Anti-Spike IgG and Interleukin-10 Immune Responses After The Second Dose of The Covid-19 Vaccine

ABSTRACT

Background:

Although there is insufficient information on the length of immune responses also how they relate with age, COVID-19 mRNA vaccines, notably those from Pfizer-BioNTech, have been rated as having a high effectiveness in generating cellular immunity. The study's objective was to look at how vaccine of Pfizer-BioNTech activated the responses of anti-spike S1-RBDIgG also IL-10 after 1 and 4months following 2nd dosage.

Methodology: The study was done after getting approval from University of Diyala/College of Medicine. 45 individual-students from college of medicine have participated. They were vaccinated with 2 doses from Pfizer-BioNTech vaccine, each dose contained 0.5ml. Each participant accepted to have 5mL of blood drawn two times, after 1-4 months in the postgraduate lab at the college of medicine (Diyala).

The samples were collected over a eight-months period, from January 2022 until Augest 2022. A serological test to mensuration SARS-CoV-2 spike protein IgG was done by using Sunlong Biotech/ SARS-CoV-2-S1-RBD. IgG ELISA Kit/ CHINA (catalogue number SL3219Hu), and IL-10 by using Elabscience Human IL-10(Interleukin 10) ELISA Kit (catalogue number E-EL-H6154) which was done in the postgraduate lab at medical college in University of Diyala. Blood was drawn twice from the same participants in the study; the first time was a month after taking 2nddose of vaccine, while the other was four months after taking 2nd dose.

Results: Male-female ratio were 18:82, with a mean age of 21 for vaccinated student participants, according to the study. The levels of S1-RBD anti-spike IgG

as well as IL-10 after the 2nd dose of the vaccine at 1-4 months. According to the independent two-sample Mann-Whitneytest revealed a significantly difference ($P< 0.05$) in S1-RBD anti-spike IgG and IL-10 levels between 1 and 4 months after the 2nd dose of vaccination.

Conclusion: anti-spikeIgG for SARS-CoV-2 and IL-10levels significantly dropped after 4 months from the 2nd dose of vaccination.

Keywords: COVID-19 Vaccine, SARS-CoV-2;S1-RBDIgG, Interleukin-10, University ofDiyala.

Introduction

The SARS-CoV2 virus infected over 200 million people, with a mortality rate of over 4 million. As a result, the COVID-19 pandemic was categorized as a globally crest-risk (1).

Most scientists and medical professionals have advocated nonpharmaceutical measures to hold the virus since pandemic's onset. In order to stop the virus from spreading, the researchers worked simultaneously on creating an efficient vaccine (2). At that time, the FDA (Food and Drug Administration) and WHO (World Health Organization) have both announced that the COVID-19 vaccine will be released in September of 2020 (3).

Pfizer-BioNTech was one of many companies that made the vaccine, which manifested between the end of 2020 and the start of 2021 and has since been administered two doses intramuscularly. The Pfizer-BioNTech vaccine contains modified RNA (mRNA) of the full length of the SARS-CoV-2 spike, which has undergone two proline mutations for better delivery. This modified mRNA is encapsulated in lipid nanoparticles. Clinical trials haveshown that the vaccine is both safe and effective (4,5).

Numerous Studies found that the mRNA vaccine from Pfizer acts as a multifunctional molecular contrivance that interposes coronavirus entry. within host cells because S1 subunit attaches to the receptor present on surface of the

host cell beforejoining the virus and hostmembrane with its subunit. When the spike-protein switches from its two structurally distinct conformations prefusion and post-fusion—it will first be activated for membrane-fusion to occur (6).

The Pfizer-BioNTech vaccine demonstrated a high level of protection by increasing the duration of IgA, IgG, and IgM antibody activation. In order to prevent RBD from binding to receptors related to ACE2, It specifically stimulates anti-S-protein receptor binding domain-IgG and neutralization activity (7,8). Furthermore, studies on the Pfizer-BioNTech vaccine discovered that it could stimulate cellular immunity by promoting T-cells as well as keeping them for a long time, providing a positive defense against COVID-19; However, interval dose has an impact on the proportional distribution of T cell subsets (9).

Studies have found that measuring the levels antibodies in serum can signalize the protection level induced by vaccination or prior infection with COVID-19 (10).

The Spike(S)-glycoprotein of the COVID-19virus is the main purpose for Immunoglobulins neutralizing also the fundamental construction of Pfizer-BioNTech vaccine, according to Piccoli L. et al. They demonstrated that RBD in plasma causes the majority of neutralizing Abs to be produced, as shown by the 90% reduction in neutralizing titter following RBD depletion (11). Research indicates that using the RBD antigen in serological tests and measuring the level of RBD-specific antibodies can serve as a reliable indicator of SARS-COV-2 immunity in patients. This supports the understanding of how COVID-19 antibodies behave over time (12).

On the other hand, a lot of controversy surrounded the stability, long-term potency, and the quality of antibody responses in COVID-19 patients. While others showed persistent antibody existence and inevitability, others showed abnormal antibody retreat or delayed and weak antibody responses (13, 14).

Because of this, the question of whether there is a period of long-term immunity exists in relation to decreasing of serum-antibodies against COVID-19. While

research indicates that infections following vaccination are linked to depressed the levels of immunoglobulin to the COVID-19 spike-protein, prompting the idea for booster doses (9, 15).

Therefore, more research is required to assess the effectiveness of booster vaccinations in order to give a complete picture of the vaccine's effectiveness. (16). However, representatives from US Department of Health and Human Services have requested that FDA approve booster shots because thebenefits of receiving COVID-19 vaccine far outweigh the risks. (17).

In each infection or vaccination response, cytokines and chemokines play a crucial role in the extending also maintenance of adaptive immunity. They are also important regulators of inflammation and innate immunity (18).

The Pfizer mRNA vaccine caused changes in cytokine/chemokine levels that included the release of molecules that respond for inflammation to both a proinflammatory role, such as VEGF-A, interleukin-6, and CRP, and an antiinflammatory function, such as IL-10 (IL-1Ra) which is essential for controlling the immune system's response to pathogens inside the body of the host, preventing further harm to the host and preserving normal tissue homeostasis. (19,20).

Our study aims to examine the levels of COVID-19 spike Immunoglobulin IgG and Intrelukin-10 in a linear cohort consisting of student from the University of Diyala who had received two doses of the (Pfizer-BioNTech) vaccine.

Materials and methods

first of all, our study was presented and approved by the ethical review committee in the College of Medicine at the University of Diyala. The study samples were 45 healthy student volunteers from the Medical College at the Universityof Diyala who got two doses of Pfizer vaccine and They had not previously been infected with COVID-19. Blood pulled as well as serological tests were carried out in the college's postgraduate laboratories.

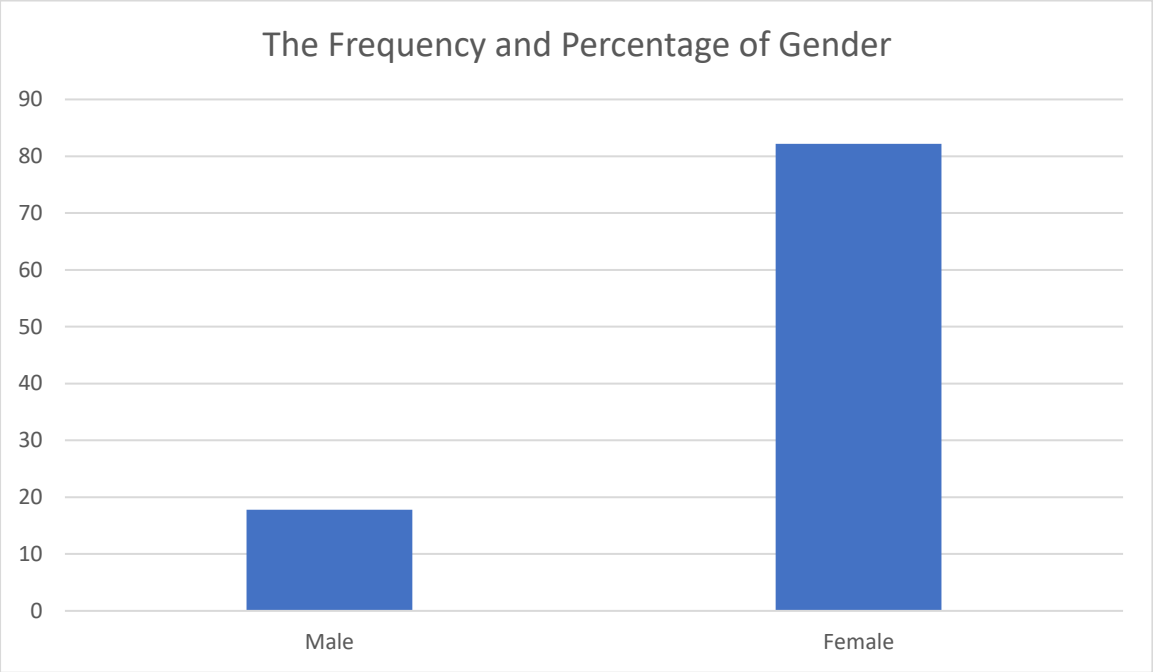
Every student who took part in our study received a follow-up by way of regular meetings for checking on their health. where volunteers suffering from seasonal influenza, chronic respiratory diseases, or other diseases were ignored.
5ml of blood were pulled and putted in gel-tube for serum separation from each participant after 30 and 120 days from taking 2nd doses of Pfizer vaccine.

The Sunlong Biotech/ SARS-CoV-2-S1-RBD IgG ELISA Kit (catalogue number SL3219Hu) and Elabscience Human IL-10 ELISA Kit (catalogue number E-EL-H6154) were used with a Microplate-Reader MindrayMR-96A/Europe ELISA-system to measure Anti-Spik IgG and Intrelukin-10 serum levels. The S1-RBD IgG was quantified using a direct ELISA method as per the manufacturer's protocol, while the IL-10 was measured using a Sandwich ELISA method. The manufacturer reported a 98.02% specificity and a 98.41% sensitivity for the S1-RBD IgG quantification kit, while the functional sensitivity of IL-10 was determined by the lowest analyte concentration that could be consistently measured with an intermediate precision CV of 20%. The statistical analysis was conducted using STATISTICA v.12 and SPSS statistical software v.26. Mean with STDV was used to express quantitative variables, while a Mann-Whitney U-test with Bonferroni's correction was employed to identify differences between groups for both continuous and categorical variables, with a significance level of P-value 0.05. The purpose of the analysis was to compare SARS anti-spike IgG plasma levels and Intrlukin-10 levels between groups with different durations of infection (1-4 months).

Results

Forty-five student volunteers from the Diyala medical college participated in the study at various stages of their studies. Depending demographic variables for gender and age, we found that male-female ratio was 18: 82, respectively. Figure-1 clarifying that.

Furthermore, the age population was 21 $\pm$2.1.



After getting the ELISA results for Anti-spike IgG as well as Intrlukin-10 levels (for 30 and 120days post Pfizer vaccination), our data have been statistically analysed in accordance of independent two-sample Mann-Whitney test, we noted that there was a significantly dropped ($P< 0.05$) in the levels of anti-spike IgG after 120days from 2nd dose of Pfizer vaccination as shown in Figure 2.

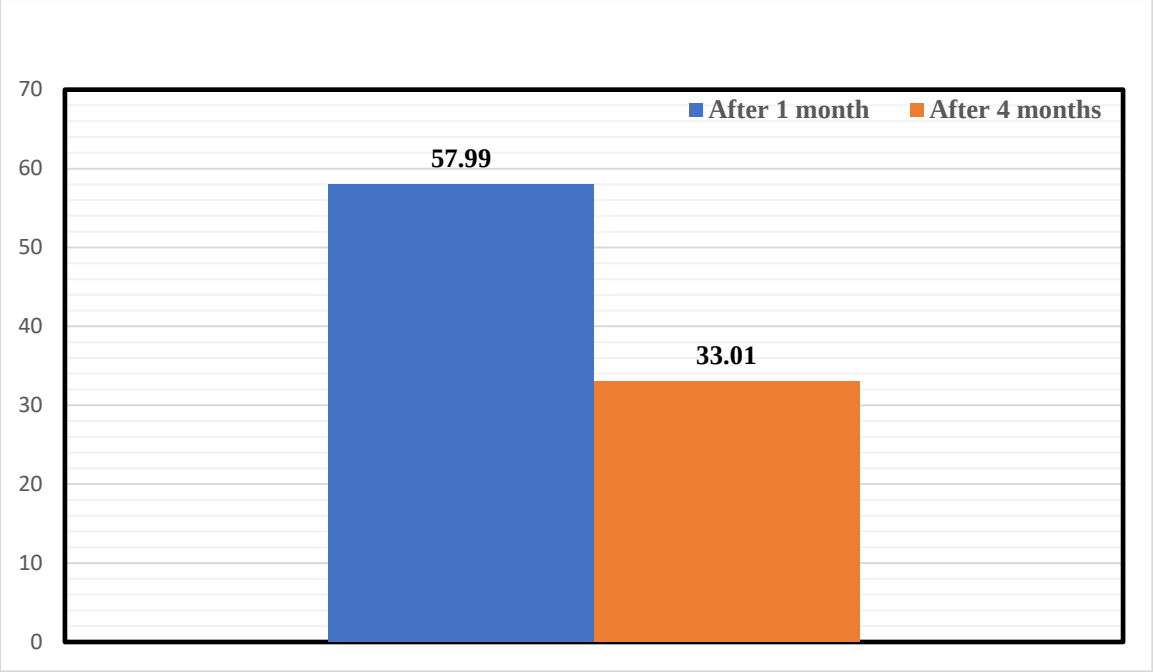


Figure 2: Anti-spike IgG level After 30 – 120 days by Independent two samples Mann-Whitney test

Furthermore, IL-10 shows a significantly decreasing of its levels after 120days for 2nd dose from vaccination according to Mann-Whitney test as in the figure 3.

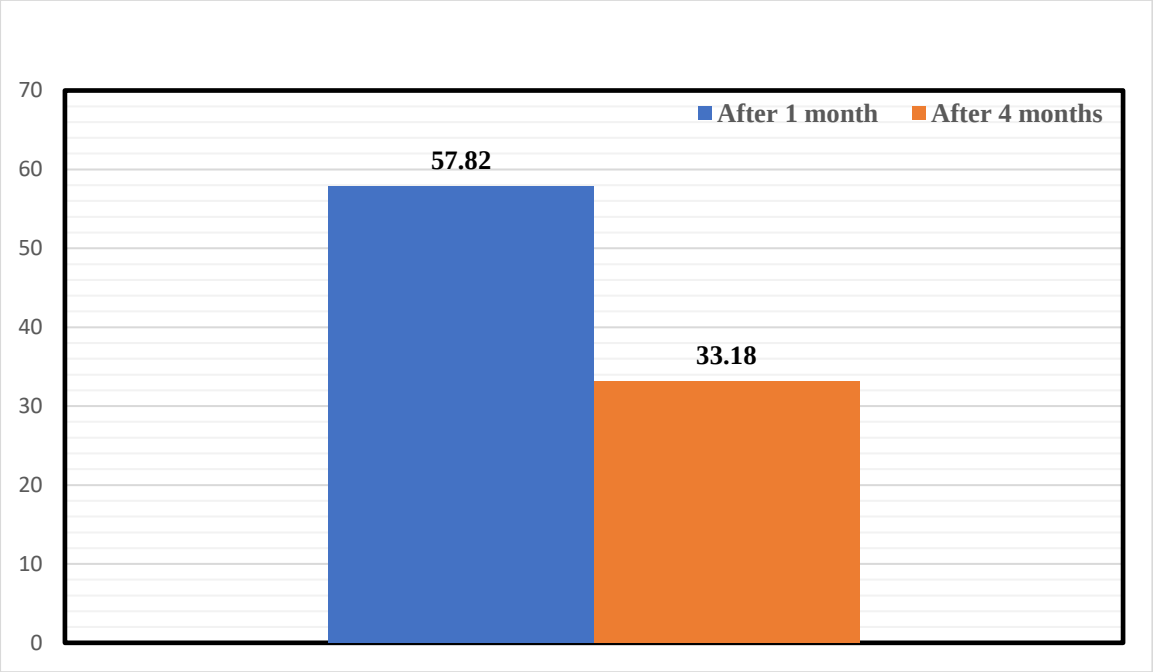


Figure 2: Interlukin-10 levels After 30-120days by Independent two samples Mann-Whitney test for

Discussion

In response to the severity and rapid spread of COVID-19 worldwide, the development of a vaccine was rapidly undertaken to control its spread among the population. This led to one of the most significant global health challenges. However, the desired levels of immunoglobulins necessary to provide protection for various populations using different vaccines remain unclear (21).

Assessing the speed and effectiveness of the immune response, particularly the levels of immunoglobulins, is one of the most important methods for evaluating vaccine efficacy. This will shed light on critical concerns related to active immunization against COVID-19 using mRNA vaccines (22).

A COVID-19 infection can efficiently stimulate the immune system and potentially trigger the body to produce potent neutralizing immunoglobulin that can recognize viral antigens and stop the virus from being contagious. As a result, the majority of SARS-CoV2 vaccines are created to stimulate the production of antibodies against the spiked SARS-CoV2 protein. Therefore, determining prevalence of anti-spike IgG levels may provide important information about developed immunity to SARS-CoV2. After vaccination, it is hoped that the maintenance of anti-spike IgG in serum will be an index of dynamic long-term immunity that serves as a clinical signal for the effective vaccine response responsible for prohibiting infection and containing or slowing the virus transition and prevalence (23).

The ability of the mRNA COVID-19 vaccine to induce a strong immune response against the spike protein, particularly the receptor-binding domain (RBD), is

significant. This is because the RBD contains several important neutralizing epitopes (24).

Research examining the response of anti-spike IgG following COVID-19 vaccination has shown that these levels gradually decline over a period of more than 100 days. In contrast, anti-spike IgA levels begin to decrease significantly after 90 days post-vaccination and inversely correlate with anti-spike IgG levels (25).

Therefore, the findings from our data align with those reported by Muller et al. Who utilized an ELISA to measurement anti-spike-IgGtiters in a cohort of 176 recipients of the Pfizer vaccine against COVID-19. They noticed IgG level for the mRNA vaccine continues to rise more than 90days after the 2nd dose of vaccine (26).

According to Terpos et al., 2021, the difference in antibody half-lives antibodies to Spike-RBD proteins typically have a stumper halflife than antibodies to complete Spike is what causes the reducing in Anti-Spike IgGs level to be significantly dropped by day 50. This is a common characteristic of the antibody specificities because the speckled varieties reflect the quality of these types of antibodies in recovery patients and other people who have received mRNA vaccinations.

Additionally, according to some references, the known serum half-lives of the different immunoglobulin isotypes 21–28 days for gamma and 5–6 days for alpha are coordinated with the decay as well as induction of specific IgGs in response to the mRNA COVID-19 vaccine (27).

One of the crucial cytokine types in the case of COVID-19 infection is IL-10, which can prevent the production of proinflammatory molecules like IL-1, 6, TNF, and INF in various cell types (28).

Studies on the COVID-19 pandemic have revealed that IL-10 can control the cytokine storm response in patients with severe COVID by blocking IL-12 and inhibiting IL-6, IL-1, and IFN (29).

Additionally, a clinical study discovered that the levels of proinflammatory and antiinflammatory cytokines, such as IL-6, IL-10, IL-4, IL-8, and TNF-, increased during COVID-19 infection (IFN-) (30). Additionally, they proposed a connection between the pro- and anti-inflammatory functions of IL-6 and IL-10, IL-2, and IL-4 (30). Due to its function and response following vaccination, IL-10 has demonstrated significance during COVID-19. The regulation of cytokines may be a sign of an effective vaccination, which leads to an effective production of antibodies (31).

Studies have shown that the first dose of vaccination in antigen-naive subjects causes inflammation and activation of the innate immune system, which results in acute and persistent effects on serum cytokine levels that can last up to a week. They continued, "Following the second immunization, broader and stronger cytokine alterations were found." Pfizer-BioNTech then caused changes to systemic cytokines like IL-10 (32).

All of the aforementioned studies as well as others in the field of vaccination immunology are consistent with our findings, which showed a marked decline in IL-10 serum levels four months after receiving the second dose of the Pfizer-BioNTech vaccine compared to those after one month. It's possible that the Pfizer-BioNTech vaccine caused an increase in IL-10, or that the vaccine most likely caused an immunological exacerbation.

Given that IL-10 is a multifunctional cytokine with regulatory and neutralizing functions that reduce the hyperinflammatory response, the increase of IL-10 during vaccination as a type of immune response role may make an equation between proinflammatory and anti-inflammatory. This is because after four months, we noticed that the levels of IL-10 decreased, which may be because the body of the vaccinated lacks inflammation.

CONCLUSION

The levels of Anti-spike IgG and Interleukin-10 in the serum experienced a notable decrease after a period of 120 days (4 months) following the second dose ofPfizer vaccine administered.