Effect of The Pfizer-Biontech Vaccine on Ifn-Γ Serum Levels and its Genetic Variations in Response to Vaccination

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KEYWORDS

Pfizer vaccine, IFN-γ, gene polymorphism

ABSTRACT

Background: Pfizer vaccine, which is a nucleoside-modified mRNA vaccine against coronavirus disease 2019 (COVID-19) is one of the first mRNA products to be approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

Aim of the study: The aim of this study is to study the impact of Pfizer vaccine on the levels of interferon gamma (IFN-γ) serum levels and its gene polymorphism after 3 months and 6 months of vaccination with Pfizer vaccine .

Materials and methods: This case-control study involved (150) subjects who were divided into three groups (each group included 50 subjects). The first group included (50) vaccinated group 3 months after second dose of Pfizer vaccine, and the second group included (50) vaccinated group 6 month after second dose of Pfizer vaccine. The third group was the control group which included (50) healthy unvaccinated subjects. The study was done during the period from May /2022 to September / 2022). Serum levels of IFN-γ were estimated by ELISA technique. The PCR technique was used to study gene polymorphism for IFN-γ.

Results: The results in figure (1) showed that the mean levels of INF-γ among the male participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (292.54, 231.48, and 15.71), respectively, with highly significant differences (HS) between the three groups ($p<0.01$). The results also showed that the mean levels of INF-γ among female participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (317.31, 228.14, and 15.74), respectively, with highly significant differences (HS) between the three groups ($p<0.01$). Analysis of rs2430561 SNP of (IFN-γ) gene using Sanger sequencing. Single "T" peak indicative of a T homozygous allele showed that Single "A" peak indicative of a A homozygous allele. Presence of the "T" and "A" peak indicative of T/A heterozygous allele.

Conclusion: Pfizer vaccine was shown to have significant impacts on IFN-γ levels and presence of single T/A alleles is indicative of heterogeneous T/A allele on analysis of rs2430561 SNP of (IFN-γ) gene among vaccinated individuals.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Most infected individuals with mild symptoms spontaneously recover, but SARS-CoV-2 infection can result in a severe acute respiratory illness requiring mechanical ventilation with ~1% mortality. To induce immunity and reduce the severity of SARS-CoV-2 infection, several categories of vaccines have been developed (1).

The primary goal of vaccination is to induce innate immunity and protective adaptive immunity against SARS-CoV-2 in the form of antibodies and specific T-cell responses (2,3).

Pfizer and the German biotechnology business (BioNTech) collaborated to create a COVID-19 vaccination utilizing messenger RNA (mRNA) technology. The Pfizer-BioNTech COVID-19 vaccine was created quickly in reaction to the worldwide pandemic brought on by the new coronavirus, SARS-CoV-2 (4).

Interferon gamma (IFN-γ) is a cytokine that plays a crucial role in the immune response to viral infections, including COVID-19. It is produced by various immune cells, such as T cells and natural

killer (NK) cells, and helps coordinate the immune response to eliminate virus-infected cells (5).

It is well known that host genetic polymorphisms play a key role in the vulnerability or resistance to different viral infections (Debnath et al; 2020 ; Ramos et al., 2020). One important factor contributing to the variety of clinical signs of coronavirus disease 2019 (COVID-19) is host genetic variations (6).

2. Methodology

The current case-control study included 150 participants who were divided into three groups (each group included 50 subjects). The first group included (50) vaccinated group 3 months after second dose of Pfizer vaccine, and the second group included (50) vaccinated group 6 month after second dose of Pfizer vaccine. The third group was the control group which included (50) healthy unvaccinated subjects. The study was done during the period from May /2022 to September / 2022). Venous blood samples were taken from all participants and a part of the blood was put in gel tubes to clot for 20-30 minutes, then centrifuged to obtain serum samples. ELISA kits were used to in vitro quantitative determination of Human IFN-γ concentrations in serum. The second part of blood was transferred into an anticoagulant tube and immediately stored at -20 C◦ to be used later for IFN-γ DNA extraction and gene polymorphism.

Statistical analysis: The experimental results were performed according to the graph pad prism version 8 where Tukey multiple comparisons were done. One and two-way (ANOVA) was preformed to investigate the significance of differences between groups. The values were expressed as (mean±standard errors) of mean (SEM) and P value<0.01 was considered statistically significant.

Results and Discussion

The results in table (1) showed that the mean levels of IFN-γ among male participants (after 3 months of vaccination, after 6 months of vaccinations, and in the control group) were (292.54, 231.48, and 15.71), respectively, with highly significant differences (HS) between the three groups (p<0.01). The results showed that the mean levels of IFN-γ among female participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (317.31, 228.14, and 15.74), respectively, with highly significant differences (HS) between the three groups (p<0.01).

Table (1): Mean IFN-γ levels among the study group

Mean IFN-γ level (pg/ml)	After 3 months vaccination	After 6 months vaccination	Controls	p-value
Males	292.54	231.48	15.71	P<0.01 (HS)
Females	317.31	228.14	15.74	P<0.01 (HS)

Figure (1) shows the Sanger sequencing analysis of rs2430561 SNP of IFN-γ gene, where Single “T” peak indicative of a T homozygous allele. Single “A” peak indicative of a A homozygous allele. Presence of the “T” and “A” peak indicative of T/A heterozygous allele.

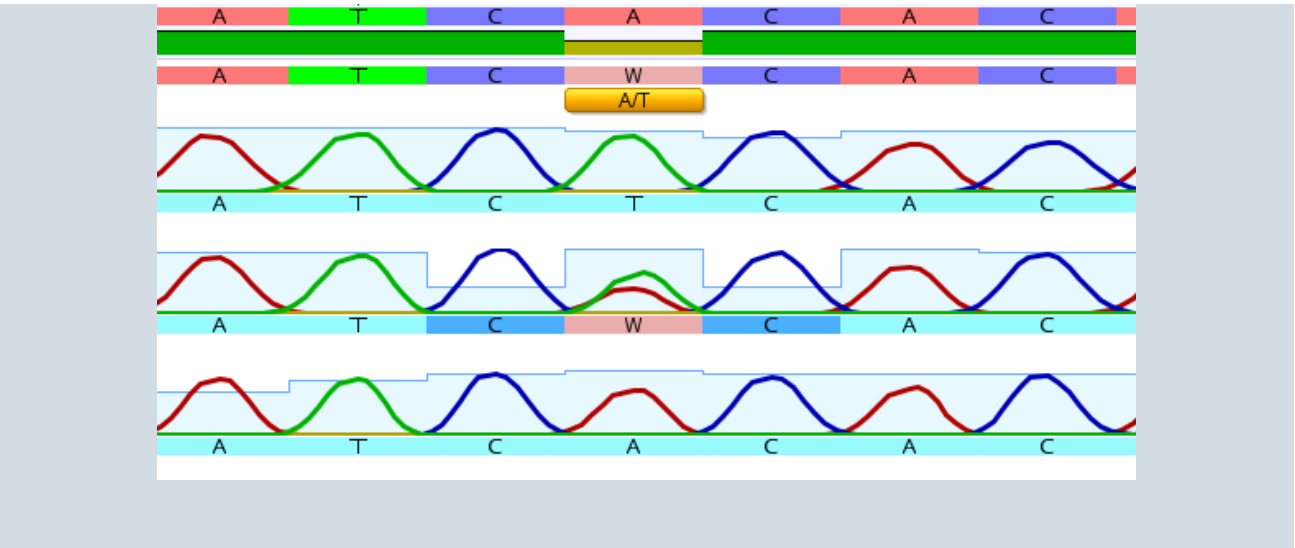

Figure (1): Analysis of rs2430561 SNP of IFN-γ gene using Sanger sequencing. Single "T" peak indicative of a T homozygous allele. Single "A" peak indicative of a A homozygous allele. Presence of the "T" and "A" peak indicative of T/A heterozygous allele

Discussion

The summary of our results showed that the mean level of IFN-γ was significantly higher in the two vaccinated groups (after 3 months and after 6 months) when compared to their levels in the control group for both males and females. The mean levels of INF-γ was higher in females than males after 3 of vaccination, and was higher in males than females after 6 months of vaccination, but without significant differences.

However, several clinical factors could influence the generation of an effective immune response as well as the maintenance of an immunological memory during time. Even if no significant differences were reported for age and sex in the main clinical trials evaluating the efficacy of mRNA-based vaccines (7,8).

With regard to INF-γ, our current study showed that this cytokine highly significantly increased in the two vaccinated groups compared to the control group and significantly increased in the 3 months group in comparison with the 6 months group.

Consistent with our results, a study conducted by (9) showed that the cytokines IFN-γ and TNF-α–producing cells were significantly higher in vaccinated healthy individuals compared with healthy controls in response to SARS-CoV-2 spike Ag.

Activated T-helper (Th) cells can indirectly kill infected cells, support B cell function and antibody response, by producing interferon-γ (IFN-γ)3,4. It has been shown that Th1 phenotype is associated with lower severity of COVID-19 disease5, thus strategies for the development of COVID-19 vaccines should also consider the possibility of activation Th cells for IFN-γ production (10).

Our result is inconsistent with studies that show T cells produced much less IFN-γ in the first two weeks following the second dose of the vaccine. This down regulation of the immune response may be due to regulatory T cells. Inversely, IFN-γ production was found to be increased after 2 weeks from the second dose to levels similar to those achieved after the first dose (11).

Another study reported that increased levels of IFN-γ were detected in all lymphocytes and in CD3+ T cells 7 days after complete vaccination. Also, an increased level of IFN-γ in serum of all vaccinated individuals was observed, both in SARS-CoV-2 naïve and previously infected individuals 7 days after full vaccination. Thus, the mRNA-based vaccine-induced Spike-specific T cells that abundantly produce IFN-γ (10).

In a study by Bergamaschi *et al.,* 2021 (12), they examined the cytokine reactions to the first and second doses of the BNT162b2 mRNA vaccine (Pfizer/BioNtech) in antigen-naive and previously infected participants, it was discovered that there was a similar pattern of cytokine expression at 24 hours after vaccination between vaccine recipients with pre-existing SARS-CoV-2 immunity who received the first vaccine dose and antigen-naive individuals after the second vaccine dose. INF-γ levels in all groups rose after the second dosage in comparison to both before and after the first dose of the vaccination. (13).

Moreover, it can also be appreciated how the induction of the IFN-induced gene signature and inflammatory factors positively associate with each other, demonstrating a coordination and a biological relevance in the vaccine-induced early immune response, as well as a direct role of innate responses to vaccination in shaping adaptive immunity (14).

IFN-gamma produced 10 days following the initial BNT162b2 vaccine dosage, While recovered people reduced their IFN-gamma production after day 10 of the second dosage, naive individuals had a rise in IFN-gamma to substantial levels (15).

Regarding the IFN γ, there were no significant differences neither among the three types of vaccines, nor between males and females for each vaccine despite that the mean level of IFN γ in females were higher than that in males in subjects vaccinated with Pfizer and Sinopharm. Significant difference in the IFN γ levels between male and females in fully vaccinated subjects was observed by (16).

Consistent with this aim, in this study we aimed to detect the biological effect of single nucleotide polymorphisms (SNPs) in (IFN-γ) cytokine genes involved in the regulation of inflammation and antibody production on the magnitude and duration of the humoral immune response induced by vaccination against SARS-CoV-2.

Our results concerning analysis of rs2430561 SNP of IFN- γ gene using Sanger sequencing, there was a presence of Single “T” peak, which is indicative of a T homozygous allele. Single “A” peak is indicative of the A homozygous allele. Presence of the “T” and “A” peak is indicative of T/A heterozygous allele.

Previously in silico studies, such as that performed by Leite et al. [17], it was shown that the allele frequencies of polymorphisms associated with high cytokine expression were correlated with the daily COVID-19 mortality rate in dozens of countries. Among them were also the alleles with high expression of IFNG +874 (T) that is correlated positively with COVID-19 mortality (17).

The results suggest an association of the IFN-γ +874T/A polymorphism with symptomatic SARS-CoV-2 infection. The literature suggests that the condition can be quite heterogeneous, as demonstrated by a recent comprehensive systematic review (Ma et al., 2021). Coincidentally, the frequency of the *T allele of rs2430561 in European populations is between 35% and 50% and is much lower in Asian countries, such as Japan (7.2%) and China (18%) (17). Thus, the T allele of rs2430561 may play a role in the development of symptomatic case, which, in association with other risk factors such as advanced age and comorbidities, may proportionally increase mortality among carriers of this allele (18).

A study conducted in Iraq hypothesized that polymorphism in IFNγ gene T/A +874 (rs2430561) may have significant effect on the way that the immune system should respond to the BNT162b2 vaccine. The study was conducted in a group of healthy subjects in Iraq who received a booster immunization following a second dose of the BNT162b2 vaccine, and these outcomes indicate that IFN-γ +874 T/A have a crucial effect on immune response to BNT162b2 vaccine and A allele may represent a risk factor for BNT162b2 vaccine unresponsiveness (19).

The same Iraqi study hypothesized that polymorphism in IFNγ gene T/A +874 (rs2430561) may have significant effect on the way that the immune system should respond to the BNT162b2 vaccine. The study was conducted in a group of healthy subjects in Iraq who received a booster immunization following a second dose of the BNT162b2 vaccine. (19).

According to sex, a highly statistically significant difference was found in the distribution of IFN-γ +874 T/A alleles between as the mutant A allele had higher frequency among male group than in female group, on the other hand, the wild T allele showed high percentage in female group than in male group. Moreover, homozygous wild genotype was highly distributed between females compared to males, while homozygous mutant genotype appeared to be more associated with male in comparison with females (19).

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