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Investigation of TNF-α and IL-4 in Pfizer/BioNTech vaccinated people: A comparative study

Mayyadah Rafeeq[1], Majid S. Jabir[2*]
[1,2]Department of Applied Sciences, Technology University/ Iraq
***Corresponding author:** Majid S. Jabir, Department of Applied Sciences, Technology University/ Iraq, Email: 100131@uotechnology.edu.iq

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ABSTRACT

Background: The necessity for a vaccine to prevent this disease has been made abundantly clear by the appearance of the new SARS-CoV-2 and COVID-19. The most reliable method of halting the spread of infectious illnesses is vaccination. Since they were first made available to the general public more than 200 years ago, vaccines have saved millions of lives.

Methods: There were eighty-one (81) participants in total in the study. Individuals ranged in age from 18 to 66 and had recently received COVID-19 mRNA Pfizer/BioNTech [BNT162b2] vaccination injections. They were given two injections of the vaccine of 30 g and 0.3 mL, twenty-one (21) days apart. Before the first vaccination, blood samples were collected. This procedure was repeated on days 7-10 after the first vaccination, and on days 7-10 after the second dose. All samples were tested for IL-4, and TNF-α using a High Sensitivity Human ELISA Kit corresponding to each marker (Elabscience/United State).

Results: There was no significant increase in IL-4 levels in all groups, TNF-α results showed increased after the first and second doses compared to before vaccination, and the increase after the second dose is greater than the first dose.

Conclusions: Our research demonstrated that vaccinations caused Th1 biases and prevented Th2 responses in all groups.

Keywords: *Covid-19, Pfizer/BioNTech, IL-4, TNF-α*

INTRODUCTION

In the latest human history, COVID-19 is a pandemic of absolutely huge magnitude. Nearly 200 million confirmed cases and four million fatalities have occurred globally in less than 18 months since the epidemic began. Because of its contagiousness, the large number of patients who come with serious illnesses, and an increased risk of mortality, necessitating specialist medical treatment in intensive care units (ICU), its growth has had catastrophic impacts in many nations [1]. A newly discovered, enveloped, nonsegmented, 30-kilobase positive-sense RNA virus of worldwide relevance is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a member of the family Coronaviridae's subfamily Orthocoronavirinae (group betacoronavirus) [2]. SARS-CoV-2 virions are spherical in form and range in diameter from 65 to 125 nm, like other coronaviruses [3] and perhaps the most noticeable characteristics are the spike projections that emerge from the virions' surfaces [4].

Their envelope includes the spike (S), membrane (M), an envelope (E), proteins, and nucleocapsid (N) protein, which surrounds the RNA, inside the virion [5]. SARS-CoV-2 combines significant pathogenicity (i.e., the ability to cause sickness) with high infectiousness. The reality that viral spread can occur in asymptomatic and presymptomatic people makes this infectivity worse. SARS-CoV-1 and MERS-CoV, on the other hand, were spread by symptomatic individuals and it could, therefore, be contained most effectively [6,7]. The "cytokine storm" event, which is brought on by the excessive production of pro-inflammatory cytokines, is associated with the COVID-19 infection. An unregulated immune response that results in ongoing activation and expansion of immune cells including lymphocytes and macrophages, as well as their production of vast quantities of cytokines, is the cause of CS. Proinflammatory cytokines such as IL-1, IL-6, IL-18, IFN-γ, and TNF-α are thought to be responsible for the clinical symptoms associated with CS [8]. According to several research studies on the cytokine profiles of COVID-19 cases, the cytokine storm was directly associated with lung damage, multiple organ failure, and a poor prognosis for those with acute COVID-19 [9–11]. Despite the fact known that COVID-19 primarily presents as a respiratory tract infection, The gastrointestinal, cardiovascular, neurological, hematopoietic, and immunological systems are among the many organs that COVID-19 affects since it behaves like a systemic illness. The development of technology that increased the capacity to generate innovative vaccines in the middle of the 20th century raised the possibility for immunization to new heights. The safest, most efficient, and cost-effective method of pandemic control is vaccination. Vaccines work by triggering the body's natural immunological response [12]. As soon as the Chinese government published the genetic sequence of the new coronavirus in early 2020, vaccine-manufacturing businesses all over the world jumped into action. Among the top brands are Sinovac, CanSino, AstraZeneca, and Moderna from the United States and a vaccine developed jointly by the United States and Germany by Pfizer-BioNTech. A novel method for creating SARS-CoV-2 vaccines is messenger RNA (mRNA) [13]. Here, we identified the IL-4 and TNF-α levels after the 1st and 2nd doses of the BNT162b2 mRNA vaccine (Pfizer/BioNtech) in vaccinated participants with diseases (hypertension, diabetes, and individuals with hypertension, diabetes, and heart disease) in comparison with healthy participants.

MATERIALS AND METHODS

Study Design and Participants

Eighty-one (81) subjects who had recently received mRNA Pfizer/BioNTech [BNT162b2] vaccinations were included in the current investigation. Samples from individuals between the ages of 18 and 66 who had previously received two doses of the vaccine spaced 21 days apart at a dose of (30 µg, 0.3 mL) were utilized in this exploratory investigation. The participants were divided into four groups, first the healthy subjects, the second with hypertension, the third with diabetes, and finally those with hypertension, diabetes, and heart disease. Between October 2021 and March 2022, vaccinations were administered to all participants. Blood samples were gathered in the manner previously mentioned. Samples collected at baseline (before the first vaccination), (Day 7-10, after the first vaccination), and (Days 7-10, after the second vaccination), were analyzed. All subjects provided both spoken and written informed consent.

Specimen Collection and Preparation

A. Specimen collection

Participants' serum samples were separated from blood samples in serum collection tubes by centrifuging at 1,000–2,000 x g for 10 min. and serum fractions were meticulously collected and frozen till use. Before analysis, frozen specimens were thawed at room temperature for 1 hour. Vortexing was done on thawed samples before analysis. Parallel experiments were performed to analyze stored samples from various time periods of the same donor.

B. ELISA technique

Two important markers were measured, including IL-4 and TNF-α. Levels of markers were measured by using High Sensitivity Human ELISA Kit corresponding to each marker (Elabscience/United State). Briefly, 50µL of standard or sample was added to each well and incubated for 90 minutes at 37℃. Then, the liquid was removed and 50µL of biotinylated detection Ab was added and incubated for one hour at 37℃. Next, the solution is aspirated from the wells and washed three times. Then, 50µL of HRP Conjugate was added and incubated for 30 minutes at 37℃. After that, the solution was aspirated from the wells and washed five times. Then, 50µL of substrate reagent was added and incubated for 15 minutes at 37℃. Finally, 25µL of stop solution was added and the OD value was determined at 450 nm immediately.

Statistical analysis

The data were analyzed using GraphPad Prism 7 [14]. Results are represented as mean± SD [15].

RESULT

The mRNA Pfizer/BioNTech [BNT162b2] COVID-19 vaccine was administered to eighty-one people. The age of the mRNA Pfizer/BioNTech groups as a whole varied from (18 to 66); 69 were women, and 12 were males. Vaccinated individuals included 45 healthy individuals, 15 with hypertension, 12 with diabetes, and 9 with hypertension, diabetes, and heart disease. IL-4 and TNF-a were analyzed for all participants (before the first vaccination), (Days 7–10, after the first vaccination), and (Days 7–10, after the second vaccination) using High Sensitivity Human ELISA Kit corresponding for each marker (Elabscience/United State) as shown in Figures 1, 2, 3, and 4 respectively. All results are displayed by GraphPad Prism 7.

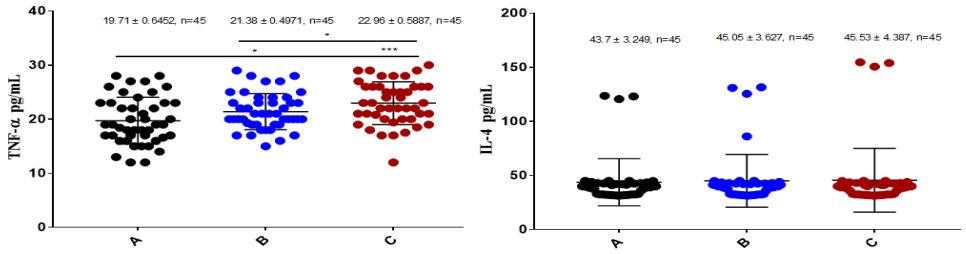

FIGURE 1: Dynamic changes in TNF-α and IL-4 levels in healthy Pfizer/BioNTech vaccinated people. A: Before the first vaccine, B: after (7-10 days) of the vaccine dose, C: after (7-10 days) of the second vaccine dose.

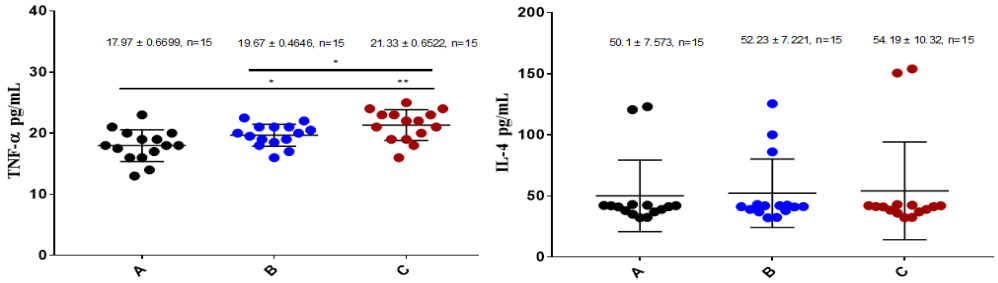

FIGURE 2: Dynamic changes in TNF-α and IL-4 levels Pfizer/BioNTech vaccinated people with hypertension. A: Before the first vaccine, B: after (7-10 days) of the vaccine dose, C: after (7-10 days) of the second vaccine dose.

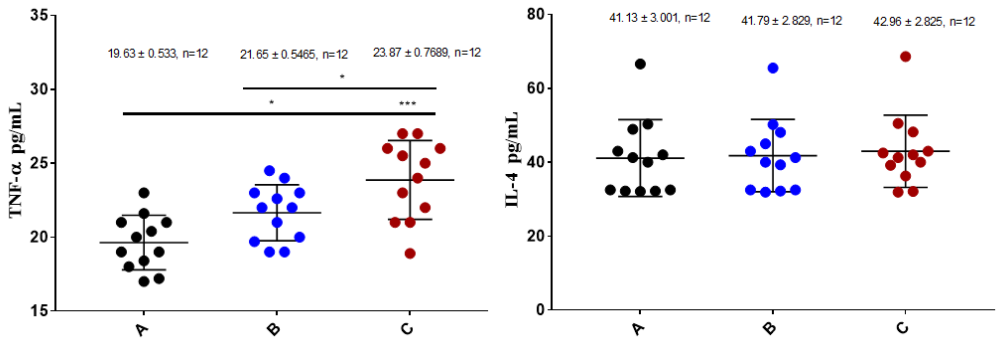

FIGURE 3: Dynamic changes in TNF-α and IL-4 levels in Pfizer/BioNTech vaccinated people with DM. A: Before the first vaccine, B: after (7-10 days) of the vaccine dose, C: after (7-10 days) of the second vaccine dose.

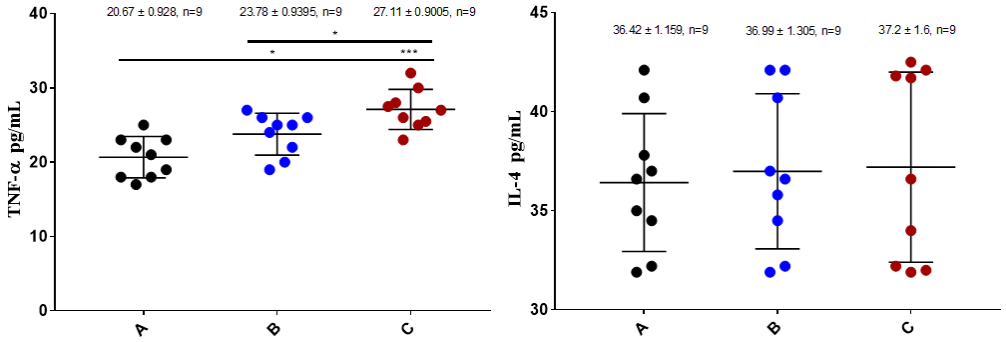

FIGURE 4: Dynamic changes in TNF-α and IL-4 levels in Pfizer/BioNTech vaccinated people with hypertension, and DM, and heart disease. A: Before the first vaccine, B: after (7-10 days) of the vaccine dose, C: after (7-10 days) of the second vaccine dose.

DISCUSSION

Many businesses are attempting to produce reliable and safe vaccines since the development of the COVID-19 vaccine is regarded as an essential and critical part of the worldwide efforts to manage this Pandemic [16,17]. Anti-SARS CoV-2 mRNA a breakthrough vaccine called BNT162b2 is being used to immunize countless numbers of individuals worldwide. It is based on a genetically modified RNA that can produce a protein in the treated people that triggers an immune response, providing the recipients of the vaccine with immunity versus SARS-CoV-2. Interleukin-4, which is primarily linked to fibrogenic inflammatory remodeling, plays an important function in the Th2 pathway, whereas Th1 cells show anti-fibrotic action by excreting gamma interferon (IFN-gamma) and interleukin 2 [18]. Given that acute COVID-19 can cause widespread alveolar destruction, which might result in causing septal fibrosis; Elevated concentrations of IL-4 in recovered individuals may lead to pulmonary fibrosis, which worsens lung function frailty. It is widely common that chronic inflammatory illnesses such as atopic dermatitis (AD), asthma, and allergic rhinitis may be caused by excessive production of Th-2 cytokines [19]. According to a report by Lu et al., COVID-19 patients had considerably greater serum levels of IL-4 than non-COVID-19 patients [20]. The higher serum levels of IL-4 may make pathological conditions worse given the significant morbidity and death rate connected to COVID-19 infections. In critically ill patients, the Th2 profile cytokine IL-4 shows an increased tendency toward illness progression [21]. IL-4 concentrations may (i) prevent immature CD4+ cells from developing into Th1

cells or (ii) suppress IFN-γ gene transcription. SARS-CoV-2 also appears to promote the release of Th-2 cytokines such as IL-4 and IL-10 that suppress Th1/Th17-mediated inflammation [22]. Our data showed no increase in IL-4 levels after vaccination in any participant group, as shown in Figures (1), (2), (3), and (4) respectively. T cells are one significant immune cell type that may be stimulated by a virus and start a cytokine storm. T cells proliferate and differentiate into distinct effector T cells after being activated by antigen-presenting cells in order to eliminate infections by generating a variety of pro-inflammatory cytokines, including IFN-γ, TNF-α, and IL-6 [22]. ICU and non-ICU patients had higher serum levels of TNF-α than the group of healthy people ($p<0.0001$) [23]. Numerous investigations revealed that people with more severe COVID-19 illness had higher TNF-α levels [24,25]. One research discovered that TNF-α and IFN- γ work in synergy to cause inflammatory cell death and higher mortality in SARS-CoV-2 infection [26], drawing attention to the destructive impact of cytokine storms in the wake of the recent worldwide COVID-19 epidemic. An increase in TNF-α may encourage viral infection and organ destruction [27]. Serious COVID-19 illness is reported to be a risk for developing the chronic obstructive pulmonary disease (COPD) [27]. In COPD, circulating TNF levels are elevated [28]. There is evidence for TNF participation in a very similar condition called idiopathic pulmonary fibrosis, even though the function of TNF in this process has not yet been identified [29]. TNF has well-documented impacts on the cardiovascular system. TNF has direct, detrimental inotropic and pro-apoptotic effects on cardiomyocytes, along with other pathways, which greatly contribute to the development of heart failure. Patients with hypertension also have higher levels of TNF. TNF and IL-6 concentrations could each function as a separate predictor of COVID-19 intensity. Furthermore, just TNF values were, not IL-6 values, great in COVID patients who also had diabetes, hypertension, kidney disease, and chronic heart failure, giving compelling evidence that TNF is a major contributing cause to these patients' severe condition [30]. The strong correlation between TNF and aging may partially account for the greater occurrence of acute COVID-19 illness in elderly people. Each of these conditions increases the chance of developing severe COVID-19 illness and its long-term consequences or death [30]. The results showed a significant increase after the two doses compared to before the vaccine, and a rise was also detected in all groups after the second dosage compared to after the first dose, as shown in Figures (1), (2), (3), and (4) respectively.

CONCLUSIONS

The vaccine polarizes the T-cell response towards type 1 immunity by inducing TNF-α production while avoiding stimulation of IL-4 cytokines that induce T-helper 2 immunity.

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