



## IL-15 and SARS-CoV-2 S1-RBD IgG serum levels decrease after 4 months of the 2nd dose of Pfizer–BioNTech vaccine administration which was given to the medical college students at Diyala University

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### Abstract

#### Background:

Fighting the Covid-19 pandemic is one of the global priorities now, and the most important type of pandemic control is vaccination. Pfizer-Biotech is considered one of the most important vaccines currently because of its high effectiveness in stimulating the immune system, despite limited data regarding the duration of the response and its side effects. The goal of this study is to assess the response of SARS CoV-2 S1-RBD IgG and Interleukin-15 after 30 and 120 days from the 2nd dose of Pfizer-BioNTech vaccine which applied on the medical college students at Diyala university.

#### Methodology:

This study began after the obtainment of the Medical College of the University of Diyala, the Medical College of AL-Iraqia University, and the Iraqi Ministry of Health approvals. It continued from October 2021 until March 2022. A total of 45 male and female participants from the College of Medicine (Diyala University) students who took the two doses of Pfizer-BioNTech and were divided into two groups: 1 month (30 days) and 4 months (120 days) after the full vaccination (two doses). A 5 ml of their blood was taken two times (30 days and 120 days after the 2<sup>nd</sup> dose of the Pfizer-BioNTech vaccine) in the postgraduate laboratories inside the Diyala Medical College.

A serological analysis to quantify IL-15 and SARS CoV-2 S1-RBD IgG has been done using BT LAB/ Bioassay Technology Laboratory/ Human Interleukin 15 ELISA Kit from CHINA and Diasino/ SARS CoV-2 S1-RBD IgG ELISA Kit/ CHINA respectively. All the lab work happened in the postgraduate laboratories inside the Diyala Medical College.

Demographic information (Age and Gender) has been collected from the participants. These participants were split into two groups depending on the time after the 2<sup>nd</sup> of Pfizer-BioNTech vaccine dose (1 month and 4 months, respectively).

STATISTICA (version 12) and SPSS (version 26) were used to input, review and data analysis. Essential approaches of percentages and frequencies were used for qualitative variables, while, average and standard deviation were used for quantitative variables. For both IL-15 and SARS CoV-2 S1-RBD IgG, less than 0.05 of a P-value was considered considerable.

#### Results:

The ratio, according to gender, was (17.8: 82.2) while the age Average was (20.9 years old). The serum data of IL-15 and SARS-CoV-2 S1-RBD IgG levels after 1 month (30 days) and 4 months (120 days) were statistically non-parametric.

Mann-Whitney test (Independent two samples), showed a considerable drop ( $P < 0.05$ ) of IL-15 as well as SARS CoV-2 S1-RBD IgG serum levels in the 4<sup>th</sup>-months group compared to the 1-month group.

#### Conclusion:

Interleukin-15 and SARS CoV-2 S1-RBD IgG serum levels significantly dropped after 120 days of the 2<sup>nd</sup> dose of Pfizer-BioNTech vaccine.



**Keywords:** SARS CoV-2 S1-RBD IgG, IL-15, Pfizer-BioNTechvaccine, students of the medical college, Diyala university.

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## Introduction

COVID-19 cases reached at the mid of August 2022 approximately 600 million cases with more than 6 million deaths while in Iraq it was approximately 2.5 million with 25000 deaths (1). In order to stop the virus from spreading, research was done simultaneously on creating an effectual vaccine(2).

Pfizer–BioNTech was one of the first vaccines to be licensed internationally between December 2020 to February 2021, and it began to be used intramuscularly with two doses (3). In January 2021, the National Committee for Drug Selection (NCDS) at the Iraqi Ministry of Health (MOH) accepted AstraZeneca, Pfizer/BioNTech, and Sinopharm vaccines for urgent use. The first batch of vaccines was received by Iraqi MOH in February 2021 and according to the MOH data, Iraq has succeeded to vaccinate only 20% of its population until now (4,5).

Pfizer–BioNTech vaccine consists of lipid nanoparticles containing nucleoside-modified RNA (mRNA) encoding the SARS-CoV-2 -luf htgnel spike, remodeled by two proline mutations to combine it in the perfusion modification. Clinical trials have shown this vaccine to be both safe and effective due to its stability, translatability, and reactogenicity as well as well-tolerated delivery technologies due to its lipid structure (6,7). Furthermore, self-amplifying mRNA vaccines have the benefit of frequently requiring a lower dose of mRNA, which also reduces (Toll-like-receptor) TLR recognition related to innate immunity (8). According to some Studies, Pfizer–BioNTech vaccine is able to induce specific types of antibodies like IgA and IgG for unlimited time by stimulating anti-S-protein receptor bound domain S-RBD-IgG with neutralization

efficiency competent to inhibit RBD binding with relative receptors (ACE2) angiotensin modifying enzyme2 (9,10).

Chemokines and Cytokines are substantial drivers of innate immunity and inflammation, also they play a vital part in the maintenance and evolution of adaptive immunity, in response to both vaccination and infection (11).

One of these cytokines is Interleukin-15 (IL-15), which is the one of most interesting and most widely tested. IL-15 is a cytokine with structural similarity to IL-2 that connect to and indicate through the common gamma chain (Gamma-C, CD132) and a complex composed of IL-2/IL-15 receptor beta chain (CD122). Mononuclear phagocytes (and some other cells) secrete the IL-15 following infection by a virus. The proliferation of the Natural killer cells such as cells of the innate immune system, which its initial role is to kill virally infected cells, has been stimulated by this cytokine (12). Studies demonstrated that IL-15 has little impact on the simple or central memory T (T-CM) cell subsets while dramatically increasing in vivo the proliferation of rhesus macaque (RM) CD4 (+) and CD8 (+) T (EM) cells (13).

Some studies showed that the cytokine/chemokine response pattern was different after the 2nd vaccination of Pfizer–BioNTech especially IFN-g, IL-15, IP-10/CXCL10, and IL-6 which rose significantly on day 23 compared to day 2 (11).

For characterizing markers related with vaccination resulting in useful antibody evolution, we choose to study the IL-15 and SARS-CoV-2 S1-RBD IgG serum levels after four months (120 days) of the 2nd dose of Pfizer–BioNTech vaccine applied on the participants.

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## Materials and Methods

Ethically, our study has been reviewed and approved by the ethical scientific Committee of the University of Al-Iraqia /college of medicine. 45 Volunteers who participated in

our study were students in the College of the Medicinein Diyala University. All volunteers took two doses ofPfizer–BioNTech. Vaccine. The study applied work happened in the post-graduate laboratories/College of Medicine/ Diyala university in the period between September 2021 to the end of March 2022. Each student suffering from weakened immunity, recurrent influenza, chronic disease, and infected previously by COVID-19 has been excluded and followed up happened regularly throughout the study.

5 ml of blood was drawn during two periods, 30 and 120 days after the Pfizer-BioNTech second dose to measure IL-15 and S1-RBD-IgG levels then whole-blood samples were placed in a gel tube for the purpose of serum **Results**

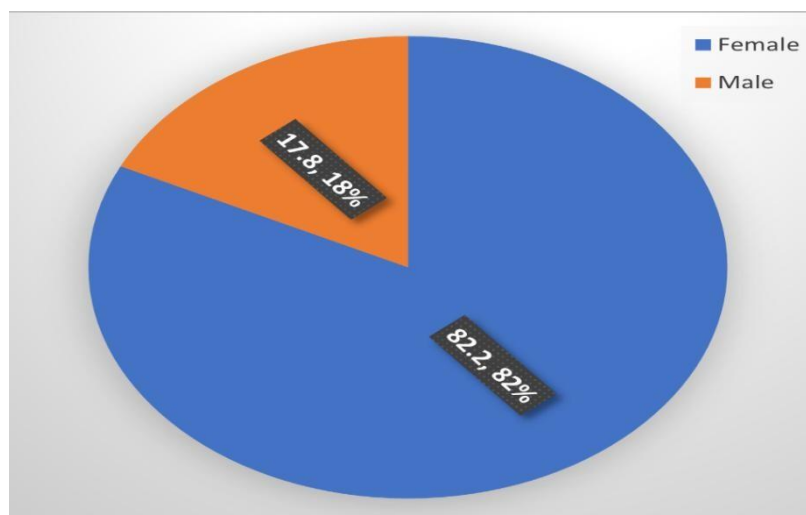
separation by centrifugation, after that, the serum was transferred into a Plain tube to be kept in a refrigerator.

To conduct the study, we utilized the BT LAB/ Bioassay Technology Laboratory/ Human

Interleukin 15 ELISA Kit/ CHINA (catalogue number E0097Hu) and Diasino/ SARS CoV-2 S1-RBD IgG ELISA Kit (catalogue number DS207704) as indirect ELISA. The results were recorded using a MindrayMR-96A Microplate-ELISA Reader system /Europe. The sensitivity and specificity of both kits and according to the manufacturer were 98.41% and 98.02%, respectively.

STATISTICA version 12 and SPSS version 26 statistical software were used to calculate the statistical analyses. A preliminary analysis using the Kolmogorov-Smirnov and Shapiro-Wilk tests was done on the distribution standard. Absolute and relative frequency expressions were used to express categorical variables.

In accordance with gender, our study included 45 participants, males and females. As mentioned above, they were medical students in the faculty of Medicine/Diyala University. The ratio of the number of males to female was (17.8: 82.2) as in figure 1, while the age mean was 20.9 2.1.



6. Figure-1: The Gender Frequency and Percentage

SARS CoV-2 S1-RBD IgG and IL-15 responses were checked by ELISA after 30 and 120 days from taking the Pfizer vaccine in the serum. Kolmogorov-Smirnov and Shapiro-Wilk tests have been used to analyze data and

investigate the response levels. Table-1,2 displays the normality of SARS CoV-2 S1-RBD IgG and IL-15 variables following the results, which appeared to be non-parametric after 30–120 days (1 and 4 months).

**Table-1:** The normality Test for COVID-19 S1-RBD IgG serum level after 30 and 120 days

<b>One-Sample Kolmogorov-Smirnov Test</b>			
<b>Variable</b>		<b>SARS CoV-2 S1-RBD IgG after 30 days</b>	<b>SARS CoV-2 S1-RBD IgG after 120 days</b>
<b>n</b>		<b>45</b>	<b>45</b>
<b>Normal Parameters<sup>a,b</sup></b>	<b>Mean</b>	<b>643.1736</b>	<b>457.3740</b>
	<b>Std. Deviation</b>	<b>258.07060</b>	<b>269.36739</b>
<b>Most Extreme Differences</b>	<b>Absolute</b>	<b>.159</b>	<b>.265</b>
	<b>Positive</b>	<b>.159</b>	<b>.265</b>
	<b>Negative</b>	<b>-.085</b>	<b>-.152</b>
<b>Test Statistic</b>		<b>.159</b>	<b>.265</b>
<b>P (2-tailed)</b>		<b>.006c</b>	<b>.000c</b>
a. Meaning distribution is Normal. b. Measured from data. c. Lilliefors Significance Correction. d. The lower bound of true significance.			

**Table2:**The normality Test for IL-15 variables after 30 and 120 days

<b>One-Sample Kolmogorov-Smirnov Test</b>			
<b>Variable</b>		<b>IL-15 after 30 days</b>	<b>IL-15 after 120 days</b>
<b>N</b>		<b>45</b>	<b>45</b>
<b>Normal Parameters<sup>a,b</sup></b>	<b>Mean</b>	<b>626.7469</b>	<b>501.5724</b>
	<b>Std. Deviation</b>	<b>161.47985</b>	<b>132.61335</b>
<b>Most Extreme Differences</b>	<b>Absolute</b>	<b>.209</b>	<b>.112</b>
	<b>Positive</b>	<b>.106</b>	<b>.095</b>
	<b>Negative</b>	<b>-.209</b>	<b>-.112</b>
<b>Test Statistic</b>		<b>.132</b>	<b>.112</b>
<b>P (2-tailed)</b>		<b>.048<sup>c</sup></b>	<b>.192<sup>c</sup></b>
a. Meaning distribution is Normal. b. Measured from data. c. Lilliefors Significance Correction. d. The lower bound of true significance.			



On the flip side, an independent two-sample Mann-Whitney test has been used in order to reveal if there is a significant difference in values for IL-15 and SARS CoV-2 S1-RBD IgG after 30 and 120 days of Pfizer–BioNTech vaccination. As in Table 3, which shows us

that there is a significant change for IL-15 and SARS CoV-2 S1-RBD IgG after 30 and 120 days of the final (2nd )dose of Pfizer– BioNTech vaccination, the P-value was less than 0.05.

**Table 3:** Independent two samples Mann-Whitney test forSARS CoV-2 S1-RBD IgG and IL-15

Variable	Vaccination	Mean Rank	Z	P-value. (2-tailed)
SARS CoV-2 S1-RBD IgG concentration	30 days	57.99	-4.536	0.000
	120 days	33.01		
IL-15	30 days	59.13	-4.951	0.000
	120 days	31.87		

### Discussion

The field of vaccination against the Covid-19 pandemic has witnessed rapid progress and high importance, with clinical release for innovative programs, particularly vaccines based on mRNA. A variety of reactions are elicited by innovative vaccine techniques like the Pfizer-mRNA vaccine, but it is largely unknown how to control their quality and quantity (14).

Studying the rate and speed of antibody response after vaccination plays a vital role for vaccine evaluation (15).

Studies found that the immune system will be effectively stimulated after COVID-19 infection which generates strong neutralizing immunoglobulins able to be recognizing and blocking viral antigens. Consequently, some of the COVID-19 vaccines have been prepared to stimulate immunoglobulins against COV-19-spike protein. For this reason, Anti S1-RBD IgG levels may supply a worthy insight for granting immunity against COVID-19 as well as a convenient indicator for effective long-range immunity and for a good clinical indicator for vaccine induction (16).

It is pivotal that SARS-CoV-2 vaccines elicit a robust immune response against spike-protein, especially its receptor-binding domain (RBD), which contains numerous neutralizing epitopes (17).

Researchers have estimated the anti-spike IgG after vaccination and found that while anti-spike IgG and IgA gradually decrease after 3 months, IgG drops faster than IgA(18).

Our study was consistent with other studies such as Muller et al. 2021, which revealed that the IgG response lasts for four months, despite its low rates (19).

Terpos et al., 2021 indicated that the drop in anti-Spike S1-RBD IgG levels will be considerable by day 50 due to differing antibody half-lives, with antibodies to Spike-RBD having a lower half-life than antibodies to full Spike. The observed discrepancies indicate the pattern of these antibodies not just in recovered patients as well as in mRNA vaccinated individuals; hence, this is a property of antibody specificities in general (20).

Modification of cytokines is a vital marker of successful vaccination that's why we focused



to analyze IL-15 serum levels in our tow period study (11).

Studies found that the first dose of vaccination can cause inflammation and the innate immune system's activation, which

led to both acute as well as more long-lasting impacts on cytokine/chemokine serum levels (up to seven days after taking dose). After the second dose of vaccination, cytokine changes were more extensive and significant, which also suggests that anamnestic responses were stimulated (11).

Therefore, one of the most crucial methods for determining whether a vaccine is effective is to monitor the rate and quickness with which the immune system responds, particularly cytokines as well as antibody levels (15).

The pleiotropic cytokine interleukin-15 (IL-15) has a wide range of biological impacts on a wide variety of cell kinds. By modifying innate and adaptive immune cells, it is vital in the development of both protective immune responses as well as inflammatory responses to microbial invaders.

Furthermore, IL-15 stimulates innate immune responses by activating NK cells, CD8+ T cells, and T regulatory cells. (21).

In fact, Pfizer-BioNTech's mRNA vaccine administration caused a systemic cytokine signature that included IL-15, a molecule crucial for inducing innate immune responses, forming adaptive immunity, and establishing immunological memory. It's significant to note that variations in IL-15 levels may have a positive correlation with antibody titers against the SARS CoV-2 Spike- RBD due to coordinate response to the vaccine (22).

Some studies have recommended that it can be made an analysis after 24 hours of taking the vaccine to know the innate and vital signs of immune response behaviors stimulated by the vaccine, one of the cytokines identified by these studies is IL-15,

where they found that there is a high stimulation of a group of cytokines, including IL-15 (22).

These data can be consistent with our study, our results proved that the serum levels of

interleukin-15 after 30 days of the second (final) dose of the Pfizer BioNTech vaccine increased significantly compared to 120 days because of the effectiveness of the vaccine in stimulating the cells of the immune system. which indicates the true effectiveness of the Pfizer vaccine in stimulating the immune system in 30 days after the 2<sup>nd</sup> dose of the vaccination. In another recent study, a specific indirect mechanism of interleukin-15 was found on macrophages, monocytes, and dendritic cells to stimulate IP10/CXCL10 by interferon-gamma which may be the cause of the increased serum level of the IL-15 after 30 days of the 2<sup>nd</sup> dose of the Pfizer- BioNTech vaccine (23).

Due to the importance of interleukin-15 in deciding and activating the immune response against viral infections, it became clear that there is a positive and effective effect of the Pfizer vaccine in activating some cytokines such as IL-15 early after the 2<sup>nd</sup> dose of vaccination.

### Conclusion

IL-15 and SARS CoV-2 S1-RBD IgG serum levels were significantly decreased after four months (120 days )of the 2<sup>nd</sup>Pfizer-BioNTech vaccination dose. In addition, cytokines/ IL- 15 could be used to assess the efficiency of mRNA vaccination strategies and to monitor vaccination efficacy.

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