Evaluation of IgG and IFN-γ generated after vaccination with Pfizer in persons residing in Kerbala province, Iraq

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ABSTRACT

The SARS-CoV2 vaccines are safe and smart way to protect ourselves. Once the body takes one or more doses of the vaccine, it produces an immune response without being fully exposed to the disease. It is important to evaluate the immune response generated against vaccine. So, the aim of the study was measuring the level of IgG and IFN-γ levels and study the differences their levels according to some risk factors like age, sex, previous infection, and BMI. that done by Cross-sectional study, where it was conducted between November 2021 and April 2022. Serum samples were obtained from 105 vaccinated persons with Pfizer. SARS-CoV-2 IgG levels and IFN-γ were detected. And the results obtain were the participants' ages ranged from (18-72) years, they were divided into 2 groups: those under 25 years old 66 (62.9%), and those over 25 years old 39 (37.1%). 56 subjects (53.3%) were males and 49 (46.7%) were females. The sample was taken at various times and weeks. 50 individuals (47.6%) had received one dose, while the others 55 (52.4%) had received two doses, Additionally, 39 subjects (37.1%) had a confirmed infections prior to receiving the vaccine whereas others did not 66 (62.9%). Furthermore, according to BMI, 63 subjects (60%) had a normal weight and 29 (27.6%) had overweight, finally this study Concluded that younger participants under the age of 25 had higher antibody and IFN γ concentrations. higher antibody level and lower IFN γ in males than females were observed. Previous infection with covid 19 seems to have no effect on antibody and IFN γ level after vaccination with Pfizer.

Keywords: COVD19 vaccine, IgG concentration, IFN γ concentration, Pfizer

الخلاصة

لقاحات SARS-CoV2 هي طريقة آمنة وذكية لحماية أنفسنا من الاصابه بالمرض حيث انه بمجرد أن يأخذ الجسم جرعة واحدة أو أكثر من اللقاح ، فإنه ينتج استجابة مناعية للفيروس دون التعرض الكامل للمرض.لذلك كان من المهم تقييم الاستجابة المناعية الناتجة عن اللقاح ولذلك فان الهدف من الدراسة هو قياس مستويات IgG و IFN ودراسة الفروق في مستوياتهما وفقًا لبعض عوامل الخطر مثل العمر والجنس والعدوى السابقة ومؤشر كتلة الجسم.حيث أجريت دراسة مقطعية بين نوفمبر 2021 وأبريل 2022. وتم الحصول على عينات مصل من 105 شخص تم تلقيحهم بلقاح الفايزرومن ثم تم الكشف عن مستويات SARS-CoV-2 IgG و IFN.اما بالنسبة للنتائج فتراوحت أعمار المشاركين من (18-72) سنة ، وتم تقسيمهم إلى مجموعتين: من هم دون 25 سنة كانو 66 مشاركا (62.9٪) ، ومن هم فوق 25 سنة كانو 39 مشاركا (37.1٪). وكان 56 مشاركا منهم (53.3 ٪) من الذكور و 49 مشاركا منهم (46.7 ٪) من الإناث وتم أخذ العينة في أوقات وأسابيع مختلفة حيث كان 50 فردًا منهم (47.6٪) تلقوا جرعة واحدة ، بينما تلقى الآخرون 55مشاركا منهم (52.4٪) جرعتين ، بالإضافة إلى ذلك ، كان لدى 39 شخصًا (37.1٪) إصابات مؤكدة قبل تلقي اللقاح بينما لم يصاب الآخرون وهم 66 شخصا مشاركا بالفايروس قبل التلقيح (62.9٪) ،اما وفقًا لمؤشر كتلة الجسم ، كان لدينا 63 شخصًا (60٪) يمتلكون وزنا طبيعيا و 29 (27.6٪) كانو يعانون من زيادة الوزن , وبناءا على ذلك كانت الاستنتاجات كالاتي : المشاركون الأصغر سنًا الذين تقل أعمارهم عن 25 عامًا لديهم أجسام مضادة وتركيزات IFN أعلى من المشاركين الاخرين .وكما لوحظ ارتفاع مستوى الأجسام المضادة وانخفاض IFN γ في الذكور عن الإناث. وعلى ما يبدو وحسب النتائج الاحصائيه أن الإصابة السابقة بفيروس كوفيد 19 ليس لها أي تأثير على الجسم المضاد ومستوى الإنترفيرون بعد التطعيم بلقاح الفايزر.

Introduction

COVID-19 is the disease emerged in December 2019 caused by SARS-CoV-2. It was severe, and has caused millions of deaths around the world as well [1]. Due to the spread of the SARA-Coronavirus-2 different vaccines strategies were developed. Immunization is a quick, safe, and efficient method of disease prevention, where the importance of this vaccine lies in protecting against subsequent exposure with this contagious virus by inducing of the immune response to produce antibodies to fight it and prevent or control the infection [2]. At the end of 2020, 259 COVID-19 vaccination studies were conducted, with 11 in phase III clinical trials [3] . In Iraq, Pfizer vaccine (which is approved by WHO for emergency use against COVID-19) was administered in 1 December 2021 [4].

Pfizer's mRNA vaccine (which has a prospective efficacy of more than 95%) is made up of a lipid-enclosed, nucleoside-modified mRNA that specifies the shape of a COVID-19 spike protein that has undergone mutation [4] . It is given intramuscularly (IM) in two 30 g doses and the second dose is applied after three weeks from the first dose [5]. Lipid nanoparticles enable delivery of COVID-19 S gene mRNA into the host cell, maintaining the integrity of the mRNA and preventing it from being mistaken with other RNA molecules, leading to expression of the COVID-19 spike protein antigen. Lipid nanoparticles are injected into the deltoid as part of the Pfizer mRNA vaccinations. These muscle cells have T cells, antigen-presenting cells, natural killer cells, and blood vessels [6].The lipid nanoparticles are ingested by the cells, allowing the cells to produce the COVID-19 spike protein [7] Eventually, proteins enter cells and uploaded into MHC-II (antigen-presenting cells) and MHC-I, which is present in all nucleated cells in the body. The MHC-II complex is only found in B cells, macrophages, and dendritic cells. These are s-protein-stimulated and attract immune system cells. Particularly in T-helper (Th) cells, a special membrane protein (TCR, T cell receptor) interacts to the viral s-protein-MHC II complex. Strongly activated T cells begin to generate cytokines such as IL-2, IL-4, and IL5. As a result of these interleukins, the body's B-cells transform into plasma cells and begin to produce many antibodies that can neutralize or eradicate the virus [8] .

The interleukins also encourage Th cells to multiply and form memory T cells. T-cytotoxic cells (Tcx cells) are a different class of cells that generate CD8 proteins by interacting with the MHC-I protein on cell membranes through their TCR. These proteins could enable the Tcx cells to produce hazardous compounds that would cause the cells to die if they became infected with the virus in the future [9]. On the other hand, Tcx cells can also create chemicals that intensify the aforementioned immune response. The body develops immunity against the virus thanks to this process, which involves the vaccination eliciting an immune response to the spike protein. This immunity should last for six to nine months [10].

Thus, the aim of this study is to evaluate the IgG and IFN-γ levels generated after vaccination with Pfizer vaccine.

Method

This study (which is cross-sectional study) was conducted in the College of Applied Medical Sciences at the University of Kerbala during the period from November 2021 to April 2022. The majority of participants were students of both sexes, with participants' ages ranging from 18 to 70. Age, sex and dose of each participant were documented, as well as other information. Five ml blood samples were drawn and serum was separated to be used. SARS-CoV-2 IgG levels were determined using the SARS-CoV-2 IgG II Quant antibody test, which use chemiluminescent microparticle immunoassay (CMIA) for the qualitative and quantitative assessment of IgG antibodies to SARS-COV-2 in human serum. Additionally, SARS-CoV-2 IFN-γ level detection was performed using human IFN-γ.

Statistical analysis

IBM SPSS VERSION 24 software was used for statistical analysis of data. Quantitative results are indicated as mean ± SD. Pearson test was used for analyzing correlations between parameters. The statistical significance level was set at $P<0.05$. ANOVA and independent sample t test were used to compere groups.

Results

Serum sample was collected from 105 subjects. The participants' ages ranged from (18-72) years, they were divided into 2 groups: those under 25 years old 66 (62.9%), and those over 25 years old 39 (37.1%). 56 subjects (53.3%) were males and 49 (46.7%) were females. The sample was taken at various times and weeks. Some of them (50; 47.6%) had received one dose, while the others (55; 52.4%) had received two doses, Additionally, (39; subjects 37.1%) had a confirmed infections prior to receiving the vaccine whereas others did not (66; 62.9%). Furthermore, according to BMI, (63; subjects 60%) had a normal weight and (29; 27.6%) had overweight, as shown in Table (1).

Table (1): Demographic Data of the vaccinated participants

Sex	Dose	Age groups N (%)		Total
		≤25	>25	
Female	1	19(69%)	5 (20%)	24 (49%)
	2	12 (48%)	13 (52%)	25 (51%)
	Total	31 (63.3%)	18 36.7%	49 46.7%
Male	1	21 (80.7%)	5 (19.2%)	26 (46.4%)
	2	14 (46.6%)	16 (53.3%)	30 (53.6%)
	Total	35 (62.5%)	21 (37.5%)	56 (53.3%)
Total	1	40 (80%)	10(20%)	50 (47.6%)
	2	26 (47.2%)	29 (52.7%)	55 (52.4%)

Female	Confirmed Previous infection	No	21 (60%)	14 (40%)	35 (71.4%)
		Yes	10 (71.4%)	4 (28.5%)	14 (28.6%)
	Total		31 (63.2%)	18 (36.7%)	49 (46.7%)
Male	Confirmed Previous infection	No	19 (61.2%)	12 (38.7%)	31 (55.4%)
		Yes	16 (64%)	9 (36%)	25 (44.6%)
	Total		35 (62.5%)	21 (37.5%)	56 (53.3%)
Total	Confirmed Previous infection	No	40 (60.6%)	26 (39.3%)	66(62.9%)
		Yes	26 (66.6%)	13 (33.3%)	39 (37.1%)
	Total		66 (62.9%)	39 (37.1%)	105

Female	BMI groups	underweight	2 100%	0	2 (4.1%)
		Normal weight	21 (75%)	7 (25%)	28 (57.1%)
		overweight	7 (46.7%)	8 (53.3%)	15(30.6%)
		obese	1 (25%)	3 (75%)	4 (8.2 %)
	Total		31 63.3%	18 (36.7%)	49 (46.7%)
Male	BMI groups	underweight	2 (100%)	0	2 (3.6%)
		Normal weight	23 (65.7%)	12 (34.3%)	35 (62.5%)
		overweight	8 (57.1%)	6 (42.9%)	14 (25%)
		obese	2 (40%)	3 (60%)	5 (8.9%)
	Total		35 (62.5%)	21 (37.5%)	56 (53.3%)
Total	BMI groups	underweight	4 (100%)	0	4 (3.8%)
		Normal weight	44(69.8%)	19 (30.2%)	63(60%)
		overweight	15 (51.7%)	14 (48.3%)	29 (27.6%)
		obese	3 (33.3%)	6 (66.7%)	9(8.6%)
	Total		66 (62.9%)	39 (37.1%)	105

Differences in IgG and IFN γ levels according to age groups

The overall antibody concentration and IFN γ in participants under the age of 25 was higher than that in people above the age of 25. The current study does not observe any significant difference in IgG and IFN γ concentration between persons younger and older than 25 as shown in table (2).

Table (2): Differences in IgG and IFN-γ levels according to age group in Pfizer vaccine

Pfizer					
IgG AU/ml (Mean± S. D)			**IFN-γ Pg/ml(Mean± S. D)**		
≤25 years	**> 25 years**	**P value**	**≤25 years**	**> 25 years**	**P value**
18329.5±11461.0	14714.2±10204.8	0.109	65.5±14.9	63.1±14.5	0.429

Differences in IgG and IFN γ levels with Sex

As shown in table (3) there was no significant difference observed in the concentrations of IgG and IFN γ between males and females' individuals, but the mean of the IgG concentration was higher in males than females, regarding the IFN γ, the mean level of IFN γ in females were higher than that in males.

Table (3): Differences in IgG and IFN-γ level with sex in Pfizer vaccine

Pfizer					
IgG AU/ml (Mean± S. D)			**IFN-γ Pg/ml (Mean± S. D)**		
Male	**Female**	**P value**	**Male**	**Female**	**P value**
17030.8±11011.8	16877.0±11305.4	0.945	62.7±14.5	66.9±14.7	0.155

Differences of IgG and IFN γ concentrations according to number of vaccine's dose

As shown in table (4), there was no significant variation between the first and second dose for IFN γ and IgG concentration, while the mean of both markers is higher in first dose than second dose.

Table (4): Differences of IgG and IFN γ concentrations according to number of vaccine's dose in Pfizer vaccine

Pfizer					
IgG AU/ml (Mean± S. D)			IFN-γ Pg/ml (Mean± S. D)		
dose 1	dose 2	P value	dose 1	dose 2	P value
18123±11062	15905±11116	0.313	66±13	64±16	0.247

Differences in IgG and IFN γ level between previously Infected and uninfected subjects

As shown in Table (5), there were no statistically significant difference in IgG and IFN-γ mean levels between vaccinated subjects with confirmed previous infection group versus vaccinated subjects without apparent previous infection in subjects vaccinated with Pfizer.

Table (5): Differences in IgG and IFN γ level between previously Infected and uninfected subjects in Pfizer vaccine

Pfizer					
IgG AU/ml (Mean± S. D)			IFN-γ Pg/ml (Mean± S. D)		
Vaccinated without confirmed previous infection	Vaccination with confirmed previous infection	P value	Vaccinated without confirmed previous infection	Vaccinated with confirmed previous infection	P value
16159.3 ±11561.2	18331.3 ±10242.7	0.354	65.6 ±14.1	63.0 ±15.7	0.53

Differences in IgG and IFN γ according to BMI

The statistical analysis of the current study revealed that the antibodies concentration is higher than in subjects with normal weight regarding the IFN γ the higher concentration observed in obese, in spite of that there was no significant deference in IFN γ and IgG concentration observed between categories of weight as shown in tables (6)

Table (6): Differences in IgG and IFN γ according to BMI in Pfizer vaccine

Pfizer			
IgG AU/ml (Mean± S. D)		**IFN-γ Pg/ml (Mean± S. D)**	
Underweight	10254.0±5650.7	**Underweight**	59.9±14.7
normal weight	19345.6±11409.7	**normal weight**	65.3±15.9
Overweight	13815.7±10416.2	**Overweight**	62.7±13.2
Obese	13921.0±9247.9	**Obese**	68.6±11.1
P value	.061	**P value**	.663

Discussion

Every year, vaccinations save millions of lives, and the COVID-19 vaccine may do the same for you. The COVID-19 vaccines are safe and effective, offering great defense against fatal sickness. WHO reports that unvaccinated people have at least 10 times higher risk of death from COVID-19 than someone who has been vaccinated. Even if you had COVID-19 before, getting the vaccination as soon as it is your turn is crucial. You can build up immunity to COVID-19 more safely by getting vaccinated than by getting sick. So, one of the most important steps toward assisting us in returning to doing more of the activities we enjoy with the people we love is the development of safe and effective COVID-19 vaccines. There are many COVID-19 vaccines that have been approved for use by WHO (given Emergency Use Listing). Despite the differences in their mechanisms, all of the themes share a common role and purpose [1].

Differences in IgG and IFN γ according to age groups show no significant differences between age groups. However, the mean of younger age group was higher, as shown in table (2). The result of the current study is in agreement with previous research showed that IgG levels caused by Pfizer immunization decreased with age, with the higher amounts seen in people between the ages of 12 and 19[11]. Also, another study documented that the geometric mean titer of anti-spike IgG was consistently lower in the older age group and declined following the second vaccination [12]. Inversely, Age-related differences in IgG antibody levels were evident in previous study, especially between participants in the younger (aged 21 to 30) and older age groups [13]. Elderly adults are also substantially more likely to have inadequate or no post-vaccination humoral response, and the values of anti-SARS-CoV-2 antibodies after vaccination are higher than in the elderly [14]. Regarding IFNγ, the result is in agreement with previous study in which the author reported that older participants produce less IFN γ from SARS-CoV-2 spike-specific T cells than younger participants did [15]. Previous studies showed a link between the age and the potency of the humoral or cellular response ([16] . In spite of the increase in age makes the immune system suffer from characteristic changes that lead to an increase in the severity and the extent of the spread of infectious diseases, as well as to a lack of complete protection after the vaccine [17], But it was becoming clear that when considering the immune health, age is just a number, where age was not a measure to how well the immune system was.

Regarding sex, as shown in table (3) higher IgG concentration in males and higher IFN γ mean levels in females was seen. Similarly in a previous study, where the anti-SARS-CoV-2 S1 IgG ELISA assay was used to monitor humeral response to COVID-19 mRNA BNT162b2 vaccine, did not show any statistically significant correlation between the sex of the individuals and the anti-spike protein antibody titers [18] . Additionally, the mean value for Pfizer vaccines showed no significant differences in IgG titer for vaccinated males and females [19]. nversely, significant difference in IgG concentration between males and females was observed previously. The anti-Spike-RBD IgG response were observed to be significantly more in females than in males after vaccination with BNT162b2[20]

Disagreement with study show significant difference in the IFN γ levels between male and females in vaccinated subjects [21] .While agree with other said the CD4+ and CD8+ T cells in female generate more robust responses to viral infections [22] .and with Takahash study reported lower T cell levels in males associated with worsening disease as compared to females. Moreover, number of activated CD8 T cells were significantly higher in females [23]. Higher activity of T cells may in turn contribute to potentially better antiviral adaptive immune response in females, which may lead to greater viral clearance. It is well established that, compared to males, females develop stronger humeral and cellular immune response to foreign antigenic stimulation, vaccination and infections than male which is considered as benefit [24] .Whereas, strong immune response generated by females to self-antigens make them susceptible to autoimmune diseases [25].

Concerning the type of the dose, the mean of both markers is higher in first dose than second dose as show in table 4. The result of the current study is in agreement with other recent study which found that the second dose of the vaccination did not improve humoral or cellular immune responses since neither anti-spike IgG levels nor specific IFN γ producing T cells significantly increased [26]. In another study, stated that despite infected patients with COVID-19 showed robust humoral and antigen-specific responses to the first dose, these responses did not improve following the second dose of the vaccine at the time points examined [27] .Moreover, Fonseca et al., reported that following receiving the second dose of the vaccine, there was no increase in anti-S IgG in the group of healthcare professionals who had previously infected COVID-19) [28]. Tormo et al., reported that IFN γ production by T cells improved over time following the second dose, reaching levels comparable to those seen following the first dose [29].The differences in the result of the current study and this study might possibly due to of sample collection. Differences in IgG and IFN γ level between previously Infected and uninfected subjects.

While when compare in IgG and IFN γ level between previously Infected and uninfected subjects there were no statistically significant difference in IgG and IFN-γ mean levels between vaccinated subject with confirmed previous infection group versus vaccinated subject without apparent previous infection in subjects vaccinated with Pfizer. As shown in Table (5), These findings were inconsistent with other previous published data in which authors were reported that in people who were vaccinated after contracting COVID-19, antibody responses after the first dose of Pfizer/BioNTech vaccine were 6.8 times higher, and T-cell responses were 5.9 times higher than in people who had never had the disease [30]. In another study, Memory CD4+ T-cell and total CD8 responses elicited by a single dose of vaccine were significantly higher in the previously infected group compared with the no prior exposure group [31].Tormo et al., found that participants who had previously been exposed to COVI-19 had fewer and slower increases in both cellular and humeral immunity markers than those who had not experienced the prior infection [29]. Vaccination after recovery from natural SARS-CoV-2 infection, or "hybrid immunity," has been reported to substantially increase both the potency and breadth of humoral response to COVID-19 [32] .This occurs as a result of the combined effect of acquired (vaccine) immunity and natural immunity, which produces stronger antibody responses than either kind of immunity alone. It provides 25 to 100 times more antibody responses than natural and vaccine-produced immunity alone.

The differences of the current study findings with other previously published data might be possibly due to the lack of confirmation for the absence of infection with COVID-19. It has been documented that there were high proportion of individuals who are infected with COVID-19 and had never develop symptoms or experience a very mild or almost unrecognizable symptoms. This proportion is difficult to quantify because it requires intensive prospective clinical sampling and symptom screening from a representative sample of individuals with and without infection [33] .However, it has been reported that more than 30% of population were infected without symptoms and 80% of population have a mild illness, much like normal flu or bad cold[34].

Regarding BMI, the antibody concentration mean is higher in subjects with normal weight whereas the IFN γ was higher in obese participants, tables (6). This result supports previous researches that found humoral response was seen in all study participants, with normal-weight groups showing higher values than pre-obesity and obese groups [35] .

Kooistra et al., found that There was a statistically significant difference in IgG values between underweight and overweight BMI and between obese subjects and normal weight; and finally, between obese and overweight groups for IgG testing [36] . Inversely, Other previous study showed that following vaccination, BMI had no real effect on RBD-specific IgG titers and simulated neutralizing titers. Also Bates et al., documented that BMI had no influence on the size and persistence of the antibody response to mRNA-based vaccinations [37]. Regarding the IFN γ level, there were significant difference among the three types of vaccines in obese subjects. The highest mean was observed in subjects vaccinated with Sinopharm vaccine. Additionally, there were highly significant difference among the four groups vaccinated with Sinopharm vaccine (the highest means was observed in underweight and obese subjects, Table (4.13). Kooistra et al., reported no potential link between BMI and the cytokine response [36] .

There is evidence that vaccination protects against severe COVID-19 to a degree comparable to that of persons who are of a healthy weight in those who are overweight or obese[38]. People who were underweight had slightly reduced vaccination effectiveness, and they also had the lowest overall vaccination uptake. When compared to the vaccinated population who were of a healthy weight, there were higher chances of severe COVID-19 outcomes in the vaccinated cohort for those who were obese or underweight [39] .

Conclusion

Younger participants under the age of 25 had higher antibody and IFN γ concentrations than older and Pfizer vaccine produce higher antibody level and less IFN γ in males than females. Higher mean of both antibody and IFN γ production was produced after the first dose but not for significant level. Previous infection with covid 19 seems to have no effect on IgG level and IFN γ concentrations after vaccination with Pfizer. Normal weight subjects might possibly respond better to vaccine and produce more antibody level whereas obese subjects' tent to mount more IFN γ concentration.

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