




The Role of SARS-CoV-2 Vaccine in Inducing S1-RBD anti-spike IgG Antibody, IL-10, and IL-1 β Responses

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Abstract

Background: The Pfizer-BioNTech vaccine has revolutionized the field of vaccines with its approach to eliciting and modulating the immune response against SARS-CoV-2, despite uncertainty about the duration of this response.

Objectives: The study aims to estimate the immunological effectiveness of the Pfizer-BioNTech vaccine in eliciting anti-spike IgG antibodies and in modulating IL-10 and IL-1 β .

Patients and Methods: The Prospective Cohort study was conducted between June 2022 and August 2023 and included 90 participants who had not previously been infected with SARS-CoV-2 and were unvaccinated (16 male and 74 female). Their ages ranged from 19 to 23 years. They were divided into two groups: 45 participants in the control group and 45 participants who received two doses of the Pfizer-BioNTech mRNA vaccine. The levels of S1-RBD (S1 subunit contains a receptor-binding domain) anti-spike IgG antibody, IL-10, and IL-1 β were measured in the baseline group and the vaccinated group at 1 and 4 months after the 2nd dose of vaccination using the Enzyme-Linked Immunosorbent Assay (ELISA) method.

Results: The male-to-female ratio was 18:82, with a mean age of 21 among vaccinated student participants. The levels of S1-RBD anti-spike IgG antibody, IL-10, and IL-1 β before and post the 2nd dose of the vaccine at 1–4 months were (nonparametric). In addition, the Mann–Whitney test revealed a significant difference ($P < 0.001$) in S1-RBD anti-spike IgG antibody and IL-10, IL-1 β levels between 1 and 4 months after the 2nd dose of vaccination.

Conclusion: The Pfizer-BioNTech vaccine induced robust immune responses, with significant increases in S1-RBD anti-spike IgG antibody levels and in IL-10 and IL-1 β levels post-vaccination.

Keywords: Pfizer-BioNTech vaccine, S1-RBD anti-spike IgG antibody, IL-10, IL-1 β , Vaccination.

Introduction

The SARS-CoV-2 virus has infected more than 200 million people, with a mortality rate of more than 4 million. As a result, the COVID-19 pandemic has been categorized as a global crest risk (1). Most scientists and medical professionals have not advocated pharmaceutical measures to control the virus since the onset of the pandemic. To prevent the virus from spreading further, researchers have worked concurrently to develop effective vaccines (2). At that time, the Food and Drug Administration (FDA) and the World Health Organization (WHO) announced that the COVID-19 vaccine would be released

in September 2020 (3). Pfizer-BioNTech was one of several companies that developed a vaccine between the end of 2020 and the beginning of 2021, and it has since been administered twice intramuscularly. This vaccine contains modified mRNA encoding the full-length SARS-CoV-2 spike protein, with two proline mutations to improve delivery. This modified mRNA is encapsulated in lipid nanoparticles. Clinical trials have shown that the vaccine is safe and effective (4,5). Numerous studies have demonstrated that Pfizer's mRNA vaccine works as a multifunctional molecular construct that prevents virus penetration. The S1 subunit binds to the receptor on the host cell surface before linking the viral and host membranes via its subunit. When a spike protein shifts from its distinct structural conformations (prefusion and postfusion), it is first activated for membrane fusion (6). The Pfizer-BioNTech vaccine demonstrated a high level of protection by increasing the duration of IgG and IgM antibody activation. To prevent the receptor-binding domain RBD from binding to receptors related to Angiotensin-Converting Enzyme 2 (ACE2), it is specifically anti-S-RBD-IgG and neutralization activity (7,8). Furthermore, studies on the Pfizer-BioNTech vaccine revealed that it could stimulate cellular immunity by promoting T cells and maintaining them for a long time, providing a positive defense against COVID-19; However, the interval does affect the proportional distribution of T-cell subsets (9). According to previous investigations, determining antibody levels in serum can indicate the level of immunity produced by vaccination or prior infection with COVID-19 (10). The SARS-CoV-2 spike (S) glycoprotein is considered the key reason for immunoglobulin neutralization and the core design of the BioNTech-Pfizer vaccine. According to (11), the receptor-binding domain (RBD) in plasma elicits the majority of neutralizing Abs, as evidenced by a 90%

reduction in neutralizing titers following RBD depletion. Research indicates that using the receptor-binding domain 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. These findings support the understanding of how COVID-19 antibodies behave over time (12). There is controversy regarding the stability, potency, and quality of antibody responses in COVID-19 patients. Some studies show persistent antibodies, while others report abnormal declines or weak responses (13,14). Infections post-vaccination link to low spike protein antibodies, suggesting a need for booster doses (9,15). It is important to assess the efficiency of booster immunizations to provide an inclusive view of vaccine efficacy. (16). However, the advantages of receiving the COVID-19 vaccination significantly exceed the risks (17). In each infection or vaccination response, cytokines and chemokines play crucial roles in the extension and 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 including the release of molecules that respond to inflammation to play a proinflammatory role, such as Vascular Endothelial Growth Factor A (VEGF-A), interleukin-6, and C-reactive protein (CRP), and an anti-inflammatory 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). The study objective is to assess the immunological effectiveness of the Pfizer-BioNTech COVID-19 vaccine by evaluating its role in inducing S1-RBD anti-spike IgG antibodies and in modulating IL-10 and IL-1 β production.

Patients and Methods

Study design and participants: This Prospective Cohort study was presented and approved by the ethical review committee of the College of Medicine at the University of Diyala under Code 2022MAS690. The study was done between June 2022 / August 2023. Samples comprised 90 healthy student volunteers from the College of Medicine, 16 males and 74 females who had never been infected with COVID-19, and were divided into two subgroups: a control group and a vaccinated group that had received two doses of the Pfizer-BioNTech vaccine. Before their participation, they underwent IgG/IgM testing using the VIDAS® SARS-CoV-2 IgG/IgM assay. To ensure that they were not previously infected or vaccinated.

Inclusion and exclusion criteria: All participants in the study were uninfected and not vaccinated with any SARS-CoV-2 vaccine. Volunteers suffering from seasonal influenza, chronic respiratory diseases, or other diseases, even during the current study, were excluded.

Blood samples collection: Blood specimen was drawn, and serological tests were carried out in the college's postgraduate laboratories in the medical faculty/ University of Diyala. Every student who participated in the study received follow-up through regular meetings to check their health. The weekly follow-up included inquiries about side effects such as fever, headache, and fatigue. Additionally, individuals with unrelated secondary infections, such as urinary, respiratory, or gastrointestinal infections. The vaccinated students had been drawn 5 ml of blood at 30 and 120 days after the second dose of the Pfizer vaccine, the blood was placed in a gel tube for serum separation, then centrifuged at 10,000 r.p.m. for 10 min.

Determination of biomarkers: Anti-Spike IgG, IL-10, and IL-1 β serum levels were measured using a semi-automated ELISA instrument (Mindray Bio-Medical Electronics Co., Ltd.). In addition, the kits that were used to determine the

levels of S1-RBD anti-spike IgG antibody, IL-10, and IL-1 β were Diasino/SARS-CoV-2 S1-RBD IgG ELISA Kit/CHINA, REF: DS207703 and ELK Biotechnology Co. Ltd., Wuhan, CHINA kits (Cat: ELK1142, and Cat: ELK1270, respectively).

Statistical Analysis

Statistical analyses were performed by SPSS statistical software v.26.0 (SPSS Inc., Chicago, IL, USA) and STATISTICA v.12. Normal distribution was assessed preliminarily by q-q plot, Kolmogorov-Smirnov, and Shapiro-Wilk tests. Quantitative variables were reported as median and interquartile range (IQR), while categorical variables were reported as absolute and relative frequencies. Differences between groups for continuous and categorical variables were estimated, respectively, the Mann-Whitney U-test (with Bonferroni's correction when needed) and the Chi-squared test.

Results

Demographic analysis: The demographic analysis included age and sex. The average age of the participants was 20.98 \pm 2.1 years. In terms of sex distribution, the male-to-female ratio was 18% male: 82 % female.

Kolmogorov-Smirnov (K-S) test: The Kolmogorov-Smirnov (K-S) test results revealed that the distribution of all parameters, including S1-RBD anti-spike IgG antibody, IL-10, and IL-1 β of this study, were nonparametric, as shown in Figure 1(A, B, C), which indicated that the data values did not follow a normal distribution.

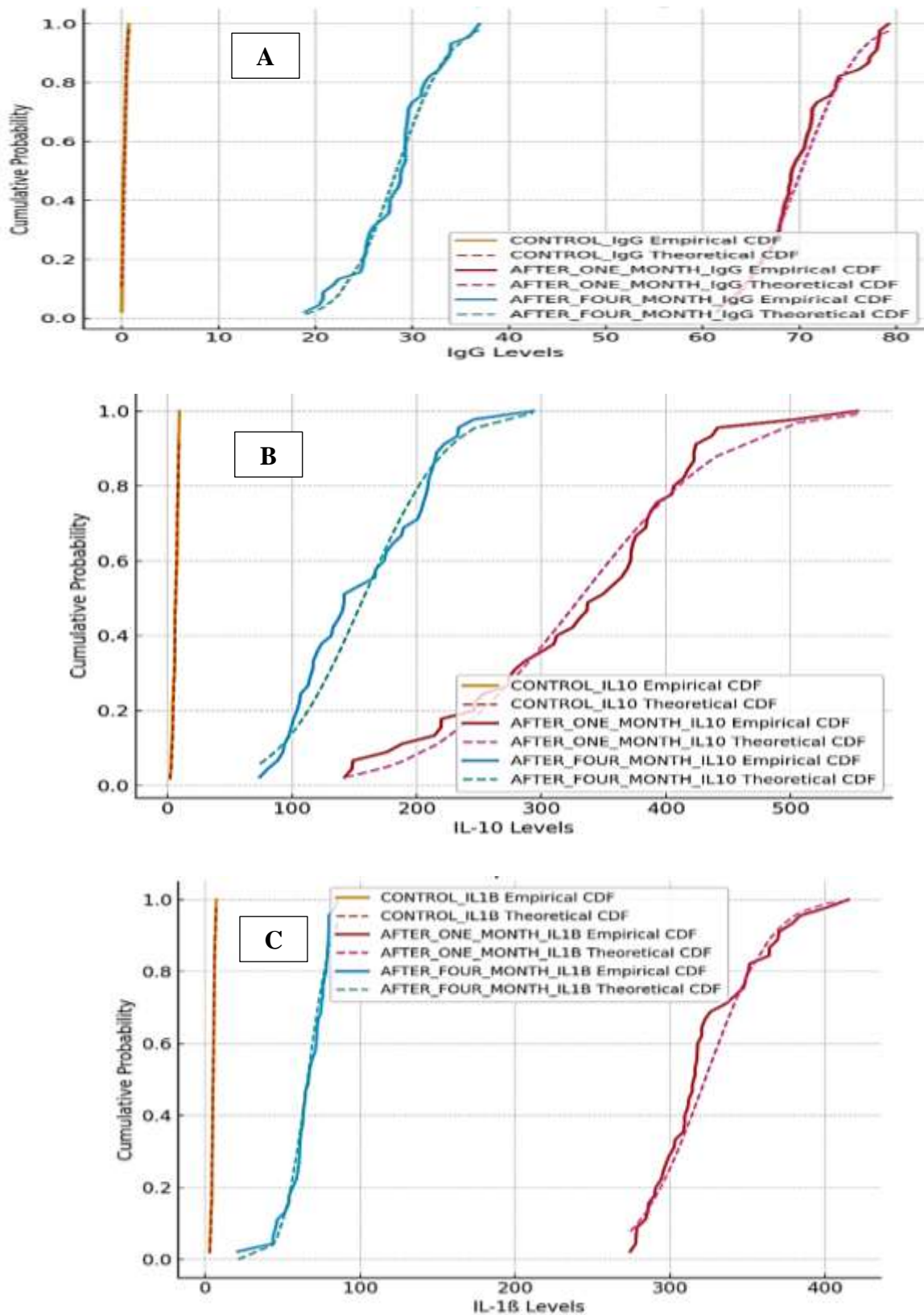


Figure 1. K-S Test CDF Comparison of normal distribution for A) S1-RBD anti-spike IgG antibody, B) IL-10, and C) IL-1 β serum level before and after 1–4 months according to one-sample Kolmogorov–Smirnov test.

Shapiro-Wilk test: On the other hand, according to the Shapiro-Wilk test, the data analysis showed that CONTROL of S1-RBD anti-spike IgG antibody, CONTROL IL-10, AFTER FOUR MONTH IL-10, AFTER ONE, and AFTER FOUR MONTH IL-1 β do not follow a normal

distribution, classifying them as non-parametric. In contrast, AFTER ONE FOUR MONTH S1-RBD anti-spike IgG antibody, AFTER ONE MONTH IL-10, and CONTROL IL-1 β follow a normal distribution, making them parametric as in Table 1.

Table 1. Shapiro-Wilk Statistic Normality test for S1-RBD anti-spike IgG antibody, IL-10, and IL-1 β .

| Variable | Timepoint | Distribution | Statistical Approach | Shapiro-Wilk Statistic | p-value |
|-------------------------------|----------------|--------------|----------------------|------------------------|---------------|
| S1-RBD anti-spike IgG | CONTROL | Non-normal | Non-parametric | 0.896 | 0.0007 |
| | After 1 month | Normal | Parametric | 0.96 | 0.131 |
| | After 4 Months | Normal | Parametric | 0.974 | 0.402 |
| IL-10 | CONTROL | Non-normal | Non-parametric | 0.936 | 0.016 |
| | After 1 Month | Normal | Parametric | 0.97 | 0.302 |
| | After 4 Months | Non-normal | Non-parametric | 0.948 | 0.034 |
| IL-1β | CONTROL | Normal | Parametric | 0.977 | 0.534 |
| | After 1 Months | Non-normal | Non-parametric | 0.932 | 0.011 |
| | After 4 Months | Non-normal | Non-parametric | 0.925 | 0.006 |

Mann-Whitney U test: The findings revealed a significant increase in the levels of these parameters one month after the administration of the second dose of the Pfizer-BioNTech mRNA vaccine. There was a sharp rise compared to baseline levels, which were close to zero ($p < 0.001$). There was a significant decrease in the levels of S1-RBD anti-spike IgG antibody, IL-10, and IL-1 β four months after the second dose of the Pfizer-BioNTech vaccine, although, the levels of S1-RBD anti-spike IgG antibody, IL-10,

and IL-1 β remain higher than control levels. The Mann-Whitney U test confirmed statistically significant differences between baseline levels and those at one month, as well as between baseline levels and those at four months. There were statistically significant differences between the levels recorded at one month and four months, with p-values less than 0.001 for both cases of the parameter's levels before, one month after, and four months after the intervention (Figure 2 A, B, C).

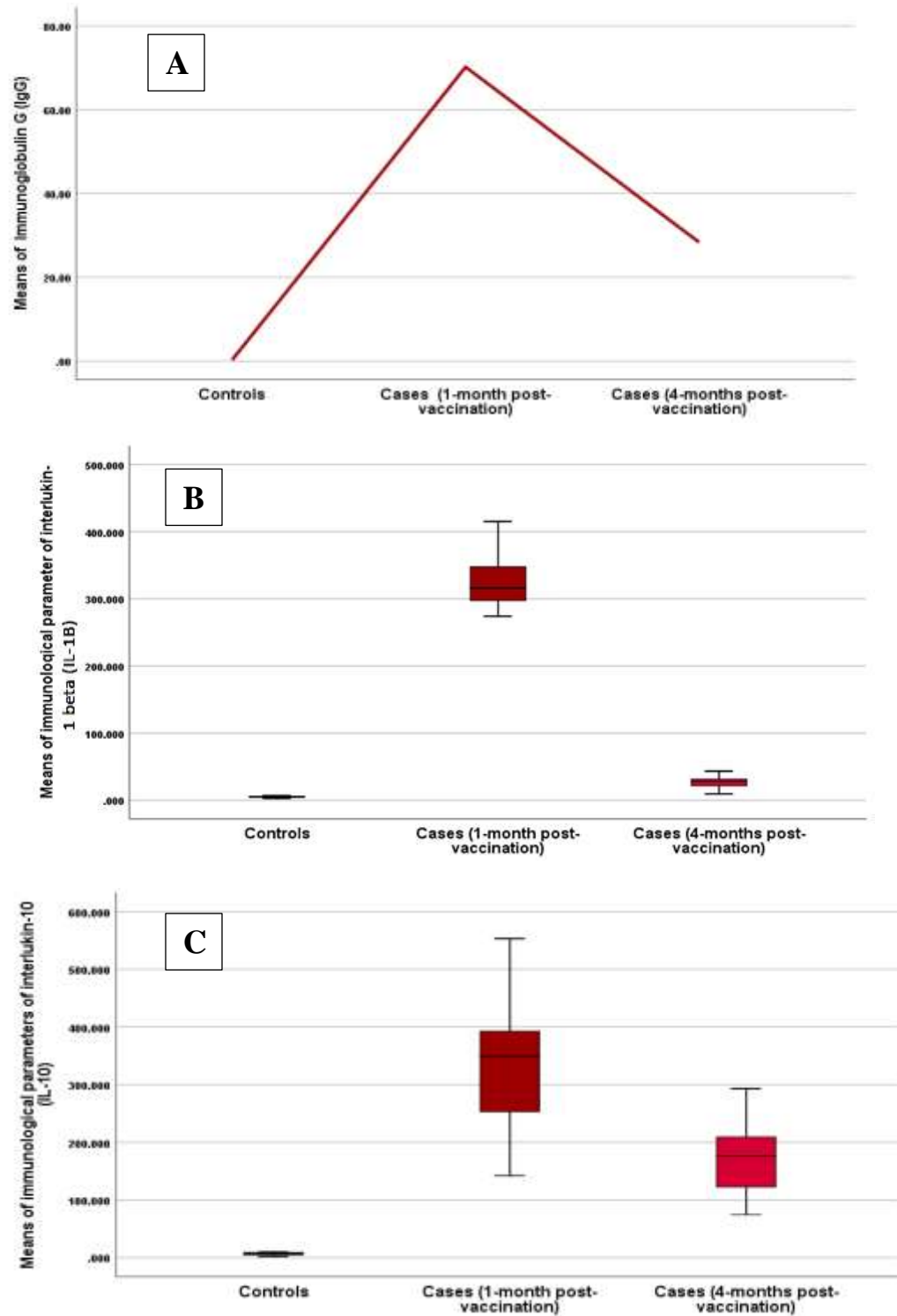


Figure 2. Mann-Whitney test for A) S1-RBD anti-spike IgG antibody, B) Interlukin-10, C) Interlukin-1 β before and after 1-4months post vaccination ($P < 0.001$).

Discussion

The rapid development of COVID-19 vaccines aimed at curbing the global spread of the virus, yet the required immunoglobulin levels for protection remain uncertain (21). Evaluating vaccine efficacy involves measuring the rate of immune response and IgG levels. The presence of anti-spike IgG post-vaccination indicates a robust immune response, supporting infection control and reducing viral transmission (23). By age group and gender, the average age of participants was 20.98 ± 2.1 years. This could be a significant factor influencing the immune response, as most studies have focused on older age groups since the vaccine primarily targets them. Given that the average age of participants in this study was 21 years, the immune response was high. According to studies on immune responses, younger individuals tend to have stronger and faster immune responses, which may explain the high levels of spike-specific IgG and the cytokines IL-10 and IL-1 β . Regarding gender, female participants constituted the majority, which may have influenced differences in the immune response to spike-specific IgG, IL-10, and IL-1 β . In general, females exhibit stronger immune responses than males due to hormonal and genetic factors, which may lead to a greater magnitude of the responses observed for the studied parameters. Additionally, immune response data may lean toward female immune patterns. The current results may have been partially affected by gender-related immune variations. The Shapiro-Wilk and Kolmogorov-Smirnov tests indicated that the immune responses were non-parametric, suggesting that individual differences may have been influenced by factors such as gender and age. IgG antibodies are produced by B cells in response to the SARS-CoV-2 spike protein. B cells initiate antibody production after administration of the Pfizer-BioNTech vaccine, enabling them to neutralize the virus and prevent its entry into host cells (24).

The immune system responds robustly, resulting in abundant production of effective, dynamic S1-RBD anti-spike IgG antibodies, which serve as a key marker of humoral immunity post-vaccination. These antibodies exhibit a precise pattern in limiting infection, which might not persist long-term. The current study observed no expression of spike IgG prior to vaccination. However, one-month post-vaccination, a significant increase in S1-RBD anti-spike IgG antibody levels was noted. As a result of B-cell activation and differentiation, specific IgG against the spike protein increased during the early stages following vaccination and reached an initial peak. Clinical studies evaluating the Pfizer-BioNTech vaccine indicated that the peak antibody response occurs within 2–3 weeks post-vaccination, consistent with the findings of the current study (25). This peak was followed by a standard decline four months after the second dose. This can be attributed to the immune system undergoing an affinity maturation process due to natural waning, resulting in the S1-RBD anti-spike IgG antibodies becoming more selective. During this phase, plasma cells begin contracting, leading to a decrease in antibody levels in circulation. However, vaccinated individuals with the Pfizer-BioNTech vaccine are likely to retain B-cell immune memory as the adaptive immune system shifts towards memory B cells (26). Nevertheless, despite the decline observed after four months, the levels remained significantly higher compared to the baseline. This could serve as clear evidence of the vaccine's efficacy, as its robust regulation of the immune system preserves antigen memory and enables a more effective response upon exposure to SARS-CoV-2. IL-10 is one of the most important anti-inflammatory cytokines, playing a vital role in regulating the immune response. It plays a critical role in modulating immune responses by suppressing pro-inflammatory cytokines (27). Before vaccination, the current study observed IL-10

levels within the lower end of the normal range. One month after the second dose of the Pfizer-BioNTech vaccine, IL-10 levels increased significantly, suggesting that vaccination may have activated Th2 cells. This activation, in turn, enhanced IL-10 secretion, which acts in a negative feedback loop to regulate excessive inflammation and promote immune activation. There may be a transient yet excessive increase in systemic inflammation, as evidenced by the significant rise in pro-inflammatory cytokine IL-1 β levels in the blood one-month post-vaccination. This supports the explanation that the observed increase in IL-10 could serve to mitigate the inflammatory response triggered by IL-1 β , thereby preventing potential tissue damage. Four months after the second dose, IL-10 levels began to decrease significantly. This can be interpreted as a progression towards a more regulated immune state induced by the Pfizer-BioNTech vaccine. The vaccination appears to have established a balance between inflammatory and regulatory signals, thereby modulating inflammation and promoting tolerance to the vaccine. IL-1 β is a pro-inflammatory cytokine that plays a critical role in initiating the inflammatory response. It is secreted by various cell types, including T cells and dendritic macrophages, and enhances inflammation, which is essential for triggering adaptive immunity (28). One month after the second dose of the Pfizer-BioNTech vaccine, we observed a significant increase in IL-1 β levels. Studies have described this rise in inflammatory cytokines as a response resembling a cytokine storm, albeit transient and milder compared to the intense response observed during SARS-CoV-2 infection. This increase could be attributed to the body's recognition of the mRNA as a foreign entity, triggering an innate immune response that is characteristic of the vaccine (29). This was followed by a significant decrease four months after the second dose, reflecting a regulated

response and the immune system's transition into a state of memory and adaptive preparedness. Despite a recorded decrease after four months, IL-1 β levels remained above baseline, consistent with findings from Miller et al. (2022) (30). They suggested that this elevation is consistent with the theory that the immune system remains primed even in the absence of infection.

Conclusion

The Pfizer-BioNTech mRNA vaccine is essential for initiating adaptive immunity, activating anti-inflammatory pathways, and dynamically producing S1-RBD anti-spike IgG antibodies, which are crucial for stimulating humoral immunity. However, booster-dose recommendations were essential to ensure sustained immune protection. Based on the findings of the current study, the booster dose strategy for the vaccine is vital for enhancing adaptive immune responses, activating anti-inflammatory pathways, and increasing the production of anti-S1-RBD anti-spike IgG antibodies.

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Ethical clearance: This study was approved by the committee of the University of Diyala-College of Medicine with code (MAS965).

Conflict of interest: None.

Use of Artificial Intelligence (AI): The authors state they did not use any generative AI tools for creating or editing the manuscript's language.

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دور لقاح فايزر-بيونتيك في تحفيز استجابة الأجسام المضادة IgG المضادة للبروتين S1-RBD ، و IL-10، و IL-1β (RIRS) في

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المخلص

الخلفية: أحدث لقاح فايزر-بيونتيك ثورة في مجال اللقاحات من خلال نهجه في تحفيز وتنظيم الاستجابة المناعية ضد فيروس سارس-كوف-٢، على الرغم من عدم اليقين بشأن مدة هذه الاستجابة.

الأهداف: تهدف الدراسة إلى تقدير الفعالية المناعية للقاح فايزر-بيونتيك في تحفيز إنتاج الأجسام المضادة من نوع IgG المضادة للبروتين الشوكي (Spike) وفي تنظيم مستويات IL-10 و IL-1β.

المرضى والطرق: شملت الدراسة ٩٠ مشاركًا لم يصابوا سابقًا بفيروس سارس-كوف-٢ ولم يتلقوا اللقاح. تم تقسيمهم إلى مجموعتين: ٤٥ كمجموعة أساسية و ٤٥ تلقوا جرعتين من لقاح فايزر-بيونتيك mRNA. قمنا بفحص مستويات الأجسام المضادة (IgG) المضادة للبروتينات الشوكية، و IL-10، و IL-1β في المجموعة الأساسية والمجموعة الملقحة بعد ١-٤ أشهر من الجرعة الثانية من التطعيم باستخدام طريقة اختبار المتمز المناعي المرتبط بالإنزيم (ELISA).

النتائج: في هذه الدراسة، كانت نسبة الذكور إلى الإناث ١٨:٨٢، بمتوسط عمر ٢١ عامًا للمشاركين من الطلاب الملقحين. كانت مستويات الأجسام المضادة (IgG) المضادة للبروتينات الشوكية، IL-10، و IL-1β قبل وبعد الجرعة الثانية من اللقاح بعد ١-٤ أشهر (غير معلمية) حسب Kolmogorov– Smirnov. بالإضافة إلى ذلك، كشف اختبار مان ويتني عن وجود فرق كبير ($P < 0.001$) في الأجسام المضادة (IgG) المضادة للبروتينات الشوكية ومستويات IL-10، و IL-1β بين ١ و ٤ أشهر بعد الجرعة الثانية من التطعيم و ما بين قبل و بعد اللقاح.

الاستنتاج: لقد أدى لقاح فايزر-بيونتيك إلى استجابات مناعية قوية، مع زيادات كبيرة في مستويات الأجسام المضادة (IgG) المضادة للبروتينات الشوكية IL-10، و IL-1β بعد التطعيم.

الكلمات المفتاحية: لقاح فايزر-بيونتيك، الأجسام المضادة IgG المضادة للبروتين الشوكي (S1-RBD)، إنترلوكين-١٠ (IL-10)، إنترلوكين-١ (IL-1β)، التلقيح.

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