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Estimation of Interleukin 6 and Tumor Necrosis Factor in Pfizer Covid Vaccinated Case

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P A P E R I N F O

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A B S T R A C T

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 interlukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) after 3 months and 6 months of vaccination with Pfizer vaccine.
Materials and methods: The study was done during the period from May /2022 to September / 2022). A 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. Serum levels of IL-6 and TNF-α were estimated by ELISA technique. Results: The results showed that the mean levels of TNF-α among male participants (after 3 months of vaccination, after 6 months of vaccinations, and in the control group) were (65.40, 47.34, and 9.45), respectively, with highly significant differences between the three groups ($p<0.01$). The results showed that the mean levels of TNF-α among female participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (67.50, 48.74, and 9.06), respectively, with highly significant differences between the three groups ($p<0.01$). In addition, the results showed that the mean levels of IL-6 among male participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (81.36, 101.88, and 51.26), respectively, with highly significant differences between the three groups ($p<0.01$). The results showed that the mean levels of IL-6 among female participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (83.05, 98.03, and 52.07), respectively, with highly significant differences between the three groups ($p<0.01$). Conclusion: Pfizer vaccine was shown to have significant impacts on IL-6 and TNF-α levels 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 [1]. To induce immunity and reduce the severity of SARS-CoV-2 infection, several categories of vaccines have been developed [2].
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 [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]. IL-6 is known to be produced early in the immune response and is involved in the activation of innate immune cells, such as neutrophils and macrophages. It helps to promote inflammation and coordinate the initial response to infections. In the context of COVID-19 vaccines, IL-6 production may be induced by the vaccine as a part of the innate immune response to the viral antigens in the vaccine [5].

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Tumor necrosis factor-alpha (TNF-α) is a secretory protein produced by various immune cells, including macrophages, T cells, and natural killer (NK) cells [6]. TNF-α plays a critical role in the immune response and inflammation. It is involved in the regulation of immune cell activation, proliferation, and apoptosis, as well as in the induction of other inflammatory cytokines [7].

2. MATERIALS and METHODS

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. This study was conducted during the period from May /2022 to September / 2022). Venous blood samples were taken from all participants and 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 (IL-6) and (TNF-α) concentrations in serum.

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.

3. RESULTS

The results in **TABLE 1** showed that the mean levels of TNF-α among male participants (after 3 months of vaccination, after 6 months of vaccinations, and in the control group) were (65.40, 47.34, and 9.45), respectively, with highly significant differences (HS) between the three groups (p<0.01). The results showed that the mean levels of TNF-α among female participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (67.50, 48.74, and 9.06), respectively, with highly significant differences (HS) between the three groups (p<0.01).

Table 1. Mean TNF-α levels among the study group

Mean TNF-α level (pg/ml)	After 3 months vaccination	After 6 months vaccination	Controls	p-value
Males	65.40	47.34	9.45	P<0.01 (HS)
Females	67.50	48.74	9.06	P<0.01 (HS)

The mean levels of (IL-6) among male participants (after 3 months of vaccination, after 6 months of vaccinations, and in the control group) were (81.36, 101.88, and 51.26), respectively, with highly significant differences (HS) between the three groups (p<0.01). The results showed that the mean levels of IL-6 among female participants (after 3 months of vaccination, after 6 months of vaccinations, and the control group) were (83.05, 98.05, and 52.05), respectively, with highly significant differences (HS) between the three groups (p<0.01), as shown in **TABLE 2.**

Table 2. Mean IL-6 levels among the study group

Mean IL-6 level (pg/ml)	After 3 months vaccination	After 6 months vaccination	Controls	p-value
Males	81.36	101.88	51.26	P<0.01 (HS)
Females	83.05	98.05	52.05	P<0.01 (HS)

4. DISCUSSION

Results showed that the mean levels of TNF-α and IL-6 were 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 TNF-α was higher in females than males after 3 and 6 months of vaccination, but without significant differences. The mean levels of IL-6 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.

In one study, no differences were instead revealed for sex, with male and female showing a comparable antibody titer 1 month after vaccination, and similar levels of antibody decay. Literature in this regard is still conflicting, reporting in some cases a higher initial antibody level in women compared to men [8], while others showed no differences due to sex [9]

The S1 subunit of the Spike protein in the vaccine produces an endothelial lesion that is amplified by simultaneous exposure to the inflammatory cytokine TNF-α and the male hormone dihydrotestosterone [10]. This condition of endothelial lesion, amplified by simultaneous exposure to TNF-α and androgens, may allow us to resolve some controversies. There is growing evidence that suggests that males have a higher risk of outcomes in the case of myocarditis [11], despite the fact that they are able to suppress the production of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and increasing the production of anti-inflammatory cytokines [12]. Since the effects of testosterone may be different under normal physiological conditions and in

pathological states [13], in the presence of an endothelial lesion and/or myocarditis these effects may be different from the physiological conditions .

In our study, TNF-α was significantly higher among the two vaccinated groups compared to the controls, and was higher in the 3 months group compared to the 6 months group. mRNA vaccines have been developed by Moderna and Pfizer-BioNTech. A cutting-edge technique called RNA and DNA vaccines employs genetically modified RNA or DNA produce a protein that safely elicits an immune response [14].

However, in one study, no significant difference in serum levels of TNF-α was detected 7 days and up to 1 month after full vaccination, which might result from specific cohort characteristics and timing of sampling [15].

Biochemical studies revealed that Spike protein triggers inflammation via activation of the NF-κB pathway and the induction of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1β [10]. After the first dose of the BNT162b2 vaccine, the human organism produces systemic inflammation which is accompanied by the upregulation of TNF-α and IL-6 after the second dose [11]. Another study reported that serum TNF-α levels did not increase after the first dose but increased significantly after the second dose. It follows that after the second dose of the vaccine, there are markedly increased concentrations of TNF-α in the serum, already only after the first day following the second vaccine dose. Finally, there would be a significant linear correlation between the levels of pro-inflammatory cytokine TNF-α and the degree of symptoms (systemic scores) occurring one day after the second dose of the BNT162b2 vaccine. For these authors, these data suggest that pro-inflammatory cytokine (TNF-α) was produced in response to the BNT162b2 vaccination, especially after the second dose [12].

In our study, IL-6 was significantly higher among the two vaccinated groups compared to the controls, and was higher in the 3 months group compared to the 6 months group .This finding agreed with [13] who found that post-vaccination PASC-like symptoms were associated with an inflammatory profile with statistically significant elevations in IL-6.

Patients with COVID-19 have significantly higher concentrations of inflammatory mediators than healthy controls [14]. Elevated concentrations of IL-6 and persistent inflammation can also last for months and might be related to the development of post-COVID-19 conditions [15]. A study presented by Karimabad et al., 2021 [16] for the coronary artery disease patients were found increase in the levels of IL-6 after second vaccination with a BNT162b2 mRNA (Pfizer/BioNTech) vaccine at day 2, and this study agreed with our finding of increased levels of IL-6 after 2nd dose of Pfizer vaccine but at (20-30) days, and this confirm the association of IL-6 with the vaccination and will be resulting in antibody development. Our study on the circulating levels of cytokines implies that cytokine modulation could be a biomarker for effective vaccination and antibody generation, and the role of these cytokines upon vaccination became more apparent after the 2nd vaccination [17].

IL-6 plays a crucial role to induce lymphocytic apoptosis that leads to the development of lymphopaenia in COVID-19 patients [18,19]. The high level of IL6 significantly downregulates the expression of human leukocyte D antigen (HLA-DR) that substantially impairs lymphocyte function, along with the depletion of CD4+ lymphocytes, CD19+ lymphocytes and natural killer (NK) cells. In addition, IL6 is considered to have an impact in the severity of COVID-19 patients, which is significantly associated with adverse clinical outcomes [20,21,22].

The severe clinical evolution of COVID-19 was related to an exacerbated host immune response, called hyperinflammatory syndrome, which occurs due to the action of the immune system in response to infection through the release of inflammatory cytokines, a phenomenon known as cytokine storm [23]. Among the main cytokines related to the clinical severity of SARS-Cov-2 infection is IL-6, which promotes a highly specific reaction of adaptive immunity by stimulating CD8+ T and B cells, which also favors the survival of phagocytic neutrophils. However, there is tissue damage by deregulation of the extracellular matrix and by attraction of pro-inflammatory macrophages and neutrophils to the tissues [24].

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Arabic Abstract

الاختلافات بين المجموعات الثلاث (ع <0.01). أظهرت النتائج أن متوسط مستويات TNF-α بين الإناث المشاركات (بعد 3 أشهر من التطعيم، بعد 6 أشهر من التطعيمات، والمجموعة الضابطة) بلغ (67.50، 48.74، 9.06)، على التوالي، مع وجود فروق ذات دلالة إحصائية كبيرة بين المجموعات الثلاث (P<0.01). بالإضافة إلى ذلك، أظهرت النتائج أن متوسط مستويات الإنترلوكين 6 بين المشاركين الذكور (بعد 3 أشهر من التطعيم، وبعد 6 أشهر من التطعيمات، والمجموعة الضابطة) بلغ (81.36، 101.88، 51.26)، على التوالي، مع وجود فروق ذات دلالة إحصائية كبيرة بين المجموعات الثلاث (P<0.01). أظهرت النتائج أن متوسط مستويات الإنترلوكين 6 بين الإناث المشاركات (بعد 3 أشهر من التطعيم، بعد 6 أشهر من التطعيمات، والمجموعة الضابطة) بلغ (83.05، 98.03، 52.07)، على التوالي، مع وجود فروق ذات دلالة إحصائية كبيرة بين المجموعات الثلاث (P<0.01). الاستنتاج: تبين أن لقاح فايزر له تأثيرات كبيرة على مستويات IL-6 وTNF-α بين الأفراد الملقحين.