The Assessment of Serum Levels of TNF-α and INF-γ for both COVID-19 Infected before and after the Vaccination of Pfizer-BioNTech (a case-control study): A REVIEW

Khalid Khalaf Abdullah[1]*& Prof. Ahmed Rushdi Abdullah[1]
1Al-Iraqia University /College of Medicine / Baghdad/ Iraq

Abstract

The virus that causes a respiratory disease called coronavirus disease 19 (COVID-19). SARS-CoV-2 is a member of a large family of viruses called coronaviruses. These viruses can infect people and some animals. SARS-CoV-2 was first known to infect people in 2019. Patients with SARS-CoV-2 infection exhibit a range of systemic manifestations that correspond to an intense inflammatory response and a cytokine storm, or rapid release of pro-inflammatory cytokines. This study aims to estimate the serum levels of TNF-α and INF-γ among people in Baghdad after one month of receiving the full Pfizer-BioNTech vaccine, SARS-CoV-2 virus infection, and SARS-CoV-2 virus infection after Pfizer-BioNTech full vaccine respectively. A total of 120 individuals participated in this study conducted at the College of Medicine / Iraqia University. They were divided into four groups, each group containing 30 individuals. After one month, the study groups were categorized into Pfizer-BioNTech (BNT162b2) full vaccination, infected with SARS-CoV-2, SARS-CoV-2 infection after full Pfizer vaccination, and control. The study showed a significant difference (P value <0.05) in the TNF-α and INF-γ serum levels of all groups compared to the control and for each group with each other except for the correlation between the infected non-vaccinated group and the infected vaccinated group which shows a non-significant difference (P value >0.05). Natural infection with SARS-CoV-2 with or without Pfizer-BioNTech vaccination can significantly increase the titers of TNF-α and INF-γ in serum.

Keywords: COVID-19, SARS-CoV-2, Pfizer-BioNTech vaccine, TNF-α , INF-γ

Introduction:

The COVID-19 pandemic has resulted in a public health emergency that has the potential to cause substantial long-term social and economic consequences. Its spread has had terrible consequences in several countries. Much attention has been paid to the pathogenic mechanism of the highly contagious COVID-19 virus. The severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) virus is the source of COVID-19. The primary manner in which that SARS-CoV-2 is spread is by airborne respiratory droplets [1].

Tiny protein molecules known as cytokines are involved in crucial immunomodulatory functions and have the aim to facilitate communication between cells. However, in some viral disorders, severe inflammation causes uncontrolled proinflammatory cytokine production, which leads to a cytokine storm [2].

TNF-α is one of the innate immune response's three primary pro-inflammatory cytokines. During an innate immune response, the primary producers of these cytokines are tissue macrophages, mast cells, endothelium, and epithelial cells [3]. TNF-α has several pro-inflammatory effects, including the development of Antigen Presenting Cells (APCs), co-stimulation of T cells, activation of the germinal center (GC), and stimulation of the synthesis of immunoglobulins. Consequently, it's conceivable that inhibiting TNF-α could lessen the efficacy of immunization reactions [4].

Interferon (IFN-γ) and other pro-inflammatory cytokines are the host's first line of defense at the entry site, and observations have shown that SARS-CoV-2 suppresses this response in a manner that is correlated with the severity of the disease [5]. During an infection, the primary source of IFN-γ

production is natural killer (NK) cell ; however, CD8 lymphocytes, and APCs such as dendritic cells monocytes, and macrophages can also produce it [6]. Additionally, the COVID-19 vaccines were effective in boosting cellular immunity, specifically IFN generated by T-helper 1 and T-cytotoxic cells, which are specific to the SARS-CoV-2 virus. [7]. The delivery of the antigen that can stimulate the immune response is facilitated in part by vaccination. Natural immunity is modeled by the prime-boost or initial vaccination dose. T cytotoxic CD8+ cells will destroy virus-infected cells and T helper CD4+ cells will be stimulated. B-cell activation will then result in the production of particular neutralizing antibodies to the virus. The immune system's memory is strengthened by the second dosage, while its preparation is aided by the first [8].

This study is aimed to evaluate the immune response after 1 month of the Pfizer -BioNTech full vaccination, COVID-19 infection, and post-Pfizer-BioNTech COVID-19 infection by achieving the following objectives:

- The estimation of the TNF-α serum levels in each group.
- The estimation of the INF-γ serum levels in each grope.

Review of literature

Materials and methods

Sampling

It is a case-control study that was performed from the first of November 2022 until the thirteenth of January 2023.

Inclusion criteria

1- The selected participants should have received the Pfizer COVID-19 (BNT 162b2) 2nd dose vaccination before 30 days of sample collection.
2- The selected participants who have been infected with COVID-19 will be included at least before 30 days of sample collection.
3- Infected- vaccinated individuals will be included after 30 days of receiving the 2nd dose of Pfizer (BNT 162b2) vaccine.
4- Healthy individuals (non- infected, non-vaccinated) were also included as a control group.

Exclusion criteria:

The volunteers who have Otitis media, pharyngitis, urinary tract infections, and gastrointestinal infections, immune response problems or any type of chronic diseases (Diabetes Mellitus (DM), Ischemic Heart Disease (IHD), Hypertension (HT), or Asthma) over the past year have been excluded from the study.

According to the above inclusion and exclusion criteria one hundred twenty (volunteers) were involved in this study, in which were divided into four groups each containing thirty participants. Male to female ratio was (50:50), with an average age ranging between (20-45 years) as shown next. The study groups' individuals were divided into:

- The Control group (C) included thirty persons who were not infected previously with COVID-19 or were not vaccinated with any vaccine-related to the Coronavirus and also did not suffer from any type of diseases.
- The Pfizer -BioNTech Vaccinated group (V) included thirty persons who did not have COVID-19 infection previously but were vaccinated with the Pfizer-BioNTech vaccine and were selected one month after the second dose.
- The COVID-19 infected group (I) included thirty individuals who were selected after one month of COVID-19 infection and were not vaccinated.
- Infected after vaccination with Pfizer-BioNTech group (IV) included thirty individuals who were vaccinated with the Pfizer-BioNTech vaccine six months to one year after the date of the second dose, and have been infected with COVID-19 one month before sample collection.

Blood sample collection

According to the study requirements, five milliliters of blood were collected from all study group participants by vein puncture which was collected into a gel tube, that remained for about twenty minutes to allow the clotting blood at room temperature. Later, the tubes were centrifugated for 10 minutes at 5000 RPM to gain serum that kept into Eppendorf tubes to avoid contamination, freezing, and melting. ELISA technique was used to estimate the serum level of the following: TNF-α, and INF-γ.

Serology

Following the protocol of the manufactured company, an indirect ELISA test was done to assess serum levels of Human TNF-α, with catalog number (E0082Hu) and Human IFN-γ catalog number (E0105Hu) with a (Paramedical/Italy ELISA system) microplate reader to calculate the results. For the sandwich, TNF-α and IFN-γ ELISA kit, six standards were made according to the instructions of the manufacturing company to be used for quantification and analysis of serum TNF-α Levels (ng/L) and IFN-γ levels (ng/ml). Data were counted by determining of mean absorbance for each duplicated measurement, and concentration serum TNF-α Levels (ng/L) and IFN-γ levels (ng/ml) were plotted for each calibrator concerning the serum levels of TNF-α for human ELISA Kit and Human Interferon Gamma ELISA Kit to obtain the mean calculation.

Ethical approval

Ethically, this study was approved by the Aliraqia Medical College Review Board inside the Medical College/Al-Iraqia University (Ethic Review No. FM.SA/327). The participants provided written informed consent for participation in this study.

Statistical analysis

SPSS statistical software version 26.0 (SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analyses. One-way ANOVA to assess whether or not there are statistically significant differences. Moreover, to assess the important differences between percentages, chi-square was used. It is deemed statistically significant when $P < 0.05$.

Results

The study's participant (volunteers) consisted of 120 individuals who were divided into four groups, each of which contained 30 people. The male-to-female ratio was (50:50), and the participants' average ages ranged from 20 to 45 years.

The data showed a significant increase ($P<0.05$) in serum titers of TNF- α and INF-γ in all groups compared to the control.

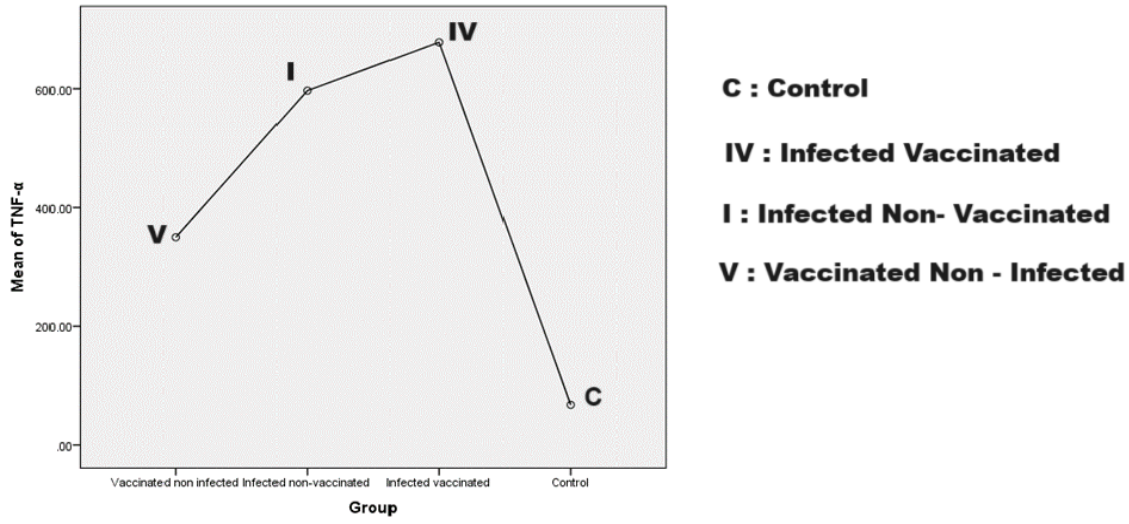

Figure (1) Serum levels of Tumor Necrosis Factor-alpha for all groups.

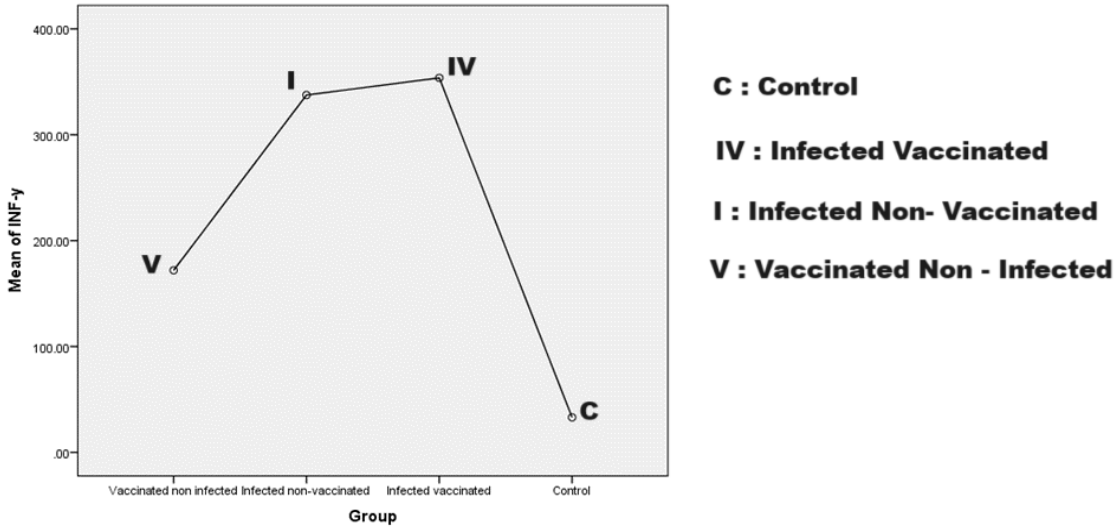

Figure (2) The serum level of Interferon Gamma in all groups.

The figures (1) and (2) showed that increase in serum levels of TNF- α and INF-γ in the vaccinated group more than the control group and in the infected group more than both the vaccinated and the control groups while the infected vaccinated group records the higher levels in all groups.

Furthermore, significant differences were seen in the outcomes (P value < 0.05) in the association between all groups except the association between the SARS-CoV-2 infected non-vaccinated (CoV) group with the vaccinated-SARS-CoV-2 infected (V-CoV) group which shows the non-significant difference (P value >0.05) as shown in tables (1) and (2).

Table (1) The association between serum levels of Tumor Necrosis Factor-alpha for all groups in the study.

Group		N	Mean	Std. Deviation	P value
TNF-α	Vaccinated noninfected	30	349.8310	± 116.60741	<0.0001*
	Infected non-vaccinated	30	596.8067	± 168.87016	
TNF-α	Vaccinated noninfected	30	349.8310	± 116.60741	<0.0001*
	Infected vaccinated	30	678.1390	± 165.19583	
TNF-α	Vaccinated noninfected	30	349.8310	± 116.60741	<0.0001*
	Control	30	67.6100	± 63.13880	
TNF-α	Infected non-vaccinated	30	596.8067	± 168.87016	0.06NS
	Infected vaccinated	30	678.1390	± 165.19583	
TNF-α	Infected non-vaccinated	30	596.8067	± 168.87016	<0.0001*
	Control	30	67.6100	± 63.13880	
TNF-α	Infected vaccinated	30	678.1390	± 165.19583	<0.0001*
	Control	30	67.6100	± 63.13880	

NS: Non-Significant, * (P< 0.05) Significant , ** (P< 0.0001) Highly Significant

Table (2) The association between of Serum levels of Interferon Gamma for all groups in the study.

Group		N	Mean	Std. Deviation	P value
INF-γ	Vaccinated noninfected Infected non-vaccinated	30 30	171.9730 337.5273	± 33.44775 ± 40.47955	<0.0001*
INF-γ	Vaccinated noninfected Infected vaccinated	30 30	171.9730 353.7350	± 33.44775 ± 32.35666	<0.0001*
INF-γ	Vaccinated noninfected Control	30 30	171.9730 33.0547	± 33.44775 ± 17.59750	<0.0001*
INF-γ	Infected non-vaccinated Infected vaccinated	30 30	337.5273 353.7350	± 40.47955 ± 32.35666	0.09NS
INF-γ	Infected non-vaccinated Control	30 30	337.5273 33.0547	± 40.47955 ± 17.59750	<0.0001*
INF-γ	Infected vaccinated Control	30 30	353.7350 33.0547	± 32.35666 ± 17.59750	<0.0001*

NS: Non-Significant, * (P< 0.05) Significant , ** (P< 0.0001) Highly Significant

Discussion

Globally, many individuals were vaccinated with the anti-SARS CoV-2 mRNA vaccine, known as Pfizer-BioNTech. The vaccine can decrease the intensity of the COVID-19 disease for those who received it, since it is founded on a genetically modified RNA that can generate a protein that resembles SARS-CoV-2 Anti-Spike S1 RBD [9]. When COVID-19 mRNA vaccines are injected, pro-inflammatory cytokines are strongly expressed and secreted, which is linked to a broad and varied activation of both immune and vascular cells.[10].

The first vaccination produced effects on serum cytokine/chemokine levels in antigen-naive individuals that were both immediate and more sustained. These effects were caused by activation of the innate immune system and inflammation. After the second vaccination, cytokine alterations were more widespread and significant, which further shows that anamnestic reactions were stimulated [11].

Systemic inflammation occurs in the human body after the first dose of the Pfizer vaccine, and TNF-α and IL-6 expression rises after the second dosage [11].

Some pro-inflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ, promote cell death in

different cell types. This cell death is associated with pathological diseases such as neurological disorders, liver damage, chronic obstructive pulmonary disease, osteoporosis, sepsis, and more. Through the direct destruction of lymphocytes, TNF-α and IFN-γ signaling may make lymphopenia worse. In fact, it has been observed that huge concentrations of pro-inflammatory cytokines, such as TNF-α, are present in the germinal centers of severe COVID-19 patients, which restricts the proper immune response[12].

Pro-inflammatory cytokine production rises in COVID-19 patients. TNF-α is produced in the respiratory tract by natural killer cells, dendritic cells, activated mast cells, lymphocytes, and monocytes/macrophages, which exacerbates the inflammatory condition. Acute respiratory distress syndrome (ARDS) is caused by a significant vascular effluence and oedema in the pulmonary micro-vasculature, this results from the cytokine pool associated with hyperactivated immune cells that is inflammatory [13] .

According to Baumgarth (2021) and Stone et al. (2019), IFN- γ is involved in regulating B cell-produced Ig isotypes and promoting the survival of antibody-secreting cells, which in turn shapes humoral responses [14],[15].

Early stages of the disease are protected by a variety of biomolecules, including IFN-γ, which is involved in a variety of pathophysiological mechanisms resulting from SARS-CoV-2 infection. IFN-γ release stimulates M1 macrophages and raises MHC antigen expression in macrophages, which helps deliver antigens to T cells and is crucial for the sustained cellular immune response [16].

This study revealed that TNF-α levels and IFN-γ levels in (the CoV group, V-CoV group, and V group) are higher compared to the (C) group (non-vaccinated, non-infected individuals), indicating that TNF-α and IFN-γ response is more prominent due to natural infection. V-CoV individuals generated higher TNF-α and IFN-γ than their V or CoV participants. This is agreed with another study by Mayyadah and Majid [9] who reported that the serum TNF-α level in a vaccinated individual with Pfizer-BioN Tech is higher than in a group of healthy individuals. Another study by Mai A. *et al* [2] supported our study which reported that the titers of TNF-α in the serum in of COVID-19 patients were significantly higher than those in healthy controls. Also, Mulchandani *et al*. [17] found that severe COVID-19 patients had significantly greater mean TNF-α levels than non-severe patients which agrees with our results.

Our study's outcomes, when paired with those of previous investigations, suggest that IFN-γ likely plays a significant role in both decrease the intensity of the COVID-19 disease and T-cell activation. SARS-CoV-2 infection and subsequent SARS-CoV-2 reinfection following vaccination [18].

Pfizer vaccine has shown specific stimulation for CD8+ and CD4+ T lymphocytes against SARS-CoV-2 and to enhance the production of immune-modulatory cytokines like interferon-γ (IFN-γ). Therefore, Dominguez-Andres et al evaluated the release of interferon-γ (IFN-γ) from peripheral blood mononuclear cells (PBMCs) in reaction to several SARS-CoV-2 strains both before to and following vaccination with BNT162b2 [19].

Conclusions

Natural infection with SARS-CoV-2 with or without Pfizer-BioNTech vaccination can significantly increase the titers of TNF-α and INF-γ in serum. Which are likely plays a significant role in both decreasing the intensity of COVID-19 disease and T-cell activation.

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