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IgG Titer of Pfizer BNT62b2 COVID-19 Negatively Correlated with Soluble Inhibitory Immune Checkpoints

Selda S. Ezzaldeen[1] *PhD*, **Haider S. Kadhim**[2] *PhD,* **Mohammed A. Tawfiq**[1] *MSc*

[1]Kirkuk Health Department, Public Health Laboratory, Kirkuk, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background The Corona virus disease 2019 (COVID-19) is a complicated respiratory syndrome caused by novel Corona virus that spread swiftly throughout the world.

Objective To study correlation between IgG spike protein-1 and soluble inhibitory immune checkpoints and among checkpoints in individuals vaccinated with booster dose of BNT162B2 vaccine.

Methods This research is cross-sectional, performed on 180 healthy adults (above 18 years old) vaccinated with BNT162B2 vaccine, 21-30 days after booster dose at community dwelling from December 2021 to April 2022. Measuring serum level of IgG toward spike protein-1 and soluble negative regulatory markers by enzyme-linked Immunosorbent assay.

Results This study showed a significant negative correlation between IgG titer and soluble inhibitory immune checkpoints, soluble CTLA-4 (r = -0.23, P value = 0.001), soluble PD-1 (r = -0.12, P value = 0.04) and soluble PD-L1 (r = -0.204, P value = 0.006), while Inhibitory immune checkpoints had a positive significant correlation among them (p value <0.001).

Conclusion This study showed a significant negative correlation between IgG titer of Pfizer BNT62b2 COVID-19 and soluble immune checkpoint markers and a moderate positive highly significant correlation among soluble inhibitory immune checkpoints.

Keywords BNT162b2, soluble inhibitory immune checkpoints, anti-S1 IgG response, mRNA vaccine

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List of abbreviations: COVID-19 = Corona virus disease 2019, sCTLA-4 = Soluble cytotoxic T-lymphocyte antigen-4, sPD-1 = Soluble programmed cell death-1, sPD-L1 = Soluble programmed cell death-ligand1

Introduction

Novel Corona disease is the illness of the multiple organs mainly "respiratory tract" caused by the virus belong to the Coronaviridae family [(1)]. The primary manifestations of this disease are respiratory symptoms and transmission through air droplets. Corona virus disease 2019 (COVID-19), on the other hand, exhibits a clinically heterogeneous manifestation that can range from a symptomless manifestation to severe symptoms that lead to numerous organ failure [(2)]. One way to stop the pandemic is a vaccination [(3)]. Several vaccines have been developed to mitigate its spread and prevent adverse consequences of the COVID-19 [(4)]. The success of a vaccine-induced immunization is influenced by several parameters regulating our immune response. One of the main factors for the variable vaccine responsiveness is the genetic variability among individuals within any given population [(5)]. The Pfizer/BioNTech

vaccine candidate, developed with Germany's BioNTech, is mRNA vaccine based on a relatively new technology, which use a piece of genetic code, messenger RNA (mRNA) for the spike protein of the SARS-CoV-2 [6]. The angiotensin-converting enzyme2 (ACE2) receptor on type 2 alveolar cells requires the S protein for virus entry, and this vaccine enables the body to mount an immunological response to get rid of the virus [7]. Inhibitory check points proteins have crucial role in self-tolerance, suppress immune response to protect healthy cells from destroying discriminately [8]. The most important diagnostic feature in individuals with severe COVID-19 infection is lymphocyte depletion, most importantly, SARS-COV-2-specific CD4+ and CD8+ T-cells. Based on the available studies, there is a possible relationship between disease severity and increased expression of some inhibitory receptors and its ligands, including soluble cytotoxic T-lymphocyte antigen-4 (sCTLA-4), soluble programmed cell death-1 (sPD-1), and soluble programmed cell death-ligand1 (sPD-L1); these receptors when bound to their ligands on antigen-presenting cells, send inhibitory signals to T cells, decreasing their activation, maturation, and proliferation through the reduction in IL-2 production [9]. Immune checkpoint inhibitors (ICIs) are drugs used to treatment of various type of cancers by blocking inhibitory immune check points proteins [10]. Using ICIs in combination with approved or experimental vaccines has proven to be a promising approach to improve vaccine immunogenicity and efficacy [11].

This study aimed to study correlation between IgG spike protein-1 and soluble inhibitory immune checkpoints and among checkpoints in individuals vaccinated with booster dose of BNT162B2 vaccine.

Methods

Design of the Study

This cross-sectional study was done in Baghdad, the capital of Iraq. The study was carried out according to the Institutional Review Board's (IRB) ethical approval on 2021/12/05 under the number 20211053 at College of Medicine, Al-Nahrain University. Before participating in the study, each subject provided informed consent and had their information obtained through a direct conservation using a questionnaire form with the following information: demographic characteristics, date of the second dose of vaccine, date of sampling, their mobile numbers, and if they are immune-compromised or have a history of COVID-19 infection.

Study population and sample collection

One hundred and eighty (180) healthy adults vaccinated with BNT162b2 (mRNA) vaccine over the age 18 years were enrolled in this study 21-30 days after the booster dose. Three milliliters of blood sample was collected between December 2021 and March 2022 through community dwellings. The serum separated and kept at -20°C to be used in serological testing.

Serological examination

The serum level of the anti-S1 IgG for all participants has been measured by using indirect enzyme-linked immunosorbent assay technique (ELISA). All that was done depending on the manufacturer's instructions, MyBioSource/USA Company. Sandwich ELISA technique has been used to measure serum level of inhibitory immune checkpoints (cytotoxic T-lymphocyte antigen-4, programmed cell death-1 and programmed cell death-ligand1) for all participants. The procedure was carried out in accordance with the manufacturer's instructions, BT LAB/China Company.

Statistical analysis

Version 16 of the statistical package for social science (SPSS) program was used for all statistical analyses. Pearson's correlation test employed to investigate the likelihood of a correlation between inhibitory checkpoint

markers and age or IgG titer or among inhibitory markers.

Results

Correlation between soluble inhibitory immune checkpoint markers and IgG titer

Pearson's correlation test was employed to explore the possible correlation between the IgG titer and inhibitory immune checkpoint markers. IgG titer demonstrated a negative correlation with each of soluble CTLA-4 (r = -0.23, P = 0.001), soluble PD-L1 (r = -0.204, P = 0.006) and soluble PD-1 (r = -0.12, P = 0.04) as shown in table (1).

Table 1. Pearson's correlation between soluble inhibitory immune check point markers and IgG titer

Variables	sCTLA-4		sPD-1		sPD-L1	
	r	P value	r	P value	r	P value
IgG titer	-0.23	0.001**	-0.12	0.04*	-0.204	0.006**

* Statistically significant, ** statistically highly significant

Correlation among inhibitory immune checkpoint Markers

Inhibitory immune checkpoints had a highly significant positive correlation among them; as sCTLA-4 had significant positive correlation with sPD-1 (r = 0.244, P value <0.001), and with sPD-L1 (r = 0.568, P value <0.001), also sPD-1 had significant positive correlation with sPD-L1 (r = 0.29, P value <0.001) as shown in table (2).

Table 2. Pearson's correlation among inhibitory immune check point markers

Variables	sPD-1		sPD-L1	
	r	P value	r	P value
sCTLA-4	0.244	<0.001**	0.568	<0.001**
sPD-1	-	-	0.29	<0.001**

** Statistically highly significant

Correlation between inhibitory immune checkpoint markers and age

Pearson's correlation was employed to investigate the correlation between the age and the inhibitory immune molecules. Age showed a negative non-significant correlation with each of soluble CTLA-4 (r = -0.037, P = 0.625) and soluble PD-L1 (r = -0.06, P = 0.426), but soluble PD-1 (r = 0.013, P = 0.862) demonstrated a weak positive non-significant correlation with age as shown in table (3).

Table 3. Pearson's correlation between age and soluble inhibitory immune check point markers

Variables	sCTLA-4		sPD-1		sPD-L1	
	r	P value	r	P value	r	P value
Age	-0.037	0.62^{NS}	0.013	0.86^{NS}	-0.060	0.42^{NS}

NS: Statistically non-significant

Discussion

The results of this study demonstrated a highly significant negative correlation between IgG titer of Pfizer BNT62b2 COVID-19 with soluble inhibitory immune checkpoint molecules CTLA-4, PD-1 and PD-L1. Low levels of serum CTLA-4, PD-1 and PD-L1 associated with high level of anti-S1 IgG following vaccination. However, this result was expected, and it indicated that inhibitory immune checkpoints have an important role in immune response after BNT162b2 vaccination, which is agreed with recent study has been demonstrated that the use of ICIs in combination with approved or experimental vaccines has proven to be a promising approach to improve vaccine immunogenicity and efficacy [(11)], another study showed that the cell-mediated immunogenicity of influenza vaccine robust in cancer patients receiving ICIs [(12)].

The presented study revealed a moderate positive highly significant correlation among soluble inhibitory molecules, this result agrees with research said that the strong positive significant correlation among studied inhibitory molecules [(13)]. PD-1 marker inhibits T cells later in an immunological response, largely in peripheral lymphoid tissues, CTLA-4 controls T-cell proliferation early in an immunological response, mainly in lymph nodes. The argument in the clinic for examining immune checkpoint combinations is supported by immuno-oncology medications' clinical profiles blocking these two checkpoints simultaneously may result in unique patterns of immune activation [(14)].

In addition, this study showed a negative non-significant correlation between age and soluble inhibitory immune checkpoints. This result agrees with Alameri and Kadhim, (2022) who reported that age not significantly associated with the immune response to the BNT162b2 vaccine [(15)], also this result agree with study that didn't find any significant difference in IgG concentration between persons younger and older than 25 years who were vaccinated with Sinopharm and Pfizer vaccines, whereas there is a significant difference between the two age groups in participants vaccinated with the AstraZeneca vaccine [(16)], while this result disagrees with Jabal et al. (2021) who reported that ethnicity and age might be significantly associated with the immune response to BNT162b2 vaccine (anti-SARS-CoV-2 spike IgG antibodies titer decrease with age) [(17)]. The study's restriction to persons under the age of 60 years may minimize the effect of age on serum levels of inhibitory immunological checkpoints [(15)].

In conclusion, this study showed significant negative correlation between IgG titer of Pfizer BNT62b2 COVID-19 with soluble immune checkpoint markers CTLA-4, PD-1 and PD-L1. Also, showed non-significant negative correlation between age and soluble immune checkpoint markers. Additionally, current study identified a moderate positive highly significant correlation among soluble inhibitory immune checkpoints.

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Author contribution

Dr. Ezzaldeen and Tawfiq contributed to the preparation of the manuscript. Dr. Kadhim contributed to the revision of the manuscript.

Conflict of interest

There are no conflicts of interest

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Correspondence to Selda S. Ezzaldeen
E-mail: seldaammar18@gmail.com
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