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(RESEARCH ARTICLE)

A comparative study of some biological variables in those vaccinated and unvaccinated with the COVID-19 vaccine in the city of Samarra

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Abstract

After the world was swept by the SARS-CoV-2 virus, it became necessary to stop and control the pandemic, so the vaccine was issued as one of the preventive measures to stop the pandemic. The effect of the Pfizer/BioNTech BNT162b2 vaccine in raising the body's immune capacity against the SARS-CoV-2 virus was studied. A comparative study was conducted on the inflammatory blood parameters, which are the total number of white blood cells (WBCs), neutrophils, lymphocytes, and C-reactive protein (CRP). These blood inflammatory parameters were compared among 3 groups (healthy, vaccinated and unvaccinated). The study was designed by dividing the sample of 90 participants (50 males and 40 females) into three groups: the first group included 30 healthy participants who were not infected with Covid-19 and were not vaccinated, the second group included 30 participants who were infected with the disease while they were not vaccinated, and the third group included 30 participants who were vaccinated with two doses and were infected again after vaccination (Breakthrough infection). The ages of the participants ranged from (21 to 60 years).

The study was conducted during the period between Dec. 2023 and May 2025, in private laboratories in Samarra city. We obtained the following results: The total white blood cell count was found to be highest in the group of unvaccinated patients (16.731 ± 4.303) and lowest in the healthy group (10.012 ± 0.601). Neutrophils were highest in vaccinated patients (7548.29 ± 798.171) and lowest in the healthy group (5891.25 ± 351.686). While the lymphocytes recorded the highest percentage in the healthy group (2779.92 ± 283.711) and the lowest percentage in the unvaccinated patients (1997.31 ± 290.039). As for the values of C-reactive protein (CRP), the highest values were recorded in the unvaccinated infected group (10.146 ± 4.752) and the lowest values were in the healthy group (1.550 ± 0.781). The ratio of neutrophils to lymphocytes was also obtained, where the highest percentage was in the unvaccinated infected (3.729), while the lowest percentage was recorded in the healthy group (1.958).

By studying these blood inflammatory parameters, it was found that the vaccine contributes to raising the body's immune capacity and reducing the possibility of infection, but it does not prevent infection completely. It also contributes to reducing the severity of infection if it occurs.

Keywords: Covid-19; Sars-Cov-2; Vaccinated; Unvaccinated; Pfizer; White Blood Cells; Neutrophils; Lymphocytes; And C-Reactive Protein

1. Introduction

At the end of 2019, the world was swept by the COVID-19 pandemic, caused by a dangerous coronavirus that severely infects the respiratory system, a severe acute respiratory infection. It is a single-stranded mRNA virus known as SARS-CoV-2 or coronavirus-2 [1,2,3]. This viral infection can be asymptomatic, moderate, severe, or fatal, which is why long-term immunization is so important to prevent recurrent infections [4]. The human respiratory system is the primary target of coronaviruses, one of the most common viruses on Earth. Patients infected with the 2019 novel coronavirus are at risk of reinfection, which is characterized by a variety of respiratory symptoms, including fever, dry cough, dyspnea, and pneumonia, as well as organ failure and acute respiratory distress syndrome, which necessitates

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hospitalization in an intensive care unit and can lead to death in some cases [5].Although diarrhea was initially observed only in small amounts in some cases, it is now becoming more prevalent among people infected with the disease [6].

COVID-19 symptoms can appear anywhere from two to fourteen days after infection, and the illness can last up to 27 days in some individuals. However, according to Chinese experts, the typical incubation period is 2–5 days, which varies depending on the patient's age, health, and clinical factors [7]. COVID-19 vaccines are considered effective and essential weapons to enhance control of this pandemic [8]. Vaccination against COVID-19 is considered the most effective means of stopping the spread of the virus, along with preventive measures used by individuals in communities [9].

The primary goal of vaccination is to stimulate a humoral immune response to stop the spread of the disease [10]. Through several mechanisms, SARS-CoV-2 vaccines enhance innate and acquired immunity. The Pfizer/BioNTech BNT162b2 vaccine demonstrated 95% efficacy against COVID-19. The Food and Drug Administration (FDA) approved two mRNA vaccines for SARS-CoV-2 with a two-dose schedule in December 2020: BNT162b2/Pfizer and mRNA-1273/Moderna [11]. Recently, vaccinations have contributed to limiting the spread of the virus. All immune manifestations, whether after infection with the virus or after vaccination, are important indicators for studying the disease and limiting its epidemic. Within three weeks of infection with SARS-CoV-2, humoral immunity stimulates the formation of antibodies to attack the spike glycoprotein (S) and nucleocapsid protein (N)[12].

The humoral response is assessed by the antibody avidity, also by the quantity and neutralization capacity of antibodies, and the antibody avidity [13]. However, recent studies indicate that COVID-19 vaccines protect against severe illness; however, post-vaccination SARS-CoV-2 infection (i.e., breakthrough infection) can occur because COVID-19 vaccines do not provide 100% protection. [14] More recently, post-vaccination infections have occurred in fully vaccinated individuals, although there is little information on risk factors, clinical symptoms and outcomes of penetrating infections compared to similar epidemiological and clinical controls. [15]. Most COVID-19 vaccines require two doses, spaced 3 to 12 weeks apart, to adequately immunize those vaccinated. The spike protein of SARS-CoV-2 is encoded by mRNA vaccines, and upon administration, it is taken up and translated into the host cell, producing the spike protein, which is then presented on the cell surface to B-cells and T-cells, triggering an immune response [16]. After the initial dose, this immune response (T lymphocytes and antibody levels) gradually declines, leaving only a small population of memory B-cells and T-cells to protect against future attacks by the same pathogen [17].

The second dose leads to a stronger immune response, which gradually diminishes over time. However, more memory B cells remain, which helps in the future to mount a more comprehensive and rapid immune response against the same disease [18]. Our primary interest in acquired immunity stems from the fact that it involves an antibody response mediated by B cells. This results in the development of specific antibodies that bind to the spike protein in order to prevent viral entry into cells and provide protection against SARS-CoV-2 infection [19]. Due to certain technological advantages, mRNA-based COVID-19 vaccines were among the first to be approved and delivered in many Western countries. These vaccines were either already approved for clinical use or in advanced stages of development [20]. In the United States, the Pfizer-BioNTech and Moderna COVID-19 vaccines received Emergency Use Authorization (EUA) in December 2020 from the U.S. Food and Drug Administration (FDA) to protect those at high risk of severe consequences [21, 22].

Studies have also indicated that one of the most important indicators of inflammation, which has shown high rates of occurrence in COVID-19 infections, is C-reactive protein (CRP). This protein is produced in the liver during acute inflammation. It is a highly sensitive biomarker of inflammation, tissue damage, and infection [23]. In the acute phase of infection, it is a relatively inexpensive and readily available method for any laboratory to detect this marker for COVID-19. Several studies have shown that CRP levels are elevated in COVID-19 patients; in critically ill patients, they have reached values 5 to 10 times higher than those found in uninfected patients [24,25]. A study of laboratory findings in COVID-19 patients also showed an increase in neutrophils (>0.7 x 10^3/µL), a decrease in lymphocytes (<0.8 x 10^3/µL), and an increase in C-reactive protein (>4.75 mg/dL), which were the most significant predictors of mortality in COVID-19 patients. Hematological abnormalities, including lymphopenia, thrombocytopenia, and leukopenia, are expected in COVID-19 patients. Lymphopenia and neutrophilia are common in COVID-19 patients. The two values are compared using the neutrophil-to-lymphocyte ratio (NLR), which can be assessed from routine blood tests by dividing the absolute neutrophil count by the absolute lymphocyte count. The NLR can help identify high-risk COVID-19 patients, as this indicator can be used as an independent risk factor for COVID-19 mortality in hospitalized patients. An increase in neutrophils indicates a severe inflammatory response, while a decrease in lymphocytes indicates immune system damage. A study of 191 patients assessing risk factors for mortality showed that the lymphocyte count in patients whose COVID-19 condition improved was higher than in those whose condition worsened. In COVID-19 patients who recovered, the highest neutrophil count and lowest lymphocyte count were found on day 7 after the onset of illness, and they continued to improve during hospitalization. Meanwhile, in patients whose condition worsened, there was a high

neutrophil count and a severe lymphocytic deficiency that persisted until the patient's death. [26, 27]. Therefore, this study aimed to evaluate some blood parameters such as lymphocytes and neutrophils from white blood cells, as well as to study the C-reactive protein index, as a comparative study between groups of patients vaccinated with the Corona vaccine and unvaccinated patients, and to compare them with healthy people.

2. Materials and Methods

Ninety blood samples were collected from the study participants between May 2023 and May 2024 at private laboratories in Samarra. COVID-19 infection was confirmed either by polymerase chain reaction (PCR) or by computed tomography (CT) scan. The samples were divided into three groups: the first group consisted of 30 healthy, unvaccinated, and uninfected samples (19 males and 11 females); the second group consisted of 30 unvaccinated infected samples (16 males and 14 females); and the third group consisted of 30 vaccinated samples who became infected after vaccination (15 males and 15 females). It is worth noting that all vaccinated individuals had received two doses of the Pfizer BioNTech/BNT162b1 vaccine.

The participants in this study ranged in age from 21 to 60 years. They were selected to be free of chronic diseases, liver disease, and cancer, and women were not pregnant or breastfeeding. The healthy group was confirmed to be previously uninfected by COVID-19 antibody tests, and their RT-PCR results were negative. Furthermore, they did not exhibit any clinical symptoms of COVID-19.

It is worth noting that written informed consent was obtained from all participants in this study before samples were taken. Sample preparation for the required analyses involved drawing 4 ml of blood from each participant. For the purpose of analyzing the white blood cell count (differential count), a Mindray automated complete blood count analyzer was used. Two ml of the drawn blood was placed in EDTA tubes to prevent clotting. The tubes were agitated several times to mix the blood with the anticoagulant, and then 20 microliters of blood were withdrawn from the tubes using the analyzer. The results were then read on the analyzer's screen.

For CRP assay samples, serum was separated from blood using a gel tube. The samples were then analyzed using a fully automated AFIAS-6 analyzer. CRP levels in blood plasma were quantified using a fluorescence immunoassay (FIA). The sandwich immunoassay technique is employed, where a pair of antibody-antigen complexes in the buffer binds to an antigen present in the sample and migrates to the test strip, where it is captured by the other binding antibody. In samples containing more antigens, the formation of more antigen-antibody complexes results in a brighter fluorescent signal, which is then read by the AFIAS analyzer to determine the CRP concentration in the samples.

Statistical analysis was performed using IBM SPSS version 26 (Armonk, New York, NY, USA). Data were presented as mean ± standard deviation for the quantitative variable to compare the means of the study groups. A p-value < 0.05 was considered statistically significant. Relationships were also examined using Duncan's new multiple range test.

3. Results and Discussion

Tests were conducted on 90 samples (30 samples from the control group – healthy individuals – 30 samples from unvaccinated patients, and 30 samples from vaccinated patients) to obtain results for hematological variables and some immunological indicators. The following results were obtained in this study:

The total white blood cell count was highest in the unvaccinated infected group (16.731 ± 4.303) and lowest in the healthy group (10.012 ± 0.601), as shown in Table 1 and Figure 1.

Table 1 The descriptive statistics and significance test value for the total white blood cell count $*10^9$/L.

Probability level	**.Sig**	**Arithmetic mean ± standard deviation**	**Variables**	
0.05	0.000	b0.601 ± 10.012	Healthy	Total white blood cell count * /10^9L
		a4.303 ± 16.731	Unvaccinated	
		b0.927 ± 10.892	Vaccinated	

*Different letters indicate the presence of significant differences, while similar letters indicate the absence of significant differences at a significance level of (0.05) in the same table.

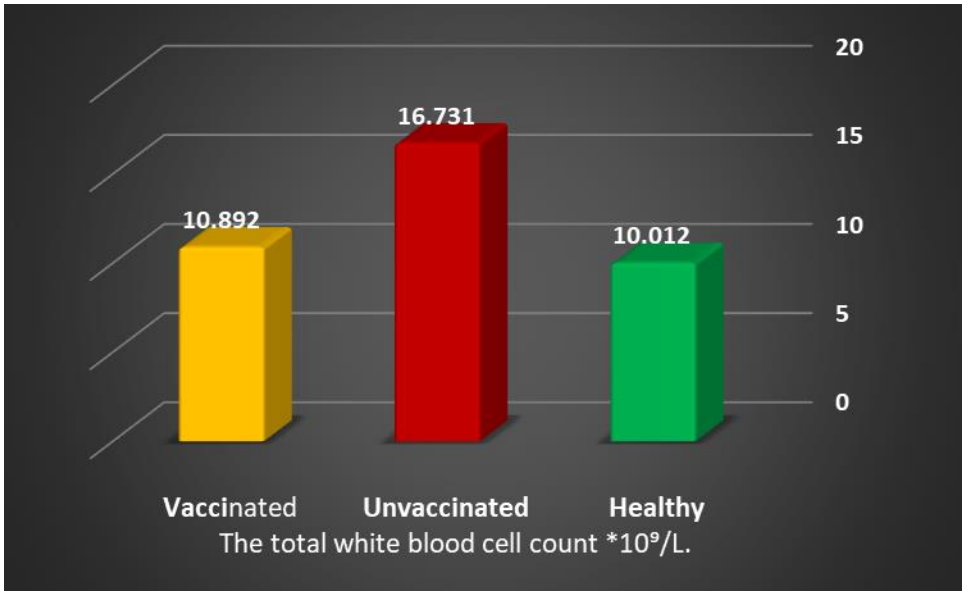

Figure 1 The significant differences between the groups with respect to the variable total white blood cell count $*10^9/L$

Some studies have indicated an increase in the total white blood cell count (TBCs) in COVID-19 patients compared to healthy individuals [33]. Other studies have documented an increase in white blood cell count in non-infected individuals as an effect of COVID-19 [34]. This is consistent with our findings.

Neutrophil counts, a type of white blood cell (calculated by differential count), were also obtained. The highest count was observed in vaccinated patients (7548.29 ± 798.171) and the lowest in healthy individuals (5891.25 ± 351.686), as shown in Table 2 and Figure 2.

Table 2 The descriptive statistics and significance test value for neutrophil count * $10^9/L$

Probability level	**.Sig**	**Arithmetic mean ± standard deviation**	**Variables**	
0.05	0.000	b351.686 ± 5891.25	Healthy	Neutrophils* $^9/10L$
		a888.126 ± 7447.46	Unvaccinated	
		a798.171 ± 7548.29	Vaccinated	

* Different letters indicate the presence of significant differences, while similar letters indicate the absence of significant differences at a significance level of (0.05) in the same table.

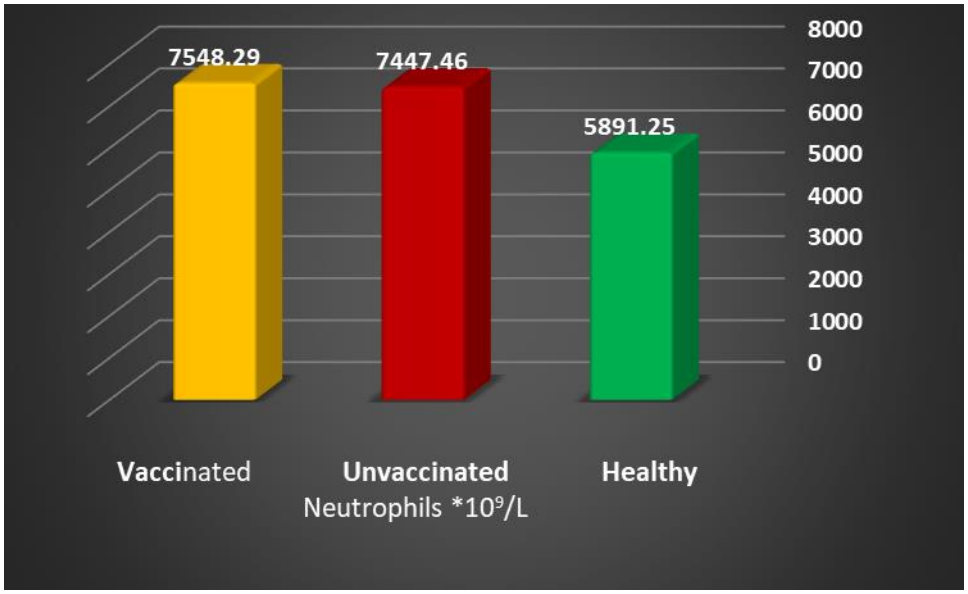

Figure 2 The significant differences between the groups with respect to the neutrophils variable $*10^9/L$

Some researchers have indicated that the neutrophil count was higher in vaccinated groups and lower in healthy groups [35]. This is consistent with our findings. Other studies have also indicated that an increase in neutrophils reflects the severity of the inflammatory response, with the highest neutrophil count observed on day 7 after the onset of illness and subsequent improvement during hospitalization. Meanwhile, in patients experiencing worsening symptoms, there is a high neutrophil count [31, 32].

Results for lymphocytes, a type of white blood cell (calculated by differential count), were also obtained, with the highest percentage among healthy individuals (2779.92 ± 283.711) and the lowest percentage among unvaccinated patients (1997.31 ± 290.039), as shown in Table 3 and Figure 3.

Table 3 The descriptive statistics and the significance test value for the number of lymphocytes $*10^9/L$

Probability level	**.Sig**	**Arithmetic mean ± standard deviation**	**Variables**	
0.05	0.000	a283.711 ± 2779.92	Healthy	Lymphocytes $*10^9/L$
		b290.039 ± 1997.31	Unvaccinated	
		a246.880 ± 2681.00	Vaccinated	

* Different letters indicate the presence of significant differences, while similar letters indicate the absence of significant differences at a significance level of (0.05) in the same table.

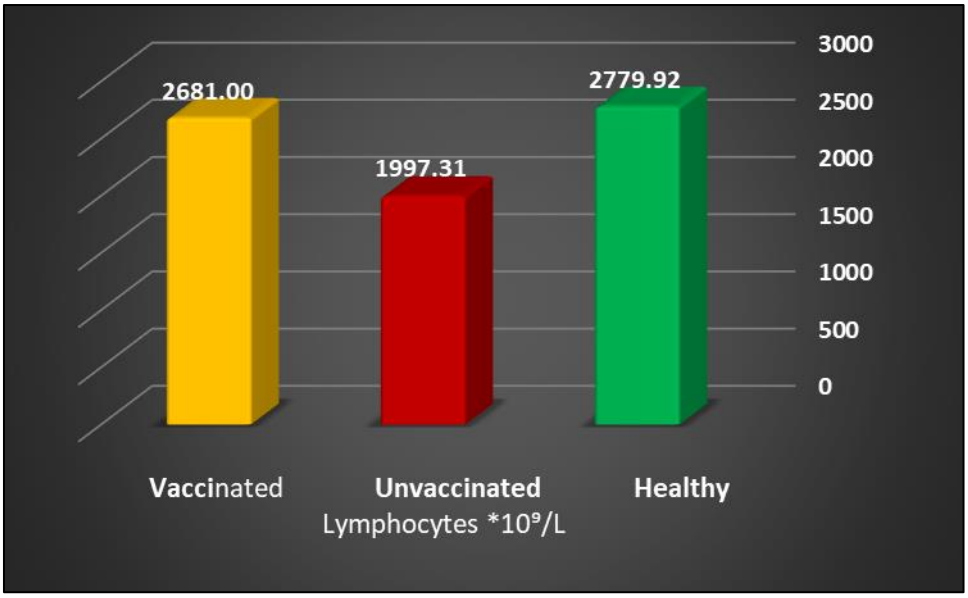

Figure 3 The significant differences between the groups with respect to the variable of lymphocytes $*10^9/L$

Some researchers have found that lymphocytes are lower in patients than in healthy individuals. In COVID-19 patients [33], the effects of COVID-19 on blood cell counts have been documented. It has been found that the number of lymphocytes and platelets decreases [34]. Lymphocytic disorders, cytokine storm suppression, metabolic acidosis, and lymphoid organ atrophy are some of the proposed causes of this phenomenon [35, 36].

T lymphocytes activate specialized B lymphocytes and memory cells to produce specific antibodies against SARS-CoV-2. Upon infection with COVID-19, the antibodies produced will neutralize the SARS-CoV-2 antigen (spike protein) under conditions of rapid viral replication, potentially leading to a strong immune response. The number of antibodies already produced may equal the number of spike proteins, so that even if an individual experiences a severe infection, the number of exhausted T lymphocytes will decrease, preventing a severe cytokine storm. This explains the high lymphocyte count in the vaccinated group [37]. This is consistent with our current study.

C-reactive protein (CRP) values were also obtained. The highest values were recorded in the unvaccinated group (10.146 ± 4.752) and the lowest values in the healthy group (1.550 ± 0.781), as shown in Table 4 and Figure 4.

Table 4 The descriptive statistics and significance test values for CRP levels in mg/L.

Probability level	**.Sig**	**Arithmetic mean ± standard deviation**	**Variables**	
0.05	0.000	c0.781 ± 1.550	Healthy	C-reactive protein mg/L
		a4.752 ± 10.146	Unvaccinated	
		b 5.607 4.585 ±	Vaccinated	

* Different letters indicate the presence of significant differences, while similar letters indicate the absence of significant differences at a significance level of (0.05) in the same table.

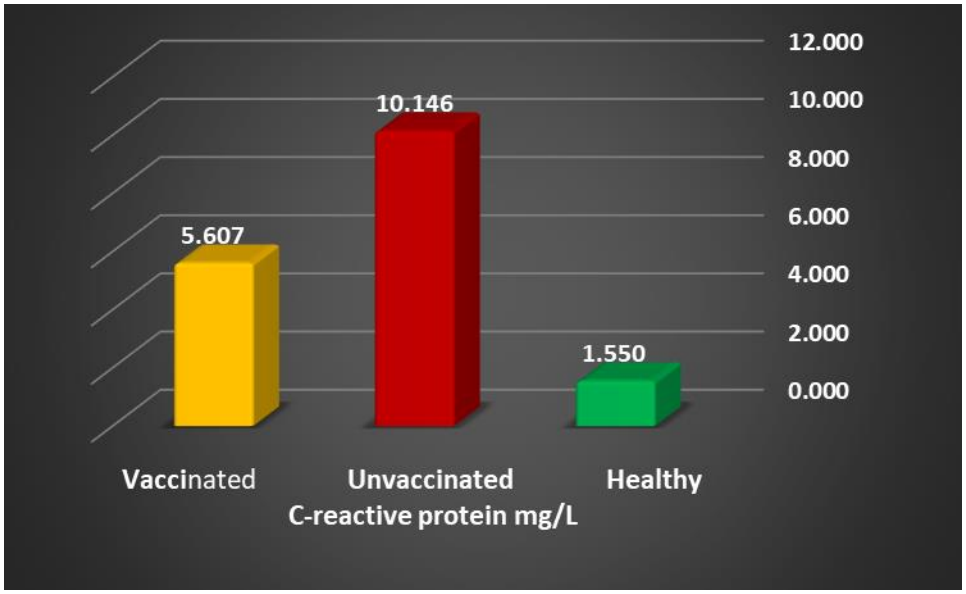

Figure 4 The significant differences between the groups with respect to the C-reactive protein (CRP) mg/L variable**.**

In COVID-19 patients, a cascade of inflammatory processes occurs. Some evidence suggests that the progression of COVID-19 is closely linked to dysregulation and excessive cytokine secretion. A study in Wuhan showed that patients who died from COVID-19 complications had elevated C-reactive protein (CRP) levels. Elevated CRP is associated with the overproduction of inflammatory cytokines in severely ill COVID-19 patients. Cytokines play a role in fighting microbes, but if the immune system becomes overactive, it can damage lung tissue [38, 39]. Another study showed that CRP levels were positively correlated with lung lesions and disease severity. CRP values >40 mg/L at hospital admission can indicate disease severity and an increased risk of death [40].

Another study indicated that C-reactive protein (CRP) levels in the vaccinated group were lower than those in the unvaccinated group, and the differences were statistically significant ($P < 0.05$). The mortality rate was also significantly lower in the vaccinated group than in the unvaccinated group [41]. Another study indicated that the mean CRP value in the vaccinated group was significantly lower than in the unvaccinated group. A previous study reported that CRP values > 4 mg/dL at the time of hospital admission can be used as an indicator of disease severity [40]. Other studies have also reported that in unvaccinated individuals, the vaccine cannot inhibit viral replication because the specific antibodies that have not yet formed cause hyperinflammation. The cytokine storm resulting from this excessive inflammatory response leads to increased CRP production and damage to target organs, especially the lungs, causing acute respiratory distress syndrome (ARDS) or death [42, 39, 43]. This is consistent with our current study.

In another study, researchers observed the greatest increase in C-reactive protein (CRP) levels in uninfected individuals who were vaccinated. This suggests that elevated CRP levels in vaccinated individuals may lead to immune system dysfunction after vaccination [44]. However, this does not align with our current study.

The neutrophil-to-lymphocyte ratio, an important indicator of infection severity, was also measured. The highest ratio was found in the unvaccinated group (3.729), while the lowest was recorded in the healthy group (1.958). (See Table 5)

Table 5 The ratio of neutrophils to lymphocytes

Neutrophil to lymphocyte ratio	
1.958	Healthy
3.729	Unvaccinated
2.815	Vaccinated

Some studies have indicated that COVID-19 infection triggers a natural immune response, leading to an increase in neutrophil counts, apoptosis (programmed cell death), and lymphocyte depletion. This results in a decrease in

lymphocyte counts, resulting in an increased neutrophil-to-lymphocyte ratio (NLR) [32, 31, 43]. In the vaccinated group, the antibodies produced neutralize the SARS-CoV-2 antigen (spike protein), allowing viral replication to be balanced by the number of antibodies developed. This results in a low number of lymphocytes undergoing apoptosis and reduced stress (the lymphocyte count remains high), thus maintaining a low NLR value even in severe cases [45, 46]. Another study reported a lower neutrophil-to-lymphocyte ratio in vaccinated individuals compared to unvaccinated individuals, and lower mortality rates in vaccinated patients compared to unvaccinated patients [47]. This is consistent with our current study.

4. Conclusions

We conclude from these results that the COVID-19 vaccine protects against the worsening of infection and contributes to reducing mortality by enhancing the immune system's ability to recognize the spike protein that causes infection. Furthermore, the COVID-19 vaccine does not prevent reinfection but reduces its severity. It also puts the immune system in a better state of readiness through memory lymphocytes, which accelerates the response and antibody production in a short period.

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