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Original Article

Assessment of Some Immunological Markers in Patients and Vaccinated Individuals with COVID-19 in Kirkuk city

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Abstract

Background: Infection with coronavirus disease-2019 (COVID-19) can trigger both innate and adaptive immune responses, resulting in large inflammatory reactions later in the disease. The initiation of immunological responses entails a complicated interaction between innate immune components, which quickly respond in a nonspecific manner, and specialized components of the immune system can recognize specific epitopes of antigens. **Objective:** The objective of this study was to assess some co-stimulating molecules in patients with COVID-19 (hospitalized and nonhospitalized) and vaccinated individuals compared with a control group in Kirkuk city. **Materials and Methods:** The immunological markers under study in which our methods tried to estimate them are CD28, CD80, and CD86. From 90 individuals of patients with COVID-19, vaccinated persons, and control group blood samples were collected and centrifuged to get the serum to carry out the immunological analysis. Through using nasopharyngeal swabs that were collected from non-hospitalized patients (patients out of the hospital), coronavirus infection was confirmed by polymerase chain reaction (PCR). Additionally, PCR tests were run on the control group to make sure they were not infected with COVID-19. **Results:** For the vaccinated group especially in comparison to COVID-19 patients, the revealed significant differences in the immunological markers among tested groups with respect to the CD28 test with (P value > 0.0001) and CD80 test with (P value > 0.0001), as well as the CD86 test appears to show a significant difference with (P value > 0.0001). **Conclusion:** This study revealed that, compared to patients with COVID-19 who were not given the vaccine, the vaccine had a role on those who received it and significantly increased some immunological markers..

Keywords: Patients with COVID-19, vaccinated individuals and immunological markers (CD28, CD80 and CD86)

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease-2019 (COVID-19). COVID-19 infection can occur in a variety of signs ranging from asymptomatic to severe enough to necessitate hospitalization. The virus spreads widely due to its simplicity of transmitting from one person to another.[1,2] Although little of the immune interaction is known about people who are asymptomatic or have a mild infection and do not require hospitalization, Recent studies have demonstrated the critical role that immune responses which are already induced against viral particles in hospitalized patients. Adaptive immune responses, primarily T cells, play a key role in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, as they do in other respiratory viral infections.[3-9]

One prominent feature of COVID-19 disease is lymphopenia during early infection,[7,10-12] whereas when patients convalesce it is back normalized.[8,11] CD4+ (T cells), CD8+ (T cells), B cells, and natural killer cells have all been shown to be affected by lymphopenia in some patients.[7,8] Innate and adaptive immunity are two main branches of the immune system that are necessary for the initiation and progress of inflammatory diseases.[13,14]

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In general, two different signals are required to drive T-cell clonal proliferation, cytokine production, and effector function in naive CD4 T cells. The T-cell receptor (TCR) interacts with a processed antigenic peptide supplied by a syngeneic major histocompatibility molecule to produce an antigen-specific signal. The interaction of T-cell surface molecules with co-stimulatory molecules produced on antigen-presenting cells generates a second signal (antigen-presenting cell [APC]).[15-17] B7-1 (CD80) and B7-2 (CD86) are effectively co-stimulatory molecules; they are ligands for the T-cell membrane proteins CD28 and CTLA-4, respectively, and have been identified as significant determinants of professional APC that play a key role in CD4-T-cell activation in humans.[18] CD28 is a crucial co-stimulatory receptor for optimal T-cell activation and differentiation. CD28 binds with B7-1 (CD80) or B7-2 (CD86) on the surface of professional APCs. The immunization process is one of the most effective and economical medical interventions for preventing infectious diseases. Through vaccination, COVID-19 can be controlled and avoided.[19] Therefore, the study tries to get the clinical value and differences of these immunological markers.

Materials and Methods

Study design

As part of the case-control study design of our study, specimens from COVID-19 patients—whether or not they required hospital admission, vaccinated individuals and the control group were collected.

Setting and time of the study

The study was conducted on inpatients at Al-Shifa-14 Hospital and Azadi Teaching Hospital, while the outpatients were in home quarantine, and samples were taken from them. The ones who had received vaccinations were taken from hospital workers and placed outside.

Study samples and methods

In this investigation, 90 samples overall, including 25 COVID-19 patients who were hospitalized and 25 non-hospitalized, as well as those who received the SARS-COV-2 vaccination and a healthy controls, were used. From outpatients and control group, nasopharyngeal swab samples were taken to perform a polymerase chain reaction (PCR) test (real-time PCR).

Blood samples were taken from each group under study and incubated in the gel tubes that were centrifuged to obtain serum. Sera were collected to use in three immunological ELASA kits involving human cluster of differentiation (CD28, CD80, and CD86) (Sunlong Biotech Company for Research, China).

Calculation of CD28 results

To represent known concentrations of human CD28 standard and the related reading OD, the log scales (*y*-axis and *x*-axis) were used. The concentration of human CD28 in the sample was calculated by plotting the sample's OD, on the *X*-axis as indicated in Figure 1. The initial concentration was determined using the statistician's equation and the standard curve.

Calculation of CD80 and CD86 results

The log scales (*x*-axis and *y*-axis) were used to show known concentrations of Human CD80 and CD86 standard and the related reading OD. As shown in Figures 2 and 3, the concentrations of Human CD80 and CD86 in the samples were measured by graphing the sample's OD on the *Y*-axis. Using the statistician's equation, the exact concentration was estimated from the standard curve.

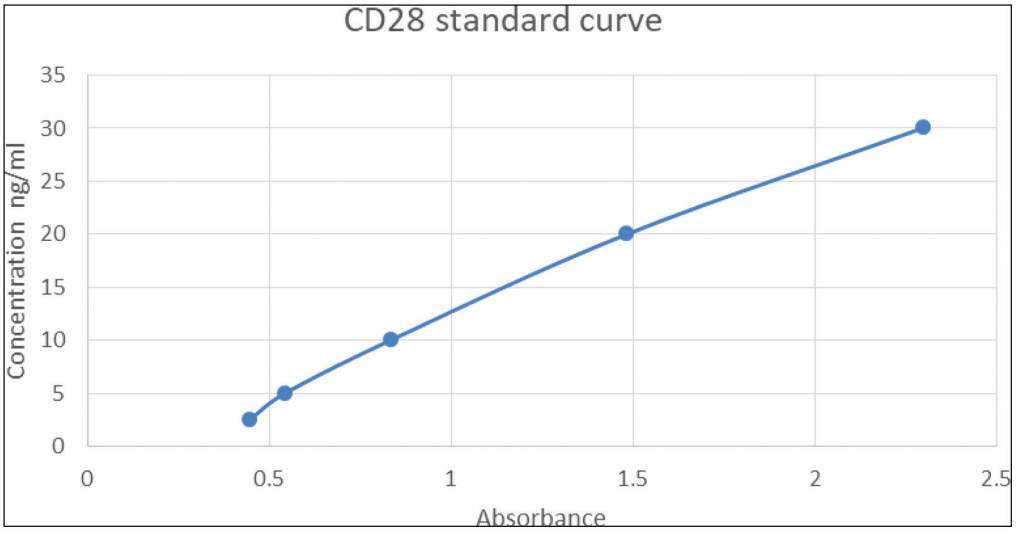

Figure 1: Standard curve of the human CD28 standard

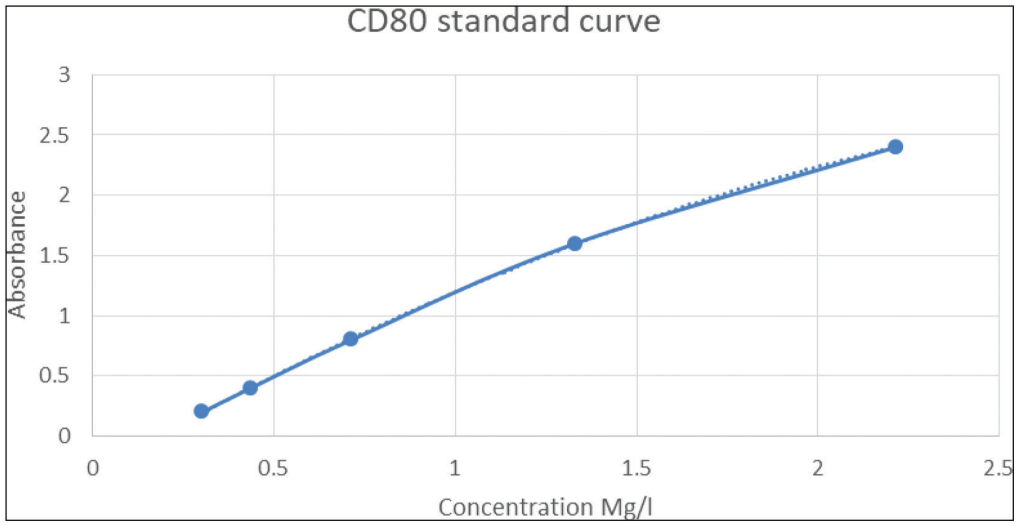

Figure 2: Standard curve of the human CD80 standard

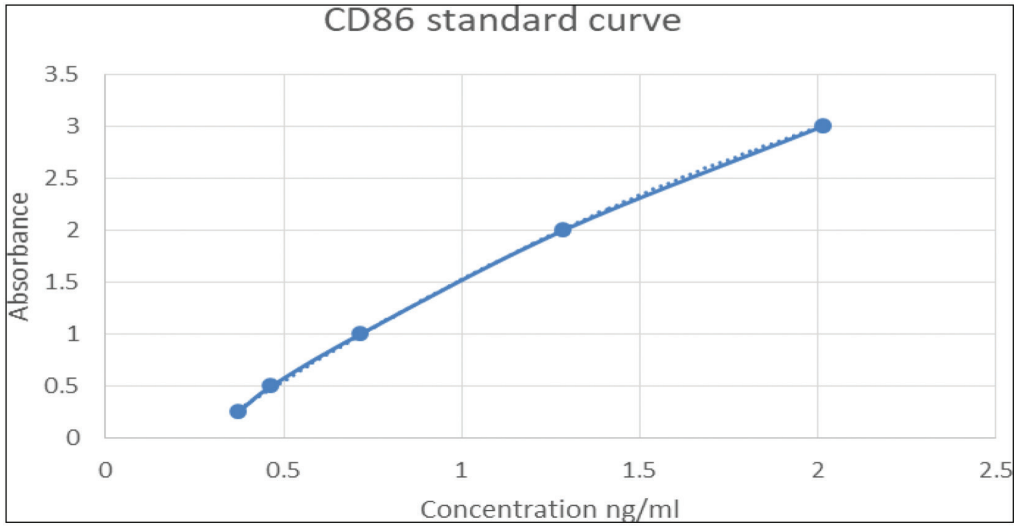

Figure 3: Standard curve of the human CD86 standard

Statistical analysis

Statistical analysis was performed using GraphPad Prism statistical software.

Ethical approval

The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 3657 (including the number and the date of November 11, 2021) to get this approval.

Results

This study involved the assessment of some immunological markers in four different groups: patients with COVID-19 (hospitalized and nonhospitalized), vaccinated , and control group. COVID-19 was found to be positive in 50 of the 90 subjects; 25 of them were in the hospital and 25 of them were outpatients. A total of 20 people were vaccinated, with a control group of 20 people. Table 1 shows percentages of gender, age, and comorbidity conditions that represent common characteristics of all groups included in the study. When it came to age, we divided it into two categories: 30 years or below (55.5%) and 31 years or above (44.4%). When it came to chronic diseases, we distributed them into two categories: those

Table 1: Common characteristics of tested groups

Demographic variable		%
Gender	Male	37.7
	Female	62.2
Age	30 years or less	55.5
	31 years or more	44.4
Comorbidities	Yes	22.2
	No	77.7

who have them (22.2%) and those who do not (77.7%). The percentage of females in the entire group was 62.2%, but the percentage of men was 37.7%.

With $P < 0.0001$, there was a significant difference between the four examined groups when it came to the CD28 test. Figure 4 shows a significant increase in CD28 in vaccinated patients (mean = 51.72) as compared with hospitalized patients with COVID-19 (mean = 8.5), nonhospitalized patients with COVID-19 (mean = 7.236), and the control group (mean = 13.32). There was also a significant difference in CD28 between the nonhospitalized and control groups, but no difference was observed between the hospitalized and nonhospitalized groups.

Relating the CD80 test, Figure 5 illustrates a significant difference respectively four groups with a P value < 0.0001. The vaccinated group (mean = 4.7) indicates a significant increase compared to the other three groups of hospitalized patients with COVID-19 (mean = 0.8003), nonhospitalized patients with COVID-19 (mean = 0.916), and the control group (mean = 0.8217), while no substantial change was seen between the latter groups.

About the CD86 test, Figure 6 shows a significant difference between four tested groups with $P < 0.0001$, where the vaccinated group (mean = 6.922) show a significant rise as compared with the three groups hospitalized patients with COVID-19 (mean = 1.133), nonhospitalized patients with COVID-19 (mean = 1.448), and control group (mean = 1.308) and no significant difference was observed between later groups.

Discussion

In order to investigate their relationship with COVID-19 among the groups under study (patients with COVID-19, individuals who had received vaccinations, and controls), it was attempted in this study to assess the proportion of some co-stimulatory molecules, which would include

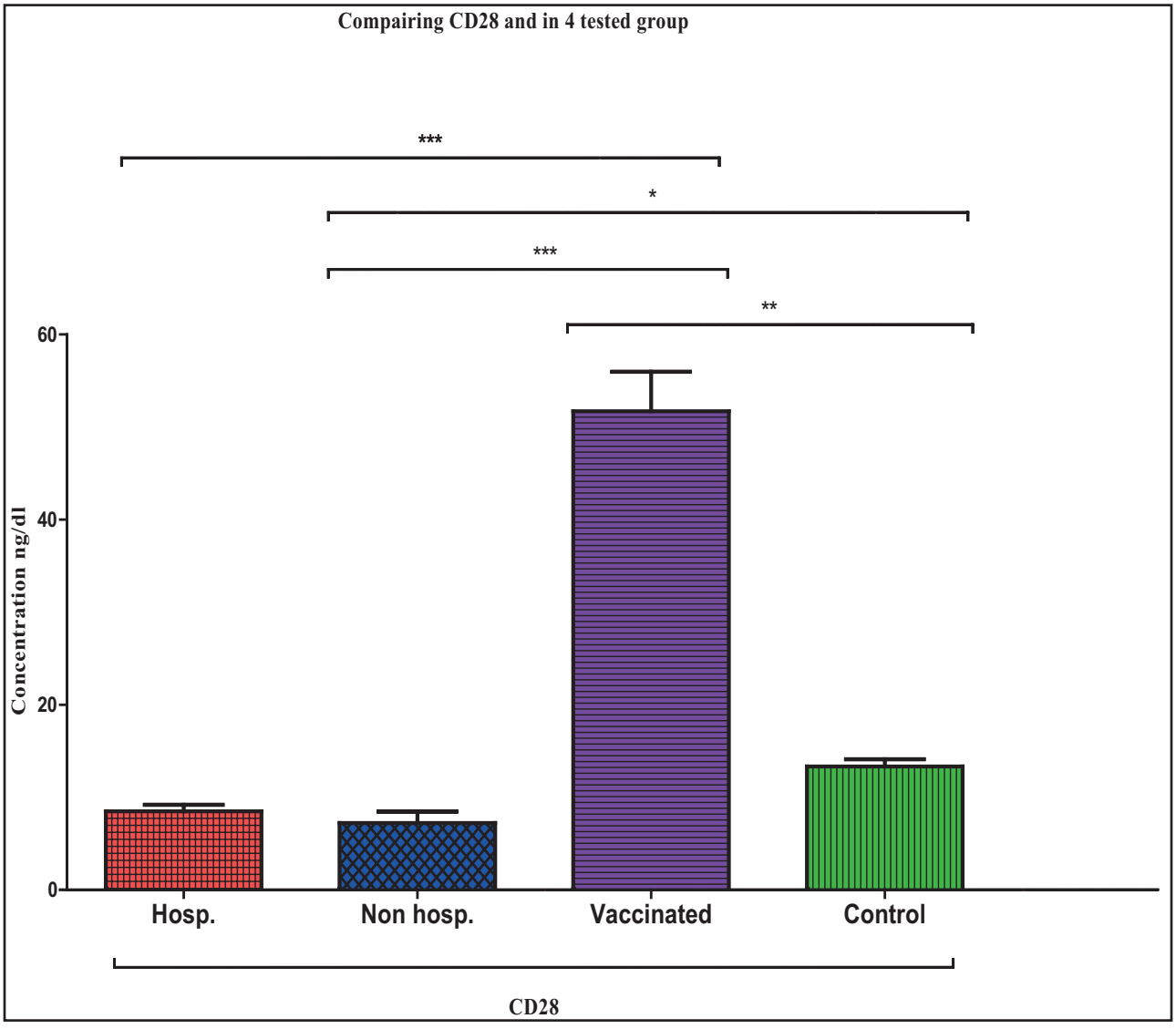

Figure 4: CD28 concentration in four tested groups using one-way ANOVA test with a significant difference between them ($P < 0.0001$)

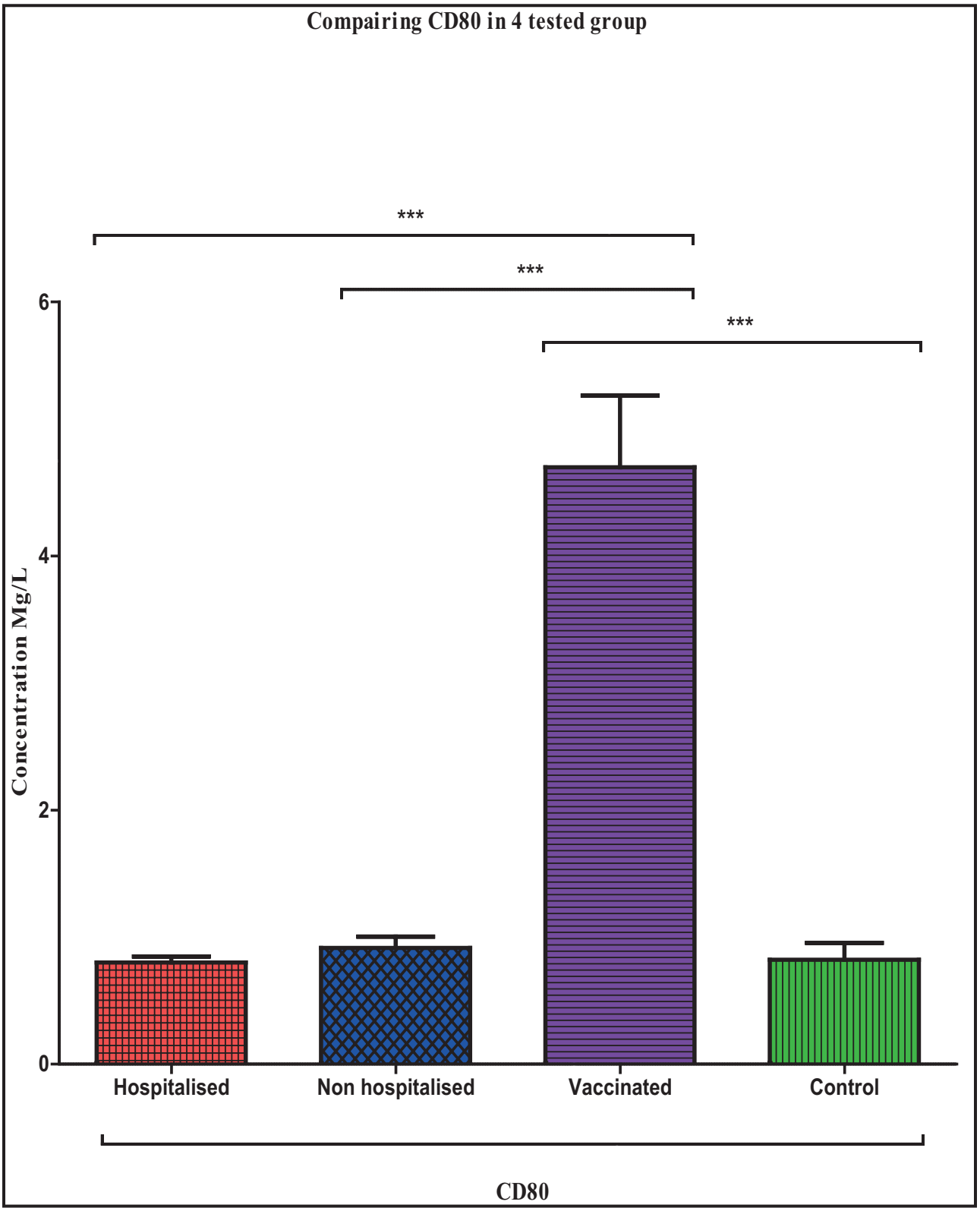

Figure 5: CD80 concentration in four tested groups using one-way ANOVA test with a significant difference between them ($P < 0.0001$)

Cluster Differentiation of Human (CD28, CD80, and CD86). The results obtained indicated that these markers had significant differences among the tested groups. In other words, these markers had a high concentration in vaccinated individuals, whereas these markers had a low concentration in patients with COVID-19. This may indicate deficiency or consumption of T cell during their infection. Patients with lymphopenia and decreased

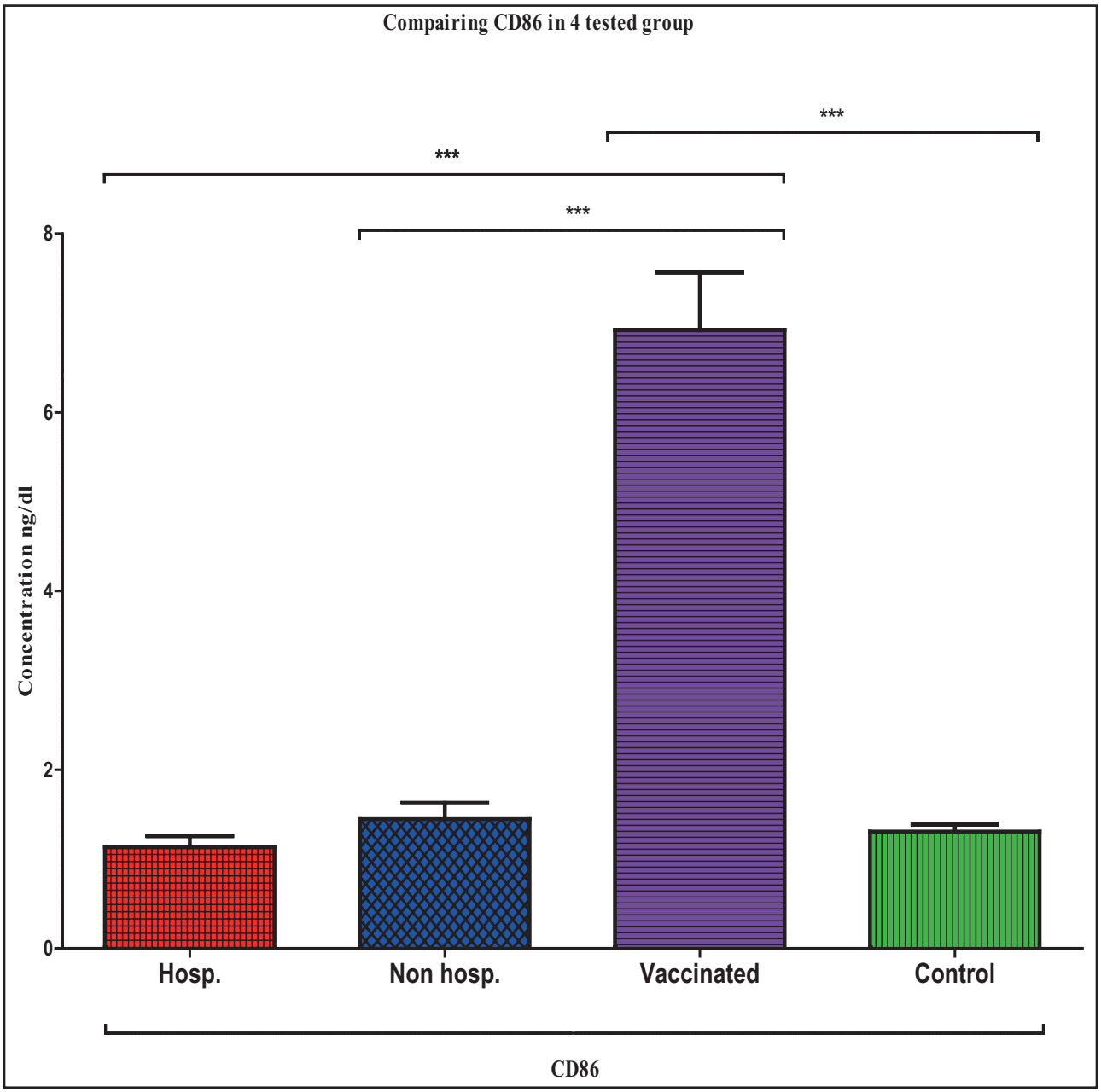

Figure 6: CD86 concentration in four tested groups using one-way ANOVA test with a significant difference between them ($P < 0.0001$)

peripheral T-cell counts were identified.[20-23] These results indicate that T lymphocytes are pulled away from the bloodstream and into the inflamed area in order to regulate viral infection. T-cell exhaustion increased and reduced functional variability indicated serious condition related to patients with COVID-19.[24] The peripheral lymphopenia seen in COVID-19 individuals may be due to lymphocyte adherence to inflamed respiratory vascular endothelium or lymphocyte recruitment to the respiratory system. The peripheral lymphopenia seen in COVID-19 individuals may be due to lymphocyte adherence to inflamed respiratory vascular endothelium or lymphocyte recruitment to the respiratory system. High levels of interleukin-6 (IL-6), IL-10, or tumor necrosis factor (TNF) may be related to lymphopenia in severe disease, either directly through these cytokines' effects on T-cell populations or indirectly through other cell types including dendritic cells and neutrophils. T-cell depletion may also result from excessive T-cell activation or high levels of pro-apoptotic molecule expression, including FAS (also known as CD95), TNF-related apoptosis-inducing ligand (TRAIL), or caspase 3. Because of this, a common characteristic of many people with severe disease and despite the fact that the processes underlying lymphopenia in COVID-19 are still poorly understood is a decrease in the number of T cells, particularly in the peripheral. It is yet unknown why lymphopenia favors T cells, possibly more especially CD8+ T cells.[25]

As for the control group, they are not vaccinated and not infected after conducting a PCR examination and making sure that they are not infected so the immune status is stable. In individuals who were vaccinated, whether it was mRNA or a viral vector vaccine, they had high immune markers concentration, and it is likely that it was caused by the vaccine that stimulates the immune system to produce specific antibodies against the virus, as has been proven in other studies.[26-30]

CD28 is a protein that is present on T cells and provides co-stimulatory signals that are essential for T-cell activation and survival; it is an effective signal for the production of several interleukins, primarily IL6; its ligands are the molecules CD80 and CD86. CD80 is an immunoglobulin in addition to that it conceder a ligand for cytotoxic T lymphocyte antigen 4 (CTLA4) and also known as (CD152), which is present constitutively on numerous T cells; present on APCs and their receptors on T cells; and present also on dendritic cells, as well as activated B cells, and macrophages in particular. Dendritic cells, macrophages, B cells (memory B cells), and other antigen-presenting cells all express CD86; it sends out co-stimulatory signals that help T cells activate and survive.[31]

CD28 and CTLA-4 are cell surface co-signaling molecules that regulate T-cell activation when their ligands B7-1 and B7-2 are engaged by antigen-presenting cells.[32]

Approximately 1 week after the onset of COVID-19 symptoms, T- and B-cell responses to SARS-CoV-2 are detected in the blood. CD8+ T cells are needed to target and kill virus-infected cells directly, but CD4+ T cells are needed to activate both CD8+ T cells and B cells. CD4+ T cells are also responsible for the generation of cytokines, which are used to stimulate immune cell recruitment. Mature dendritic cell is an antigen-presenting cell that interacts with CD28 on T cells to deliver antigens from any pathogen to T-cell MHCs. It also expresses large levels of CD80 and CD86.[33] Although angiotensin converting enzyme-2 (ACE2) does not express on T cells, SARS-CoV-2 may penetrate cells that do not express ACE2 via other receptors.[34]

Individuals with critical COVID-19 infection have abnormally stimulated T cells (hyper-activated), unable to specialize into specific subsets of T cells. The stimulation and differentiation may also be repressed prematurely of activation at an early stage, contributing to infection and pathogenesis of the virus.[35] Proliferation and apoptosis regulate T-cell levels during homeostasis.[36] As an outcome, T-cell decrease in COVID-19 could be caused by either lower proliferation or increased apoptosis.

Conclusion

In contrast to patients with COVID-19 and the control group, this study found that the vaccinated group had higher levels of the co-stimulatory proteins CD28, CD80, and CD86. This may be because the vaccination boosts immune function in the absence of the virus itself. Therefore, future studies are recommended on immune markers that may affect the activation of the T cell or help find ways to activate the immune system and help the patient to face corona disease.

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Conflicts of interest

There are no conflicts of interest.

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