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DIFFERENCES IN IFN-GAMMA AND SARS-COV-2 SPECIFIC IgG LEVEL IN mRNA BNT162B2 (PFIZER-BIONTECH) VACCINATED NAIVE AND COVID-19 RECOVERED INDIVIDUALS FROM ZAKHO DISTRICT, DUHOK, IRAQ

DILMAN IMAD MOHAMMED[*] and **AZAD AHMED SULAIMAN**[**]
[*]Dept. of Medical Laboratory Sciences, College of Health Sciences, University of Duhok, Kurdistan Region-Iraq
[**]Dept. of Pathology and Microbiology, College of Veterinary Medicine, University of Duhok, Kurdistan Region-Iraq

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ABSTRACT

COVID-19 has caused massive public health threats worldwide, leading to the development of effective vaccines to control the epidemic. Real-world evidence confirms the efficacy of vaccinations against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This study aimed to evaluate and compare the titer of anti-SARS-CoV-2 IgG antibodies against the S1 subunit of the virus's spike protein as a marker of the humoral response and the concentration of interferon-gamma as an indicator of cellular response in naive and recovered individuals. The study included 176 individuals, 88 (50%) were naïve, and 88 (50%) had a previous history of exposure to SARS-CoV-2 with or without symptoms. The serum concentration of IgG and IFN-γ were measured two weeks after the second dose of the BNT162B2 mRNA vaccine in naive and recovered individuals using enzyme-linked immunosorbent assays. The concentration of anti-SARS-CoV-IgG and IFN-γ was significantly higher in recovered participants compared to naive. Moreover, the concentration of anti-SARS-CoV-IgG and IFN-γ was significantly associated with severe cases. Our results suggested that the second dose of the BNT162b2 mRNA vaccine provides a protective humoral and cellular immune response to almost all individuals. While inducing a more robust immune response in recovered individuals, indicates that a single dose of the vaccine may be sufficient for symptomatic recovered individuals.

***KEYWORDS*:** Covid-19, Anti-SARS-Cov-2 IgG, IFN-γ, BNT162B2 Vaccine, Naive, Recovered.

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), was caused by the novel human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Sette & Crotty, 2021). The disease first appeared in the city of Wuhan, China. The World Health Organization (WHO) announced public health on January 30, 2020, and it classified the outbreak as a pandemic on March 11, 2020 (Long *et al*., 2020). More than 757 million cases of COVID-19 have been verified and over 6.85 million deaths have been reported worldwide as of February 21, 2023 (WHO Coronavirus (COVID-19) Dashboard, 2023).

The clinical presentation has been incredibly varied, ranging from asymptomatic disease to acute respiratory distress syndrome (ARDS), multiorgan failure, and death (Ivanova *et al*., 2021).

COVID-19 has caused massive public health threats worldwide, leading to the development of effective vaccines to control

the epidemic. Vaccines, including mRNA, vector-based, and protein-adjuvant vaccines, have been developed to minimize severe disease, hospitalization, and death (Krammer, 2020).

The most effective vaccine is based on an mRNA platform that provides rapid production. The mRNA vaccine is developed via Pfizer BioNTech (Pfizer Manufacturing Belgium NV, Purus, Belgium, Germany) or Moderna (Moderna Biotech Spain, S.L., Madrid, Spain) (Zalewska *et al*., 2022). Indeed, Experiments using SARS-CoV-2 mRNA vaccine candidates have stimulated strong immune responses against SARS-CoV-2 in animal models (Corbett *et al*., 2020) and in humans (Lozano-Ojalvo *et al*., 2021) and were successful in extensive clinical trials (Thomas *et al*., 2021). Countries that have implemented the mRNA

vaccination approach have already seen a decrease in the spread of the virus, and research laboratories have shown a positive response to it (Sahin *et al*., 2020).

The Pfizer BNT162b2 mRNA vaccine was the first to gain approval (Cocomazzi *et al*., 2021). It consists of a lipid nanoparticle-formulated nucleoside-modified mRNA that encodes the spike protein and is authorized for use as a two-dose primary course 21 days apart, which has been demonstrated to induce a spike protein (S) specific humoral and cellular immunity associated with 95% efficacy (Agrati *et al*., 2021).

The vaccination campaign launched in Iraq in March 2021. Three COVID-19 vaccines have been licensed for use in Zakho/Duhok: Sinopharm, AstraZeneca, and Pfizer-BioNTech.

Since vaccination remains the best protective method against SARS-CoV-2, understanding immune responses to the vaccine is essential because protection against disease severity and infection depends on coordinated activation of both adaptive immunity's humoral and cellular arms (Zalewska *et al*., 2022). Therefore, research has focused on measuring humoral and cellular responses to the SARS-CoV-2 spike after vaccination (Lange *et al*., 2021). The humoral immune response is assessed based on the presence of specific immunoglobulin (Ig) directed against the spike (S) protein epitopes, IgGs are the most common class of immunoglobulins found in blood and other body fluids and play a major role in the development of antibody-mediated immunity (Tretyn *et al*., 2021). Moreover, cellular response is evaluated by measuring the concentration of interferon-gamma (IFN-γ), a multipotent protein released by lymphocytes (T cells) in response to some COVID-19 antigens. IFN-γ is essential for developing innate and adaptive immunity and defence against COVID-19 (Zalewska *et al*., 2022).

A second dose of BNT162b2 vaccination has been shown to generate a cellular and humoral immune response in almost all individuals. Vaccinated recovered subjects promote stronger immune responses than vaccinated naive subjects due to naturally acquired immunity (Ivanova *et al*., 2021). Thus, several studies have suggested that only a single dose may be sufficient to induce a robust immune response in recovered individuals (Ebinger *et al*., 2021). However, the second dose is important for elderly and immunocompromised people (Wei *et al*., 2022). Nevertheless, there is no indication if individuals who have previously been exposed to SARS-CoV-2 should be vaccinated or how often they should receive the vaccine.

This study aimed to evaluate the cellular and humoral immune responses 14 days after the second dose of the mRNA-based BNT162b2 (Pfizer-BioNTech) vaccine in individuals previously infected and non-infected with COVID-19 at Zakho district.

2. MATERIALS AND METHOD

2.1. Study participants

In this study, 176 participants were conveniently selected from Zakho district (88 with and 88 without history of SARS-CoV-2 infection) between 4 December 2021 and 10 April 2022. The majority of individuals involved were volunteers, employees, and medical professionals. All study subjects completed a questionnaire, including name, address, phone number, age, gender, history of COVID-19, symptoms of infection, date of RT-PCR test, history of vaccine doses being administered, and coexistence of chronic diseases. The age of individuals varied from 17 to 70 years, and all subjects were vaccinated with 2 doses of (BNT162b2 mRNA vaccines). The pre-vaccination COVID-19 exposure status was determined by a combination of medical history and PCR tests. All subjects with a positive SARS-CoV-2 PCR test in their medical history were considered seropositive. The study design was prospective population-based.

2.2. Classification of SARS-CoV-2 Infection Severity

The severity of COVID-19 was classified into mild, moderate, severe and critical group based on National Health Commission's Diagnosis and Treatment Plan for COVID-19 (Zhang *et al*., 2020).

- Mild group: involves individuals who have cough, fever, lethargy, sore throat, myalgia, and headache without shortness of breath, dyspnea on exertion, or abnormal imaging oxygen saturation.
- Moderate group: characterized by lower respiratory disease during clinical assessment or imaging, with SpO2 ≥90%, but no oxygen supply is necessary.
- Severe group: individuals show SpO2 <90%, oxygen supplementation, high-flow oxygen, or intubation required.
- Critical group: individuals suffer from respiratory failure that requires mechanical ventilation, shock with other organ failures, and requiring intensive care.

In this study, recovered individuals were classified into two groups: non-severe group included mild and moderate cases and severe group included severe and critical cases according to the aforementioned criteria.

2.3. Serum collection

Blood samples were collected from the veins of 176 participants to obtain serum 14 days following the second dose of the BNT162b2 mRNA vaccine. Blood samples were collected in sterile clot activator (Jiangxi Exquisite Technology Co., Ltd) vacutainer tubes and centrifuged at 3000 RPM for 10 minutes to separate serum and then divided into two aliquots of 300 µl directly placed in an Eppendorf tube and stored at -70°C till examination to measure IgG antibodies and IFN-γ concentration by ELISA.

2.4. Quantification of human SARS-CoV-2 spike protein IgG by ELISA

The tests were performed using an anti-SARS-CoV-2 spike protein S1 IgG (S1-IgG) ELISA kit (Sunlong Biotech Co., LTD) as per manufacturers' instructions. The test reaction wells were coated with the S1 domain of the SARS-CoV-2 spike protein, enabling specific detection of IgG antibodies against SARS-CoV-2. In the first step, reaction wells were incubated with 100 µl of patient serum for 90 min at 37°C and washed 2 times with a washing solution. The bound antibodies were incubated for 60 min at 37°C with 100 µl of biotinylated antibody and washed 3 times. Then, 100 µl of enzyme conjugate was added to each well, and incubated at 37°C for 30 min and washed 5 times.100 µl of substrate/chromogen solution was added to each well and incubated at 37°C for 30 min. During the last stage of the procedure, 100 µl of stopping solution was added to each well. Within 10 minutes of adding the stop solution, an optical density reading was taken at 450 nm using a microplate reader. The results were expressed in (U/mL). An eight-point standard curve (200–3.12 U/mL) was performed in parallel.

2.5. Quantification of human interferon-gamma by ELISA

The concentration of IFN-γ in the serum of the participant was measured using a human interferon-gamma ELISA kit (Sunlong Biotech Co., LTD), as per manufacturers' instruction. In the first reaction step, standard dilutions were added in duplicate into 10 wells, concentrations of standards (72pg/ml, 48pg/ml, 24pg/ml, 12pg/ml, and 6pg/ml). 10 µl of serum samples diluted in a 40 µl sample buffer were added to the coated reaction wells to bind interferon-gamma and were incubated for 30 min at 37°C. Then, the wells were washed 5 times with a washing buffer. In the next step, 50 µl of Horseradish Peroxidase (HRP)-conjugated antibody specific for IFN-γ was added and incubated again for 30 min at 37°C. The plate was washed 5 times with washing buffer. Then 100 µl of TMB substrate was added to each well and incubated for 15 min at 37°C. At the end, 50 µl of stop solution was added, and optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of IFN-γ. The results were expressed in pg/ml.

2.6. Statistical analysis

The data was entered into a Microsoft Excel sheet, cleaned, managed, exported, and analyzed by SPSS software (IBM SPSS Statistics 23) and Intellect us Statistics [Online computer software]. The Shapiro-Wilk tests were conducted to determine whether the distributions of age, IFN-γ Pg/ml, S1-IgG U/ml, were significantly different from the normal distribution. Univariate outliers were examined for age, IFN-γ Pg/ml, and S1-IgG U/ml, variables.

3. RESULTS

3.1. Characteristics of Study Group

The current study indicated that the number of male participants (n=103, 58.52%) was more than the female number (n=73, 41.48%). The observations for age had an average of 33.87 years (SD 10.03, 95%CI 32.35 to 35.38, Mdn 33.0, IQR 14.0). The most frequently observed age group was 25 - 44 years (n = 120, 68.18%). The study group included naive and COVID-19-recovered individuals with symptomatic and asymptomatic recoveries. Subjects with no previous COVID-19 infection were defined as naive and those infected before vaccination were classified as recovered. The number of COVID-19 recovered and naive individuals was equal (88) as shown in (Table 1).

Table (1): Frequency table for participants groups, and SARS-CoV-2 infection severity (n=176).

Variable	*n*	*%*
Gender		
Male	103	58.5
Female	73	41.5
Age group		
Under 25 Years	33	18.8
25 - 44 Years	120	68.2
45 - 64 Years	21	11.9
65 years and more	2	1.1
Group		
COVID-19 recovered	88	50.0
Naive	88	50.0
SARS-CoV-2 Infection Severity		
Non-Severe	70	79.5
Severe	18	20.5

3.2. Anti-SARS-CoV-2 spike protein IgG level in serum

Concentrations of IgG antibodies against the spike protein S1 were evaluated for all study participants, almost all had detectable IgG antibodies. As shown the highest level of anti-S1 IgG was (188.15 U/ml). The observations for S1-IgG U/ml had an average of 109.61 (SD = 26.87, 95%CI 105.57 to 113.64, Mdn 105.05, IQR 38.45) as presented in (Figure 1).

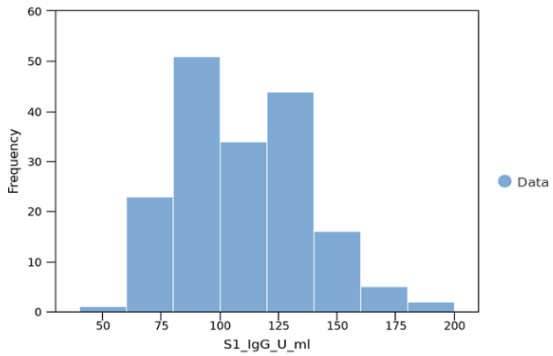

Fig. (1): Concentration of anti-SARS-CoV-2 spike protein IgG in sera of participants using ELISA. Data represent the average concentration of S1 IgG in the serum of all participants

3.3. IFN-γ level in serum

IFN-γ concentration in the sera of all participants was measured by ELISA and a detectable level (pg/ml) was observed in all samples. The most frequent level of IFN-γ was (48.31 pg/ml). The IFN-γ Pg/ml observations had an average of 19.05 (SD 8.13, 95% CI 17.83 to 20.27, Mdn 17.0, IQR 11.54) (Figure 2).

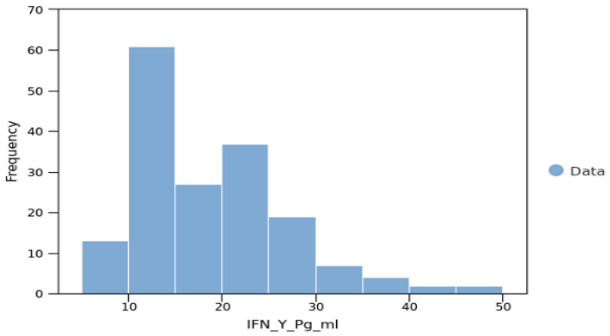

Fig. (2): Concentration of IFN-γ in sera of participants using ELISA. Data represent the average concentration of IFN-γ in the serum of all participants.

3.4. SARS-CoV-2 infection status

Most of the participants in this study showed non-severe symptoms (79.55%), while, participants with severe symptoms were (20.45%).

3.5. Concentration of S1-IgG among COVID-19 recovered and naive group

There were 88 observations in group COVID-19 recovered and 88 observations in group naive. The two-tailed Mann-Whitney U test result was significant based on an alpha value of .05, U = 7140, p < .001. The mean concentration of S1-IgG for group COVID-19 recovered was (125.64 U/ml) and the mean concentration for group Naïve was (51.36 U/ml). This indicates that the concentration of S1-IgG for the COVID-19 recovered group was significantly higher than the S1-IgG concentration for the Naïve category. (Table 2) presents the result of the two-tailed Mann-Whitney U test. (Figure 4) presents a boxplot of the ranks of S1-IgG U/ml in both groups.

Table (2): Two-Tailed Mann-Whitney Test for S1-IgG U/ml by Group.

COVID-19 recovered			Naïve			
Variable	Mean Rank	*n*	Mean Rank	*n*	*U*	*p*
S1-IgG U/ml	125.64	88	51.36	88	7,140.00	< .001

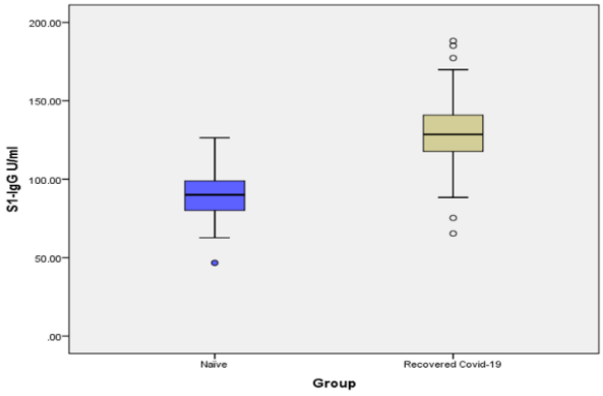

Fig. (4): Comparisons of S1-IgG antibody levels (U/ml) in naïve and COVID-19-recovered individuals. Results were analyzed using a two-tailed Mann-Whitney U test, p < .001. Data represent the mean S1-IgG concentration of all participants. Blue indicates the concentration of naïve, and yellow indicates the concentration of recovered individuals.

3.6. Association of the S1-IgG level to the participant's age

All participants were grouped according to age and S1 IgG concentration. Data were analyzed using the Spearman correlation test and indicated no significant correlation between the concentration of IgG (U/ml) antibodies and the age of the participants (Figure 5). (Table 3) result of the correlation was examined based on an alpha value of 0.05. A regression line has been added to assist the interpretation.

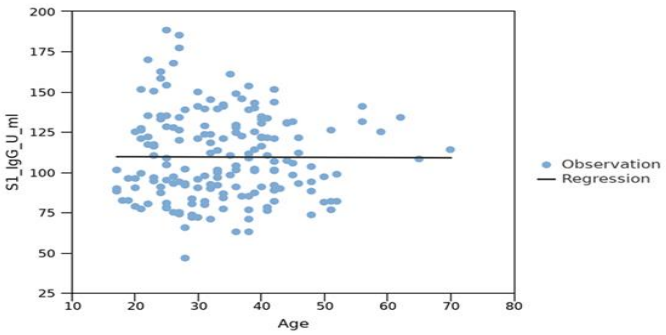

Fig. (5): Correlation of S1 IgG concentration to participant's age. Data were analyzed using the Spearman correlation test, P=0.824. Data represent the mean S1 IgG concentration of all participants.

Table (3): Spearman correlation test of S1 IgG according to the participant's age.

Combination	*r*	95.00% CI	*N*	*p*
Age-S1-IgG U/ml	.02	[-.13, .16]	176	.824

4.7. Concentration of S1-IgG among severe and non-severe group

The result of the Two-Tailed Mann-Whitney Test was significant based on an alpha value of .05, $p < .001$, indicating that the mean concentration of S1-IgG in participants with severe symptoms (150.72 U/ml) was significantly higher than the levels of S1-IgG in participants with non-sever symptom (125.05 U/ml). (Table 4) presents the results of the Two-Tailed Mann-Whitney Test. (Figure 6) presents boxplots of the ranked values of S1-IgG among non-severe and severe groups.

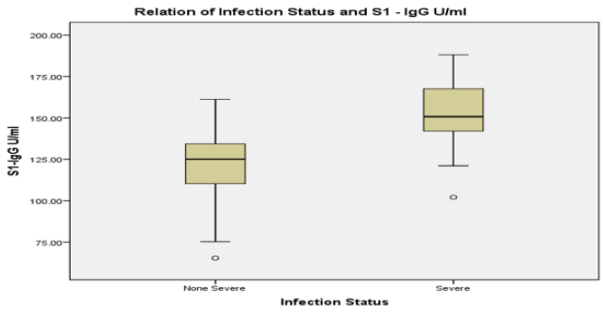

Fig. (6): Comparison of S1 IgG level among non-sever, and severe cases. Results were analyzed using a two-tailed Mann-Whitney U test, $p < .001$. Data represent the mean S1-IgG concentration of the non-severe and severe groups.

Table (4): Two-Tailed Mann-Whitney Test for S1-igG U/ml by Group.

None-severe			Severe			
Variable	Mean Rank	*n*	Mean Rank	*n*	*U*	*p*
S1-IgG U/ml	125.055	70	150.725	18	4.821	< .001

4.8. Concentration of IFN-γ among COVID-19 recovered and naive group

There were 86 observations in the COVID-19 recovered group and 86 observations in the naive group. The mean concentration of IFN-γ in the COVID-19 recovered group (114.19 Pg/ml) was significantly higher than the mean concentration of IFN-γ in the naïve group (58.81 Pg/ml) (Figure 7). Results were analyzed using a two-tailed Mann-Whitney U test and indicated a significant difference in IFN-γ level between both groups based on an alpha value of 0.05, $U = 6079.5$, $p < .001$ (Table 5).

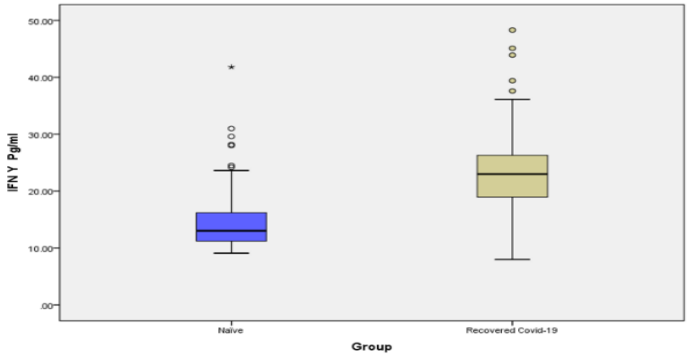

Fig. (7): Comparison of IFN-γ level in naïve and COVID-19 recovered individuals. Results were analyzed using a two-tailed Mann-Whitney U test, $p < 0.001$. Data represent the mean IFN-γ concentration of all participants. Blue indicates the concentration of IFN-γ in Covid-19 recovered individuals and yellow indicates the concentration of IFN-γ in naive individuals.

Table (5): Two-Tailed Mann-Whitney Test for IFN-γ Pg/ml by Group.

COVID-19 recovered			Naïve			
Variable	Mean Rank	*n*	Mean Rank	*n*	*U*	*p*
IFN-γ Pg/ml	114.19	86	58.81	86	6,079.50	< .001

3.10. Association of the IFN-γ level to the participant's age

All participants were grouped according to age and IFN-γ concentration. Data were analyzed using the Spearman correlation test and indicated no significant correlation between the concentration of IFN-γ and the age of the participants (Figure 8). The result of the correlation was examined based on an alpha value of 0.05. A regression line has been added to assist the interpretation (Table 6).

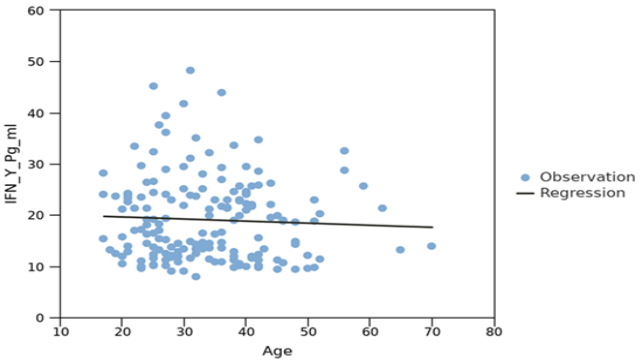

Fig. (8): Correlation of IFN-γ concentration to participant's age. Data were analyzed using the Spearman correlation test, p=0.488. Data represent the mean IFN-γ concentration of all participants.

Table (6): Spearman Correlation test of IFN-γ Pg/ml according to participant's age.

Combination	*R*	95.00% CI	*n*	*p*
Age-IFN-γ Pg/ml	-.05	[-.20, .10]	172	.488

3.11 Concentration of IFN-y among severe and non-severe groups

The result of the Two-Tailed Mann-Whitney Test was significant based on an alpha value of .05, p < .001, indicating that the mean concentration of IFN-γ in participants with the severe symptom (72.06 pg/ml) was significantly

higher than the mean levels of IFN-γ in the non-sever group (35.94 pg/ml). (Table 7) presents the results of the Two-Tailed Mann-Whitney Test. (Figure 9) presents boxplots of the ranked values of IFN-y Pg/ml by the levels of SARS-CoV-2 Infection status.

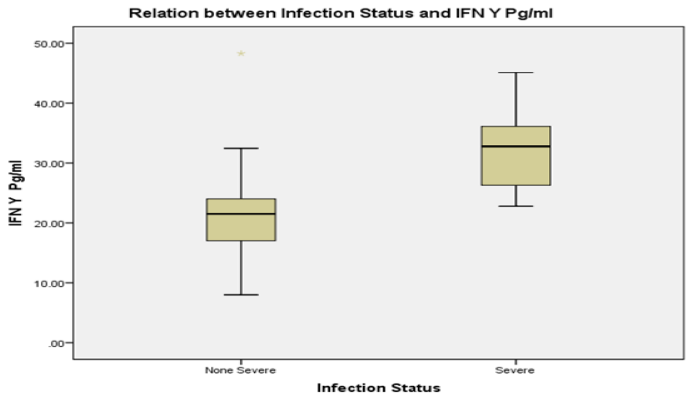

Fig. (9): Comparison of IFN-γ level among severe, and non-severe participants. Results were analyzed using a two-tailed Mann-Whitney U test, p < .001. Data represent the mean *IFN-γ* concentration of non-severe and severe groups.

Table (7): Two-Tailed Mann-Whitney Test for IFN-y Pg/ml by Group.

	None-severe		**Severe**			
Variable	Mean Rank	*n*	Mean Rank	*n*	*U*	*p*
IFN-y Pg/ml	35.94	68	72.06	18	5.457	< .001

4. DISCUSSION

Many investigations have been carried out since the discovery of SARS-CoV-2 to comprehend how the immune system reacts to the new infectious agent. Two mechanisms of specific immunity to fight pathogens are implemented within the body, humoral (antibody-mediated) and cellular (T-cell-mediated).

In this study, both humoral and cellular responses elicited by the BNT162b2 vaccine were examined and compared in naive and COVID-19-recovered subjects, also the association of these responses to the age and infection severity was evaluated.

The enzyme-linked immunosorbent assay was applied to detect the anti-SARS-CoV-2 spike protein S1 IgG antibodies two weeks following receiving the second dose of the BNT162b2 vaccine. The number and quantity of antibodies in the blood are frequently used to assess the humoral immune response to a specific virus. Most research has focused on this sort of reaction to SARS-CoV-2 because it is easier and less expensive to evaluate than cellular responses (Gudbjartsson *et al*., 2020; Hasan *et al*., 2021; Oliveira-Silva *et al*., 2022). Estimating IgG antibody levels is critical since these are the main class of antibodies produced by intramuscular immunizations and are critical in neutralizing viruses and avoiding reinfection, and levels generated by the COVID-19 mRNA BNT162b2 vaccine were both high and long-lasting, lasting several months after vaccination, indicating possibly extended protection from exposure (Tripp *et al*., 2023; Urbanowicz *et al*., 2021).

The results of the current study revealed that all of the participants in both groups (naive and COVID-19 recovered) had a positive anti-spike IgG level. Suggesting that a second vaccine dose had significantly enhanced the humoral response in most individuals. This is consistent with previous studies, which found that the second dosage of the BNT162b2 vaccine boosted the anti-spike IgG in almost all individuals (Wei *et al*., 2022). A large study conducted in Israel found that 14 days after receiving the second dose of the vaccine, 99.9% of those who received the BNT162b2 vaccine developed neutralizing IgG antibodies against SARS-CoV-2 (Lustig *et al*., 2021). These findings indicate that the BNT162b2 vaccine is highly effective and mounts a robust immune response.

Interestingly, our finding indicated that the level of anti-spike IgG in the COVID-19 recovered group (Median 128.54 U/ml) was significantly higher than the naive category (Median 90.09 U/ml). This could be due to the naturally acquired immunity by specific memory B cells generated by the infection, confirming that these memory B-cells identify the S antigen in the vaccine and produce robust IgG antibodies. As predicted, recovered individuals have developed a more rapid and lasting response to the BNT vaccine than naive individuals. Similar results have been reported by several studies to compare humoral immune responses between naive and recovered individuals, and it was found that after two doses of the mRNA (Pfizer or Moderna) vaccines, IgG antibodies were higher among

participants who had previously tested positive for COVID-19 compared to naïve subjects (Demonbreun *et al*., 2021; Lozano-Rodríguez *et al*., 2022; Urbanowicz *et al*., 2021; Zhong *et al*., 2021).

Multiple observational studies found that recovered patients may respond significantly to a single dose of the BNT162b2, Sputnik V, or ChAdOx1 vaccine due to naturally acquired immunity, but naive subjects required both doses to produce potent immune responses (Claro *et al*., 2021; Gelanew *et al*., 2022; Krammer *et al*., 2021; Mulligan *et al*., 2020; Prendecki *et al*., 2021; Walsh *et al*., 2020). In addition, results of a large study conducted by Ebinger *et al*. confirmed that spike-specific IgG antibody levels induced by a single vaccination dose in subjects with past SARS-CoV-2 infection were analogous to those without prior infection reported after two doses of the Pfizer vaccine (Ebinger *et al*., 2021). However, Wei *et al*., demonstrated that previously infected subjects had fairly low IgG levels following a single BNT162b2 dose compared to those who received two BNT162b2 doses without prior infection, mainly in the elderly (Wei *et al*., 2022). This indicates the importance of the second vaccine dose, especially for the elderly person.

Our results showed no significant correlations between the concentration of IgG antibodies and the age of the participants. The same results were observed in a study conducted on 45,965 people who received either the ChAdOx1 or BNT162b2 SARS-CoV-2 vaccination (Wei *et al*., 2021). Furthermore, multiple studies found that older individuals had lower IgG levels as compared to young following the second dose of the BNT vaccine (Fraley *et al*., 2022; Levin *et al*., 2021; Vassilaki *et al*., 2021). This observation could be explained by the delayed immunological response seen in older people.

In addition, we indicated that the mean level of S1-IgG antibodies was significantly higher in severe cases as compared to non-sever. Several studies have found that antibody response was higher in symptomatic recovered subjects following both doses of BNT vaccination and suggested that symptomatic recovered individuals need one dose of vaccine to produce sufficient antibody titer without the need for the second dose (Buonfrate *et al*., 2021; Levi *et al*., 2021; Pozzi *et al*., 2023). Nevertheless, other studies highlighted that the second dose of the vaccine is crucial for all people, especially for older and immunocompromised individuals (Lustig *et al*., 2021; Wei *et al*., 2022).

However, the cellular response to the BNT162b2 vaccine has not been extensively examined. Therefore, we sought to examine the cellular response following the second dose of the vaccine. Our study measured IFN-γ to examine the reliability of detecting the T-cell-mediated immune response using a human IFN-γ ELISA kit. Evaluation of IFN-γ as an expression of lymphocyte activity may be easier to use than the more difficult methods of measuring the CD4+ and CD8+ cellular response.

All of the participants (naive and recovered) in the current study showed a positive IFN-γ result after receiving the second vaccine dose. These results were in line with previous findings (Sedegah *et al*., 2022; Seraceni *et al*., 2022).

IFN-γ secretion has an effective defense against SARS-CoV-2. Several investigations have established that low IFN production is closely linked to failure to manage an initial SARS-CoV-2 infection and a high risk of deadly COVID-19 outcomes (Chen & John Wherry, 2020; Zhang *et al*., 2020).

In this study, we found a significant difference in IFN-γ levels between naive and recovered groups after the second dose of the BNT162b2 vaccine. The IFN-γ level in COVID-19-recovered individuals was significantly higher than in naive. Similar findings have been reported using different methods for measuring IFN-γ concentration and indicated that recovered individuals had a much stronger IFN-γ response following vaccination than vaccinated participants without a history of infection (Sedegah *et al*., 2022; Tormo *et al*., 2022). Different research also revealed that the T-cell responses induced by a single dose in previously infected participants were equal to those induced by two doses in naive individuals (Angyal *et al*., 2022; Prendecki *et al*., 2021).

The results of several studies evaluating T-cell responses against SARS-CoV-2 have been rather ambiguous due to the various methods of measuring this response. For comparison, a study conducted on 47 subjects found no significant differences in IFN-γ levels between the naive and recovered group after two doses of ChAdOx1 (Oxford/AstraZeneca; ChAd) vaccine, in which IFN-γ was assessed through IFN-γ release assay (IGRA). Nonetheless, higher medians were visible in the recovered individuals (Zalewska *et al*., 2022).

Our results indicated that the mean IFN-γ concentration (pg/ml) in participants with severe

symptoms was significantly higher than in non-severe groups. The same result has been found by others (Holder *et al*., 2022).

In addition, we indicated that there was no significant correlation between the concentrations of IFN-γ among the different ages of the participants similar results have been found by (Prendecki *et al*., 2021).

Understanding various components of the adaptive immune response to coronavirus infection, in addition to a better knowledge of the variables that determine an individual's immunity over time, is crucial to successfully combating SARS-CoV-2.

5. CONCLUSIONS

For constructing an effective vaccine, understanding the protective immune response against COVID-19 at different times after vaccination, and between individuals who are infected before vaccination with those who are not infected is very important. In the current study, the humoral and cellular data (measured by IgG antibody titer and T-cell interferon-gamma production) at 14 days after a second dose of BNT162b2 vaccination was unequivocally positive in all subjects.

Individuals with previous SARS-CoV-2 infection had stronger IgG and IFN-γ responses than naive individuals. Therefore, it can be assumed that the hybrid immune response to SARS-CoV-2 (infection with vaccine) appears to be higher than that induced through vaccination alone. The severity of infection before vaccination was significantly correlated to high IgG and IFN-γ concentrations. No association was observed between the immune response and the age of participants.

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