Effect of covid-19 vaccine (Pfizer) on ANbs, IgG serum levels for uninfected and previous infected individuals

Alaa Hazim Oudah[1]*, Thikra Abdullah Mahmood[2], Sabah. N. Mohammed[3]
[1,2]Faculty of Medicine, University of Kufa, Najaf, Iraq.
[3]Consultant / Clinical immunologist, Najaf, Iraq.
Email: alaaalzamili19@gmail.com

Abstract
Background: The World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a public health emergency on January 30, 2020. Mass vaccination programs are needed to boost population immunity and prevent COVID-19 pandemic impacts. In this research, we used the Pfizer BioNTech vaccine, which Iraqis prefer above others. Pfizer and BioNTech's BNT162b2 was 95% effective in preventing COVID-19 symptoms and almost 100% effective at preventing severe COVID-19 or hospitalizations, and the US FDA authorized its first EUA on December 11, 2020. Methods: This cohort study was conducted in Al-Manathira Health Center in Al-Najaf Al-Ashraf Governorate from (October 2021 to May 2022). 75 samples (55 males and 20 females) with or without previous infections were collected, and these samples were divided into five groups according to age (18-50 and over). We collected three venous blood samples from the vaccinated. Results: A serological analysis to measure SARS-CoV-2 S1-RBD IgG and neutralizing antibodies NAbs to SARS-CoV-2 was done by using the CLIA technique SARS-CoV-2 kits, China, which was carried out in the Najaf Specialized Laboratory for Pathological Analysis. In this study, neutralizing antibody (NAbs) and immunoglobulin G (IgG) levels after two doses of Pfizer/BioNTech vaccine increased at a level of 0.05 ($p = 0.00001$). Conclusions: This study concluded that there were high and gradually increasing concentrations of neutralizing antibodies (NAbs) and IgG after two doses of the Pfizer / BioNTech vaccine.
Keywords: COVID-19, mRNA vaccine (Pfizer), antibodies response (NAbs, IgG).

1. Introduction

Since the SARS-CoV-2 RNA virus spread quickly and the first case was discovered in Wuhan, China, in December 2019, early efforts have been undertaken to create the first COVID-19 vaccine ever. As a result, the World Health Organization (WHO) declared a global pandemic on March 11, 2020, which has since impacted more than 200 nations worldwide (1).
Pfizer, Fosun Pharma, and German-based BioNTech (short for Biopharmaceutical New Technologies) worked together to develop the candidate mRNA platform BNT162b2. BioNTech created and evaluated four modified mRNA-based (modRNA) vaccine, candidates. These candidates are intended to be given in two doses three weeks apart and direct immune cells to produce numerous copies of the full-length SARS-CoV-2 spike protein upon insertion into the host cell cytoplasm (2). High SARS-CoV-2 antibody titers and robust antigen-specific T-cell responses (CD8+ and Th1-type CD4+) were induced by two 30 g of the Pfizer vaccine (BNT162b2), according to studies carried out in the United States and Germany among healthy men and women. At 7 days following the second dose, BNT162b2 was 95% effective in preventing COVID-19 (3).
The existence of neutralising antibodies is a robust correlate of vaccine efficacy, despite the absence of an established protective threshold. However, it is difficult to measure neutralising antibodies on a broad scale. The invention of binding assays aimed against the spike protein of SARS-CoV-2 shown an excellent association with neutralising antibodies and permits a large-scale evaluation of the immunogenicity of SARS-CoV-2 vaccines over time (4,5).
This study aimed to Evaluate the Immune Response (NAbs, IgG) of Individuals Attending PHC Centers to pfizer covid 19 Vaccines.

2. Methods

2.1. Vaccinated individuals

During the period from (October 2021 to May 2022), random specimen blood was collected from 75 vaccinated individuals (55 males and 20 females) uninfected and previously infected with COVID-19 , who attended primary health care centers in Najaf city. The old age range of individuals vaccinated is between (18-50 and more) years old. Vaccines are given by intramuscular injection (MI).

2.2. Study design:

Cohort study was conducted in Al-Manathera Health Center in Al-Najaf Al-Ashraf Governorate.

Inclusion Criteria:

1- Individuals who receive the vaccine of BNT162b2 (Pfizer-BioNTech) are adults (18-50 and more)years old.

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Exclusion Criteria

1-Autoimmune and immune deficiency diseases.
2-patient Diagnosed with Cancer.
3-pregnant women.
4-People who have a temperature of (37 ℃) or more or have low oxygen levels.
5-Chronic renal disease.
6-cardiovascular diseases.
7-who have allergies to certain substances, as well as medications, foods (such as eggs), latex, or any component of a vaccination.
8-Who has a brain-related neurological condition or has had a problem brought on by a vaccine.
9- Who uses steroids, cancer-fighting medications, or have you had radiation therapy.

2.3. Collecation of sample:

To conduct medical tests, blood samples were drawn from individual as follows:
•1st collection: Blood samples were obtained from vaccinating attendance individuals before receiving The first dose of Pfizer/mRNA BioNTech's BNT162b2 vaccination.
•2nd collection: Blood samples were obtained after the administration of the first dose (21-45 days) of the, mRNA BNT162b2, vaccine (Pfizer/BioNTech).
•3rd collection: Blood samples were obtained after (21 to 120 days) The second dose of, Pfizer/mRNA BioNTech's BNT162b2 vaccination.
collected in sterile serum tubes (gel tubes) and left for half an hour at room temperature. Then, centrifuged at 4000rpm for (10) min. Then was divided into 2 aliquots in the Eppendorf tubes(1.5-0.5) ml serum which was stored at -40 C° till used for an immunological assay of As the microparticles that were used in the quantitative detection of those neutralizing antibodies (NAbs) and also IgG antibodies that are against the protein spike measured by CLIA technique.

2.4. Statistical Analysis

After testing the normal distribution of data, A nova test was applied for the difference between means of their finding of vaccinated cohort study by SPSS program version 25.

2.5. Approval of the Ethical Committee

The approval was taken by the Research Ethics Committee after presenting the research project to the committee at the College of Medicine, University of Kufa, numbered (009647801241456) ,date 2/3/2022.

3. Results

3.1. Relationship of Immune response for NAbs and IgG with Age groups

Relationship of Immune response for NAbs and IgG with Age groups.There are not significant different(P-value = 0.99) between age groups and immune response for three collection (pre vaccinated C1, first dose C2, and second dose C3), in table (1).

Table (1) : Relationship between Immune response for NAbs and IgG depending of Age groups (No. of cases =30)

Primers		NAbs response			IgG response		
		C1	C2	C3	C1	C2	C3
Age group (years)	<20 No.=5	2 (40.0) %	5 (100.0) %	5 (100.0) %	3 (60.0) %	5 (100.0) %	5 (100.0) %
	20-29 No.=13	5 (38.5) %	13 (100.0) %	13 (100.0) %	6 (46.2) %	13 (100.0) %	13 (100.0) %
	30-39 No.=8	1 (12.5) %	8 (100.0) %	8 (100.0) %	1 (12.5) %	8 (100.0) %	8 (100.0) %
	40-49 No.=0	0 (0.0) %	0 (0.0) %	0 (0.0) %	0 (0.0) %	0 (0.0) %	0 (0.0) %
	More than No.=4	0 (0.0) %	4 (100.0) %	4 (100.0) %	2 (100.0) %	4 (100.0) %	4 (100.0) %
Total		30 (100.0) %					
P-value = 0.99 (not significant) > 0.05, X2 = 1.523, df = 2							

3.2. The dynamics of the response of neutralising antibody(NAbs) and immunoglobulin G (IgG)to the vaccine

The NAbs, and IgG response detected in serum samples is a typical indicator of vaccination effectiveness. No antibodies are induced before the vaccine administration (C1), except for those with the previous infection. When collecting (C2) after administration of the first dose of the vaccine that stimulates the production of antibodies within 21-45 days of our study, there was arithmetic mean difference and a significant difference at the 0.05 level (p-value = 0.00001).

Table (2) : Comparisons between Antibodies neutralizing NAbs, Immunoglobulin G (IgG) for three collection(C1,C2,and C3)for two administration of vaccine.				
Outcome variables	Vaccinated cohort (N= 30)			P value
	C1 Pre-vaccination	C2 After 1st dose	C3 After 2nd dose	
Neutralizing antibodies (mean±SE) (µl/mL)	.379 ±.15248	20.610 ± 2.208	31.600 ± 1.724	0.00001*
IgG (mean±SE) (AU/mL)	5.829 ± 1.917	14.223 ± 1.717	25.167 ± 1.678	0.00001*

3.3. Relationship between Immune response markers (NAbs, IgG) and previous infected and not infected individuals vaccinated

This table shows (3) no significant difference (p-value > 0.05) for three collections (C1, C2, and C3) neutralizing Antibodies with infected and not infected individuals vaccinated. At the same time, in IgG level was found significant(p-value ≤ 0.05) only in C1was .005 and not significant for two collections (C2 and C3).

Table (3): Relationship between Immune response markers and previous infection and not infected individuals vaccinated.					
Immune response markers		Not infected N=17	previous infection N=13	P value	95% Confidence Interval of the Difference
Neutralizing Antibodies (Mean ± SE) (µl/mL)	C1	.254 ± .146	.542 ± .297	.358	-.91988 , .34350
	C2	19.452 ± 3.421	22.123 ± 2.539	.558	-11.90160 , 6.56133
	C3	29.805 ± 2.065	33.946 ± 2.885	.241	-11.214 , 2.933
IgG (Mean ± SE) (AU/mL)	C1	1.508 ± .612	5.801 ± 1.404	.005	-7.17815, -1.40846
	C2	13.729 ± 2.329	14.869 ± 2.631	.749	-8.35069 , 6.07105
	C3	22.529 ± 2.076	28.615 ± 2.541	.072	-12.744 , .572
	C2	56.00 ± 12.565	64.384 ± 15.664	.676	-49.032 , 32.263
	C3	46.000 ± 9.107	61.000 ± 16.941	.413	-52.011 , 22.010

4. 4. Discussion

Due to the severity and quick global spread of COVID-19, the virus has emerged as one of the most significant global health issues. Most of the time, vaccines work by imitating an illness. As a result, it is virtually certain that all efforts to halt or reduce the spread of SARS-CoV-2 will depend on widespread COVID-19 immunisation (6). The majority of SARS-CoV-2 vaccines aim to promote the production of antibodies against the spiked SARS-CoV2 protein. So, keeping an eye on the number of NAbs and anti-S1-RBD IgG levels could tell us a lot about how SARS-CoV-2 acquired immunity develops (7).

There is no discernible difference between the age groups in this study's analysis (table.1) of the immunological response to NAbs and IgG, which is similar with the findings of the Romanian review (6).The cause might be that they only treated one age group or only followed up briefly following vaccination doses. Additionally, our research included a larger age range and a longer follow-up period after collecting three sets of blood samples before to after first, and after the second dosage.

The results of this study (table.2) were about neutralizing antibodies before and after administration of the first and second doses. The result was negative before the first dose (379 ± 248µg/mL). However, when the first dose of the vaccine was taken, the NAbs began to increase significantly more than before vaccination (20,610 ± 2.208 µg/mL). After the second dose of Pfizer was administered, the concentrations of NAbs gradually increased, reaching a higher proportion than the first (31,600 ± 1.724 µg/mL). The results of this study were in agreement with those of a study conducted in the United Kingdom (8).

It may be deduced that the humoral immune response, particularly involving NAbs, was triggered by the BNT162b2 mRNA vaccination. The major histocompatibility complex (MHC) molecules will be processed and linked with the S protein produced by transfected cells for the activation of T CD4+ cells after their injection. The latter will promote the activation of T CD8+ cells and B cells to create neutralizing antibodies (NAbs). The effectiveness of the Pfizer BioNTech vaccine accounts for enhanced immunity after the first and second doses.

This study found a slight increase in IgG concentration before injection due to previous infection (5.829 ± 1.917AU/mL). After the first dose, the percentage of IgG increased significantly more than it was previously (14.223 ± 1.717 AU/mL), but after the second dose, this ratio increased even higher than the previous one (25.167 ± 1.67 AU/mL). An earlier infection prior to the first dosage is what is causing the rise in IgG concentration before the vaccination. However, the immune system's activity was clearly increased after receiving the first dosage of the vaccination. The rise after the second dose was greater than it was both before and after the first dose of the vaccine. This demonstrates that

immunity is acquired by vaccination rather than infection, as shown by research conducted in Italy that found a decline in IgG levels and no discernible change after two doses of the Pfizer vaccine (9)

The objective of the cohort study was to compare the levels of immunoglobulin G and neutralizing antibodies at three-time intervals before and after the first dosage (21–45 days) and after the second dose (21-120 days). Significant and elevated amounts of immunoglobulin G and neutralizing antibodies were observed. These findings support the idea that, in the event of a vaccine shortage, different jurisdictions should take into account a significant interval between the first and second doses in order to protect a larger section of the populace. The second dose of the SARS-CoV-2 vaccine will not be given out in the United Kingdom for up to 12 weeks. Canada, on the other hand, extended this time frame to 16 weeks.

In this study, we found a significant difference between the uninfected condition (1.508 ± 0.612) prior to receiving the first dose of vaccine BNT162b2 (Pfizer) and those who were exposed to the previous infection. These findings indicate the relationship between NAbs, IgG and the status of vaccinees in terms of the previous infection and non-infection after three collections (C1, C2, and C3). When calculating the immunoglobulin concentration, a substantial difference of (5.801± 1.410) is found, however this difference is no longer there after receiving the two doses of the vaccination. Following immunisation, we did not see a significant change in the NAbs concentrations between previously infected and uninfected individuals. This finding is in line with a study's findings from the United Kingdom, which showed that patients with prior illnesses did not exhibit a consistent pattern of antibody behaviour after infection or vaccination (10). The findings of this research were in line with those of the Polish study (11).

After the vaccine was given twice, both groups developed high amounts of NAbs and IgG in response to vaccination. Since it is generally known that natural infection alone only offers a fleeting defence, there was no evident impact of the prior infection on antibody levels. According to the US Department of Health and Human Services and the Centers for Disease Control and Prevention, the average person's immunity to a natural illness is thought to last 90 days, despite the fact that this is not known for sure. In order to further clarify this, the World Health Organization advises that people who have already contracted the disease wait three months before receiving the vaccine. This is another indication that the prior infection had no impact on the level of antibodies produced after receiving the vaccine, according to the WHO. The results of our study, which showed that two people with high levels of IgG and NAbs before taking the vaccine and when they took the first dose of the vaccine experienced strong and similar symptoms of natural injury, suggest that people may be afraid of vaccination taken soon after an infection because it may result in a risk of hyper-immune response. In order to lower their chance of contracting SARS-CoV-2 in the future, it is recommended that all eligible individuals get the immunisation. This includes people who have already contracted the virus (12).

5. Conclusions

1-Corona vaccine Pfizer/BioNTech has a specific effect in stimulating the immune response and raising the titer of neutralizing antibodies that contribute to preventing the binding of the virus by neutralizing and preventing the binding of the protein S (subunit S1 containing RBD).

2-The Pfizer/BioNTech vaccine stimulates the production of IgG antibodies at high and noticeable levels after two doses and gradually.

3-There is no noticeable effect of the previous infection by raising the levels of antibodies after the two doses, as it had a negligible effect that gradually disappears after two doses of the vaccine.

6. Recommendations

Neutralizing antibody test should be performed before vaccination to detect active SARS-CoV-2 infection and avoid an overloaded immune response before taking the vaccines.

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