 infected individuals. The plasma levels of SARS-COVID-19 Anti-Spike IgG after the 2nd dose of the Pfizer-BioNTech vaccine in group 1 (one month) and group 2 (four months) were statistically non-parametric. Once the independent two samples Mann-Whitney test was used, a significant difference (P<0.05) was observed in SARS-COVID-19 Anti-Spike IgG plasma levels after 30 days of the 2nd dose of the Pfizer-BioNTech vaccine administration, compared to the 120 days of administration. In conclusion, SARS-COVID-19 Anti-Spike IgG levels significantly increased in group 2 (four months after the 2nd dose of the Pfizer-BioNTech vaccine), compared to group 1 (one month after the 2nd dose of the Pfizer-BioNTech vaccine).

**Keywords:** SARS-COVID-19 Anti-Spike IgG, Pfizer-BioNTech vaccine, Medical students, Al-Iraqi University

## 1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic shows until now a high infection rate with a hazardous rate of deaths that reached approximately six million and a half (1), which pushes for a cosmopolitan searching effort to gain a vaccine that is necessary to overcome this pandemic (2, 3). Infectious disease research is fraught with difficulties in vaccine development, protective immune monitoring, and toxicity which are considered challenging approaches. This involves novel and effective vaccine development, as well as an effective therapy, to stimulate a rapid protective response in the community (4). By the time of September 2020, the World Health Organization and the Food and Drug Administration (FDA) declared multiple vaccines for SARS-CoV-2 based on different vaccinology platforms (5, 6). In January 2021, the Iraqi Ministry of Health (MOH) and the National Committee for Drug Selection accepted three types for urgent use, namely Sinopharm, AstraZeneca, and Pfizer/BioNTech. The Iraqi MOH received the first vaccine batch in February 2021 (7). The most popular vaccine nowadays is Pfizer–BioNTech, the first licensed vaccine fully approved by the FDA. It is a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine that codes for SARS-CoV-2 spike protein membrane-anchored and perfusion stabilized (8). This vaccine is administered intramuscularly in two separate doses. Studies have shown that this vaccine induces neutralizing antibody response (4, 6).

Since the S protein is essential for the entry of the virus, it is considered a suitable target for COVID-19 vaccines. The angiotensin-converting enzyme-2 (ACE2) is a receptor for the profusion state of the receptor binding domain (RBD), and it is found in the S1 subunit, while the S2 subunit holds the cleavage site needed for viral and cellular membrane fusion (9). The Pfizer-BioNTech vaccine protects through IgM, IgG, and IgA antibodies. Precisely, it provokes anti-Spike IgG, which inhibits the binding of RBD to ACE2 receptors by its neutralizing action (10). Therefore, the presence of antibodies that can be measured in serum may indicate a past immune response toward prior infection, or it could be due to vaccination (11).

Studies showed that SARS-CoV-2 Anti-Spike-specific IgG levels increased soon after 21 days of the first vaccine dose administration, while after the 2nd dose of vaccination, the serum levels of IgG increased and reached their peak on day seven of the 2nd vaccination. Furthermore, it remained at a high serum level for >100 days after (12). This study mainly aimed to measure the Anti-Spike S1-RBD IgG in the students of Al-Iraqi University after one month (30 days) and four months (120 days) of the fully Pfizer-BioNTech vaccination.

## 2. Materials and Methods

### 2.1. Study Population

Volunteer students from Al-Iraqi University were the subjects of our study. They were completely vaccinated with Pfizer–BioNTech. The study was done inside the lab of the Higher Education affiliated to the Microbiology Department, Faculty of Medicine, Al-Iraqi University, from October 2021 until February 2022.

In total, 90 volunteers who had no history of previous COVID-19 infections, recurrent influenza, or any chronic diseases were selected. Follow-up meetings were done for all volunteers who participated in this study. They were divided into two groups; Group A contained 45 samples one month (30 days) after the 2nd vaccine administration, and Group B had 45 samples but four months (120 days) after the 2nd vaccine administration. A 5 ml blood sample was collected from each participant and poured into a gel tube, then centrifuged to gain serum which was transferred into a plain tube that was stored in the refrigerator. This process happened after 30 days and 120 days following the full vaccination with the Pfizer-BioNTech mRNA vaccine.

### 2.2. Serological Analysis

To analyze the COVID-19- S1-RBD (Anti-Spike) IgG serum levels, we used the SARS-CoV-2 S1-RBD IgG enzyme-linked immune-sorbent assay

(ELISA) Kit (Diasino®: Cat.No. DS207704, China). The manufacturer company protocol depended on the indirect ELISA to measure the serum levels of Anti-Spike IgG. Micro-plate reader Biotech (USA) ELISA system was used for final calculations. The ELISA kit quantification sensitivity was 98.41%, while the kit specificity was 98.02%. To quantify and analyze the antibody concentrations (DU/ml), 6 standards of SARS-CoV-2 S1-RBD kit were used. Values of SARS-CoV-2 S1-RBD IgG serum levels of more than 10 DU/ml were considered positive results. Results were measured by determining the mean absorbance of each duplicated measurement. Then, the mean calculation was made by plotting the common logarithm of absorbance against concentration in DU/ml for every calibrator.

### 2.3. Statistical Analysis

STATISTICA (version 12) was used for the statistical analysis in addition to SPSS statistical software (version 26). The distribution standard was analyzed preliminarily by Kolmogorov-Smirnov and Shapiro-Wilk tests. Conclusive data showed relative, as well as absolute frequencies. On the other hand, quantitative variables were shown as mean±standard deviation. The non-parametric Kruskal made the distinction between the groups for persistent, as well as categorical variables- the Wallis test, which deals with more than two groups, and the Mann-Whitney U-test, which deals with Bonferroni's correction when necessary, in order to find out the differences between one and four months of SARS anti-spike IgG plasma levels (P=0.05).

## 3. Results

The two groups were students who volunteered from Al-Iraqi University. Demographic data, such as age and gender were studied. The mean age was 25.8±2.35 years old, and the gender distribution in one and four months can be seen in tables 1 and 2.

**Table 1.** The Frequency and Percentage of Gender after one month

| Gender | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|
| Female | 17 | 37.8 | 37.8 | 37.8 |
| Male | 28 | 62.2 | 62.2 | 100.0 |
| Total | 45 | 100.0 | 100.0 | |

**Table 2.** The Frequency and Percentage of Gender after four months

| Gender | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|
| Female | 25 | 55.6 | 55.6 | 55.6 |
| Male | 20 | 44.4 | 44.4 | 100.0 |
| Total | 45 | 100.0 | 100.0 | |

ELISA data results of the SARS-CoV-2 S1-RBD IgG were non-parametric and analyzed depending on Shapiro-Wilk and Kolmogorov-Smirnov tests. The results of the independent two samples Mann-Whitney test presented a significant increase (P<0.05) in the serum levels of SARS-CoV-2 S1-RBD IgG after four months of the vaccination, compared to one month after the 2nd dose of Pfizer-BioNTech vaccine (as presented in table 3).

**Table 3.** Independent two samples Mann-Whitney test for SARS-CoV2 S1-RBD IgG serum level after 1 and 4 months

| Variable | Time since vaccination | Mean Rank | P-value. (2-tailed) |
|---|---|---|---|
| Anti-spike IgG | 1 Month | 39.58 | .031 |
| | 4 Months | 51.42 | |

## 4. Discussion

COVID-19 has become a severe cosmopolitan health issue because of its rapid spread and high severity worldwide. Therefore, vaccines have been quickly developed to control the disease spread among people. However, antibody levels required to protect the population remain blurred (13, 14). Therefore, resources and efforts to monitor the rate of the immune response need to be invested. This will help develop effective and safe vaccines (15). Antibody levels were monitored and evaluated to test the effectiveness of the vaccine. This will sort out the issues concerning immunization by vaccination.

Most COVID-19 vaccines are designed to provoke robust antibodies immune response contra the viral spike-RBD, which has many epitopes, to mimic natural infection with the virus. Accordingly, measuring anti-S1-RBD IgG level prevalence could provide an extra-valued vision of immunity toward SARS-CoV-2. Later after vaccination, increased levels of IgG are wished to be a promising index responsible for long-term immunity stimulated by the vaccine, which in turn is accountable for slowing down the transmission rate, thereby preventing the infection (16).

The investigation of the rate and speed of antibody response after vaccination plays a vital role in vaccine evaluation (15). Our study results showed a significant increase in anti-S1-RBD IgG levels in the serum of the volunteers after 120 days following the 2nd dose of the Pfizer–BioNTech vaccine, which runs with (17) who revealed that the IgG response lasts for four months despite its low rates. However, Ali, Alahmad (18) stated that there would be a decline in IgG serum levels a few months after the 2nd dose of the vaccination. Moreover, Bergamaschi, Terpos (19) indicated that a drop in the serum level of the SARS-CoV-2 S1-RBD IgG could be seen in 50 days due to different antibody half-lives, especially since SARS-CoV-2 S1-RBD IgG has a low half-life, compared to the antibodies against the whole Spike. However Wisnewski, Luna (12) confirms that SARS-CoV-2 spike-specific serum IgG further increases and remains elevated more than 100 days after, which in turn makes the persistence of antibodies a positive index of efficient long-term immunity following vaccination.

Anti-Spike S1-RBD IgG serum levels are increased significantly after 120 days following the 2nd dose of mRNA-Pfizer-BioNTech vaccination. Furthermore, studies concerning the vaccinology-immunology of the COVID-19 Pfizer-BioNTech vaccine are necessary to know more data about the duration of protection that this vaccine can give, as well as its efficacy against different strains of the SARS-COV-2 virus.

## Authors' Contribution

Study concept and design: A. A. W. K.
Acquisition of data: A. A. W. K.
Analysis and interpretation of data: A. R. A.
Drafting of the manuscript: A. R. A.
Critical revision of the manuscript for important intellectual content: A. M. A.
Statistical analysis: A. M. A.
Administrative, technical, and material support: A. A. W. K.

## Ethics

The Committee of Ethical Review accepted the study in the Faculty of Medicine at the University of Al-Iraqi.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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