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*CORRESPONDENCE
Thowiba Yousif Jameel,
mostafadolamee@gmail.com

†PRESENT ADDRESS
Thowiba Yousif Jameel,
Department of Biology, Faculty of Science, Tikrit University, Tikrit, Iraq

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Hematological and inflammatory changes following Pfizer-BNT162b2 mRNA vaccination in young healthy adults

Mustafa Abdulkareem Salman[1,2], Thowiba Yousif Jameel[2*†], Abdurrahman Ayvaz[3] and Ahmed Rushdi Abdullah[4]

[1]Department of Anatomy, College of Medicine, University of Diyala, Diyala, Iraq, [2]Department of Biology, Graduate School of Natural and Applied Science, Erciyes University, Kayseri, Türkiye, [3]Department of Biology, Faculty of Science, Erciyes University, Kayseri, Türkiye, [4]Department of Microbiology, Medical College, Aliraqia University, Baghdad, Iraq

Introduction: COVID-19 infection causes significant changes in certain hematological markers and increases inflammatory severity, such as NLR, LMR, PLR, and Ferritin. In contrast, the Pfizer-BNT162b2 mRNA-based vaccine does not induce high levels of inflammation.

Methods: A prospective cohort study, conducted at the University of Diyala from January 2022 to January 2023, assessed the immune response to the Pfizer-BNT162b2 mRNA vaccine in 45 healthy, unvaccinated medical students aged 18 to 23. The study evaluated Complete Blood Count, proinflammatory cytokines, ferritin, and anti-IgG antibodies at the time of the second dose of vaccination and 4 weeks after the second dose of the vaccine. Biomarkers were analyzed using CBC instrumentation, ELISA, and Cobas e411 assays, with ethical approval granted for the study.

Results: Hemoglobin levels remained stable, while red blood cell (RBC) volume and RDW-CV significantly decreased. RBC indices (MCH, MCHC, HCT) increased, and total leukocyte counts slightly declined with a notable granulocyte rise. The platelet count remained stable; however, the plateletcrit decreased, while MPV and PDW increased. NLR and PLR levels increased significantly, whereas LMR levels decreased. No significant change occurred in PHR, SIRI, or AIRI. Inflammatory markers (TNF-α, ferritin, CD256/APRIL) and anti-spike IgG levels significantly increased after 1 month. Anti-spike IgG levels showed no strong correlations with SIRI, AIRI, PLR, PHR, or MLR, though significant intercorrelations were found among hematological ratios (e.g., PLR with PHR, SIRI, AIRI; LMR with AIRI).

Conclusion: Pfizer-BNT162b2 mRNA vaccine had a significant impact on the organized redistribution of immune cells.

KEYWORDS

Pfizer-BioNTech, BNT162b2, hematological ratios, TNF-α, CD-256 APRIL, anti-IgG spike protien

Introduction

The World Health Organization (WHO) declared COVID-19 a pandemic in March 2020, after its emergence in Wuhan, China, in late 2019 (Onyeaka et al., 2021). The virus spreads via respiratory droplets, causing symptoms such as cough, fever, fatigue, and breathing difficulties with damaging tissues (Toussie et al., 2022). Global efforts led to the rapid development of vaccines, including Pfizer-BNT162b2 mRNA, Johnson & Johnson, and AstraZeneca. These vaccines, approved by the FDA, were vital in addressing the pandemic and marked a major medical achievement (Costanzo et al., 2022).

Studies have indicated the pivotal role of SARS-CoV-2 in the immune response underlying the severity of the disease and its clinical manifestations. This leads to a strong and uncontrolled response known as a cytokine storm, which affects various components of the body, including the blood. Numerous studies have linked hematological abnormalities such as agranulocytosis, lymphopenia, and thrombocytopenia to SARS-CoV-2 infection (Esmaeili and Esmaeili, 2024).

Meanwhile, other studies have highlighted the clinical benefits of GLR (Granulocyte-to-lymphocyte ratio), PLR (Platelet-to-lymphocyte ratio), LMR (Lymphocyte-to-monocyte ratio), and PHR (Platelet-to-hemoglobin ratio) due to their vital roles in understanding COVID-19 infection, as well as in assessing its implications after COVID-19 vaccination. Studies have indicated that blood markers can provide or predict immune response pathways, coagulation disorders, and inflammation associated with infection. Additionally, they play a crucial role in offering insights into immune system activity and the severity of inflammation after vaccination (Zuin et al., 2022).

Some studies have suggested that GLR often increases due to lymphopenia and neutrophilia in severe cases of COVID-19. This serves as evidence of heightened inflammation, leading to immune system dysfunction as a result of exposure to the cytokine storm (Kasten-Jolly and Lawrence, 2022).

On the other hand, some studies have found that GLR may temporarily increase after COVID-19 vaccination, accompanied by a rise in granulocytes, which is considered an indication of innate immunity against pathogens (Karimi et al., 2021).

Moreover, numerous studies have highlighted the correlation between PLR levels and SARS-CoV-2 infection. These studies found that elevated PLR is associated with disease severity, as higher platelet counts are linked to poor outcomes and prolonged hospitalization (Wang et al., 2020). Regarding vaccination, studies have shown that PLR is at its lowest levels in vaccinated individuals compared to unvaccinated ones. They suggested that vaccination may reduce platelet activity and lymphocyte depletion (Seyit et al., 2020; Karimi et al., 2021).

In the same context, a decrease in LMR in cases of SARS-CoV-2 infection has also been associated with disease severity and admission to intensive care units. Studies have indicated that this decrease may lead to an impairment of immune capabilities, hindering an effective adaptive response against COVID-19 (Seyit et al., 2020). Meanwhile, studies have shown that LMR decreases at the onset of COVID-19 vaccination due to the dominance of monocytes in response to vaccine antigens.

PHR reflects an important combination for understanding platelet pathways, oxygen transport capacity, and its impact on disease severity. Few studies have highlighted its significance in both COVID-19 infection and post-vaccination contexts, as PHR integrates with inflammatory dynamics (Ozbeyaz et al., 2022). Researchers have indicated that PHR levels remain relatively stable in vaccinated individuals compared to unvaccinated ones. However, those who exhibit persistently irregular PHR levels often do so as a result of immune stress or a prior infection.

SIRI (Systemic Inflammation Response Index) and AIRI (Aggregate Inflammatory Response Index) are novel biomarkers that have been increasingly utilized during COVID-19 infection due to their critical role in providing a composite measure of the inflammatory response during infection or after COVID-19 vaccination. These indices primarily rely on the use of lymphocytes, neutrophils, and monocytes, and they serve as tools to reflect the balance between pro-inflammatory and anti-inflammatory immune cells (Citu et al., 2022).

Moreover, AIRI functions as an extension tool of the SIRI index by incorporating additional hematological parameters, providing a more comprehensive view of systemic

Abbreviations: NLR, Neutrophil-to-Lymphocyte Ratio; LMR, Lymphocyte-to-Monocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; RDW-CV, Red Cell Distribution Width – Coefficient of Variation; HCT, Hematocrit; MCH, Mean Corpuscular Hemoglobin, MCHC, Mean Corpuscular Hemoglobin Concentration; MPV, Mean Platelet Volume; PDW, Platelet Distribution Width; PHR, Platelet-to-Hemoglobin Ratio; SIRI, Systemic Inflammation Response Index; AIRI, Aggregate Index of Systemic Inflammation; CD256/APRIL, A Proliferation-Inducing Ligand; TNF-α, tumor necrosis factor alpha; GLR, Granulocyte-to-Lymphocyte Ratio.

inflammation. Many studies have found that elevated SIRI is significantly associated with the severity of COVID-19 infection and dysregulated immune response.

In contrast, AIRI is less commonly used as an inflammatory marker due to its integration with other inflammatory indices, reflecting the overall systemic immune inflammation. Some studies have suggested that a slight increase in AIRI results from immune system activation.

In the context of SARS-CoV-2 vaccination, studies have shown a slight, short-term increase in SIRI levels, along with the body mounting an immune response against the antigen. Similarly, research indicates that AIRI levels may rise post-vaccination as evidence of a strong immune response. However, some other studies have suggested that AIRI serves as a marker for excessive inflammation, particularly in individuals with comorbidities or those suffering from IMIDs (Immune-mediated inflammatory disorders) after vaccination (Hosseninia et al., 2023).

Materials and methods

Study design and participants

This prospective cohort study was performed in the Department of Biology at the College of Medicine, University of Diyala in Baqubah, Iraq, between January 2022 to January 2023. The participants were college students who had no previous or current COVID-19 infection. The participants were vaccinated with two doses of the Pfizer-BNT162b2 mRNA vaccine. The hematological and inflammatory laboratory data were collected before vaccination and after 4 weeks from the second dose of the vaccine.

Inclusion and exclusion criteria

The eligible participants were adults, healthy, aged 18–23 years, of both genders. Although the study's 18–23 age range might not fully reflect the Pfizer-BNT162b2 vaccine's target population worldwide, it was selected to reduce confounding variables like comorbidities and medication use. Future research should incorporate a wider age range. Those willing to receive the COVID-19 vaccine and those accepted to undergo blood tests were included in the study. Participants with blood disorder, a history of acute or chronic systemic infection, autoimmune disease, those using medicines acting on the blood cells, and pregnant women were excluded from the study.

Vaccination of the participants

The Pfizer-BNT162b2 mRNA vaccine (Comirnaty BNT162b2) is recommended in our community. It is an mRNA vaccine developed by Pfizer, Inc. (USA) and BioNTech SE (Germany). The primary vaccination series consists of two doses, administered 21 days apart, with each dose containing 0.3 mL of mRNA vaccine. The immune response is represented by an increase in IgG antibodies specific to the spike protein, which usually peaks about 2–3 weeks after the first dose.

Determination of the hematological and inflammatory biomarkers

Blood samples were obtained from the participants before and 1 month after vaccination. The blood samples were drawn into anti-coagulant tubes for determination of the complete blood count and the portions into plain tubes for determination of immunologically related biomarkers in sera. The sera were separated by centrifugation at 10,000 r.p.m. for 10 min to determine the anti-spike IgG antibodies, ferritin, tumor necrosis factor alpha, and CD 265 APRIL. The complete blood count for each participant was determined using the Sysmex XP-300™ Automated Hematology Analyzer/Japan. Serum inflammatory markers were measured using Cobas e411/Hoffmann-La Roche Ltd./Germany for ferritin. Enzyme-linked immunosorbent analysis (ELISA) was used to measure the levels of tumor necrosis factor alpha and CD256 (APRIL) by using ELK Biotechnology Co. Ltd., Wuhan, China, and BT LAB kit bioassay technology laboratory/China, respectively.

Determination of the hematological and inflammatory ratios and indices

The Granulocyte-to-lymphocyte ratio (GLR), PLR, and LMR were calculated by simple division number of blood cells (numerators to denominators).

The PHR is equal to $\frac{Platelet\ count}{Hemoglobin \times 10}$.

The SIRI is equal to $\frac{Neutrophil\ count \times monocyte\ count}{Lymphocyte\ count}$ (Sannan, 2023).

The AIRI is equal to $\frac{Neutrophil\ count \times monocyte\ count \times platelet\ count}{Lymphocyte\ count}$ (Sannan, 2023).

Statistical analysis

The results are presented as numbers, percentages, and means ± standard errors. The significant differences between the data before and after vaccination were determined using a two-tailed paired t-test. The bivariate correlations between the included variables were done using a two-tailed Spearman's correlation test. The prediction of immune response was tested by using a multivariate linear

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TABLE 1 Changes in the hematological indices in vaccinated patients.

Blood profile and ratios	Baseline	After 1 month	P-value
Red cell count × 10^{12}	4.465 ± 0.065	4.569 ± 0.057	0.012
Hemoglobin (g/dL)	13.05 ± 0.15	13.05 ± 0.169	0.985
Hematocrit (%)	41.495 ± 0.668	42.766 ± 0.563	0.008
Mean cell volume (fL)	90.84 ± 0.716	88.916 ± 0.685	0.010
Mean corpuscular hemoglobin (pg)	32.162 ± 0.148	34.258 ± 0.186	<0.001
Mean corpuscular hemoglobin concentration (g/dL)	29.131 ± 0.163	31.478 ± 0.287	<0.001
Red cell diameter width-CV (%)	14.467 ± 0.123	13.767 ± 0.159	<0.001
White cell count × 10^9/L	7.7289 ± 0.171	6.756 ± 0.161	<0.001
Granulocyte (%)	54.844 ± 0.636	62.316 ± 0.949	<0.001
Lymphocyte (%)	31.78 ± 0.555	32.29 ± 3.505	0.272
Monocyte (%)	8.638 ± 0.478	8.458 ± 0.367	0.606
Platelet count × 10^9/L	258.6 ± 4.846	269.089 ± 9.142	0.203
Plateletcrit (%)	0.243 ± 0.006	0.212 ± 0.006	<0.001
Mean platelet volume (fL)	9.093 ± 0.130	9.658 ± 0.126	<0.001
Platelet diameter width (fL)	14.502 ± 0.078	15.391 ± 0.081	<0.001
Granulocyte-to-lymphocyte ratio	1.753 ± 0.041	1.950 ± 0.042	<0.001
Platelet-to-lymphocyte ratio	106.49 ± 4.018	130.063 ± 4.911	<0.001
Lymphocyte-to-monocyte ratio	4.230 ± 0.257	4.115 ± 0.170	<0.001
Platelet-to-hemoglobin ratio	1.995 ± 0.046	2.087 ± 0.083	0.536

The results are presented as mean ± SE. The p-value was calculated using a two-tailed paired t-test.

regression analysis using the anti-spike IgG antibodies as the dependent variable, while the hematological ratios and inflammatory response indices were used as independent predictors. The multivariate regression analysis (model 1) test (including the tolerance and variance inflation factor estimates) was performed to show the effect of the sample size on the prediction of the AIRI (as a dependent variable) by other biomarkers as independent variables. A bootstrap analysis (using a sample size of 500) was done to show the effect of sample size on the intercorrelation between biomarkers. All statistical analysis was performed using IBM SPSS Statistics for Windows (version 26, IBM Corp., Chicago). A p-value of <0.05 is a significant cutoff value.

Results

Table 1 shows the effect of two doses of Pfizer-BNT162b mRNA on the profile of complete blood count and the hematological ratios derived from these counts. There were no significant differences in the levels of HB, while the volume and diameter of red cells were significantly decreased by 2.1% and 4.8%, respectively. Red cell variables related to hemoglobin, including Hct, MCH, and MCHC, increased dramatically as the red cell count increased. The total leukocyte count is significantly decreased, while the percentage of granulocytes is significantly increased by 13.7%. There were no significant changes in the percentages of lymphocyte and monocyte values. The alterations in the blood platelet indices included non-significant changes in the blood platelet count, a significant increase in the PDW and MPV, and a significant reduction in the PCT. Hematological ratios derived from the blood profile are characterized by a significant increase of GLR and PLR by 11.4% and 22.2%, respectively, while a minor reduction in the LMR (2.7%) was observed. The PHR value, as a worse prognostic marker, was non-significantly increased. Table 2 shows that the significant increase in the anti-spike IgG is associated with a significant increase in the ferritin, tumor necrosis factor alpha, and CD 256 APRIL values. The inflammatory indices derived from granulocyte, monocyte, lymphocyte, and platelet counts represented by SIRI and AIRI, were non-significantly suppressed. Bivariate (Spearman's) correlations showed a significant relationship between platelet count and PLR ($r = 0.53$, $p < 0.001$), PHR ($r = 0.935$, $P < 0.001$), and AIRI ($r = 0.346$, $p = 0.020$) (Table 3). PLR is significantly and positively correlated with SIRI ($r = 0.408$, $p = 0.005$), and AIRI ($r = 0.582$, $p = <0.001$). Similarly, the LMR is also significantly and positively correlated with SIRI, AIRI, and CD256 APRIL (Table 3). The intercorrelations between these variables are not impacted by the sample size, as shown in Table 4. Interestingly, the PHR value is correlated substantially with AIRI but not with SIRI. The changes in the inflammatory indices represented by SIRI and AIRI are not predictors for the levels of anti-spike IgG antibodies, as a marker of immune response to the vaccination, as shown by a multivariate linear regression analysis (Figure 1). Figure 2 shows that the PLR, LMR, and PHR are non-significant predictors of immune response evoked by vaccination.

TABLE 2 Inflammatory and immune response biomarkers after 1 month of vaccination compared with baseline.

Biomarkers	Baseline	After 1 month	P-value
Anti-spike IgG antibody (DU/mL)	0.276 ± 0.032	70.17 ± 0.690	<0.001
Systemic inflammatory response index	1.699 ± 0.102	1.518 ± 0.118	0.159
Aggregate inflammatory response index	431.3 ± 24.8	408.6 ± 33.2	0.548
Ferritin (μg/L)	13.352 ± 0.149	128.73 ± 2.311	<0.001
Tumor necrosis factor-alpha pg/mL)	12.219 ± 0.247	570.23 ± 12.776	<0.001
CD 256 APRIL (pg/mL)	12.427 ± 0.398	310 ± 11.611	<0.001

The results are presented as mean ± SE. The p-value was calculated using a two-tailed paired t-test.

TABLE 3 Bivariate (Spearman's) correlations between the inflammatory and hematological indices.

Variables	PDW	Plat	PCT	GLR	PLR	LMR	PHR	SIRI	AIRI	Ferritin	TNF-α	CD256	As-IgG
RDW	−0.209 (0.169)	−0.059 (0.698)	−0.146 (0.339)	0.050 (0.743)	0.083 (0.587)	−0.221 (0.145)	−0.097 (0.528)	−0.007 (0.965)	−0.018 (0.906)	−0.219 (0.148)	0.153 (0.316)	−0.153 (0.315)	0.055 (0.721)
PDW		0.046 (0.764)	0.210 (0.167)	−0.060 (0.694)	−0.296* (0.049)	−0.132 (0.387)	0.009 (0.953)	−0.209 (0.168)	−0.213 (0.161)	−0.080 (0.601)	−0.141 (0.357)	−0.175 (0.250)	−0.209 (0.168)
Platelet			0.192 (0.206)	−0.005 (0.972)	0.53 (<0.001)	0.252 (0.095)	0.935 (<0.001)	−0.038 (0.803)	0.346 (0.020)	−0.098 (0.520)	−0.030 (0.847)	−0.026 (0.865)	0.096 (0.532)
PCT				−0.060 (0.695)	0.066 (0.667)	−0.077 (0.613)	0.165 (0.278)	−0.115 (0.451)	−0.031 (0.842)	0.198 (0.192)	0.043 (0.779)	−0.121 (0.428)	−0.068 (0.665)
GLR					0.214 (0.158)	0.032 (0.883)	−0.018 (0.907)	0.154 (0.313)	0.105 (0.494)	0.084 (0.585)	−0.262 (0.082)	0.020 (0.899)	−0.089 (0.560)
PLR						0.357 (0.016)	0.434 (0.003)	0.408 (0.005)	0.592 (<0.001)	−0.123 (0.422)	−0.028 (0.856)	−0.039 (0.801)	0.117 (0.445)
LMR							0.213 (0.159)	0.346 (0.020)	0.431 (0.003)	−0.165 (0.277)	−0.183 (0.228)	0.304 (0.043)	0.241 (0.111)
PHR								−0.073 (0.636)	0.295 (0.049)	−0.110 (0.471)	0.073 (0.635)	0.022 (0.886)	0.151 (0.323)
SIRI									0.904 (<0.001)	0.004 (0.979)	−0.189 (0.213)	−0.013 (0.933)	0.289 (0.053)
AIRI										−0.053 (0.731)	−0.128 (0.408)	−0.047 (0.762)	0.275 (0.068)
Ferritin											−0.195 (0.199)	0.072 (0.639)	0.046 (0.767)
TNF-α												−0.065 (0.672)	0.088 (0.567)
CD256													0.188 (0.216)

The results are presented as Spearman's correlation coefficient (p-value). RDW, red dimeter width; PDW, platelet diameter width; PCT, plateletcrit; GLR, granulocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PHR, platelet-to-hemoglobin ratio; SIRI, systemic inflammatory response index; AIRI, aggregate inflammatory response index; TNF-α, tumor necrosis factor-alpha.

Discussion

Complete blood count (CBC) has become a widely utilized indicator for assessing disease severity and its potential complications. Moreover, recent studies have associated COVID-19 with changes in CBC parameters as a result of the immune response (Abu-Ismail et al., 2023). These studies have also linked such changes to the COVID-19 vaccine, confirming

TABLE 4 The results of logistic regression analysis (Model 1) using the aggregate inflammatory response index (AIRI) as the dependent variable and the other variables as predictors.

Independent variables	β-Coefficient (95% C.I)	p-value	Collinearity diagnostic		Bootstrap for β-coefficient (n = 500)	
			Tolerance	VIF	Bias ±SE	p-value
RDW	−0.47 (−3.00, 2.07)	0.709	0.735	1.360	0.068 ± 1.27	0.691
PDW	−16.8 (−45.8, 12.1)	0.245	0.597	1.675	4.927 ± 18.23	0.367
Platelet	1.70 (0.85, 2.58)	**<0.001**	0.051	19.644	−0.038 ± 0.567	**0.020**
PCT	176.4 (−153.2, 506.1)	0.283	0.813	1.230	−29.4 ± 217.9	0.455
GLR	−13.6 (−60.5, 33.2)	0.557	0.851	1.176	−3.045 ± 26.2	0.613
PLR	0.13 (−0.58, 0.83)	0.713	0.275	3.633	0.011 ± 0.340	0.677
LMR	−8.82 (−22.9, 5.2)	0.210	0.576	1.735	-0.008 ± 8.256	0.301
PHR	−28.3 (−111.1, 54.6)	0.491	0.070	14.361	1.474 ± 51.65	0.531
SIRI	253.9 (233.5, 274.3)	**<0.001**	0.568	1.760	1.958 ± 12.2	**0.002**
Ferritin	−0.569 (−1.51, 0.37)	0.225	0.704	1.421	0.035 ± 0.512	0.273
TNF-α	−0.034 (−0.211, 0.143	0.701	0.645	1.551	0.008 ± 0.097	0.719
CD256	0.075 (−0.115, 0.265)	0.425	0.679	1.472	−0.001 ± 0.108	0.493
As-IgG	−0.916 (0.54, −0.39)	0.538	0.770	1.299	−0.138 ± 1.649	0.567

VIF, variance inflation factor, which corresponds to the inverse tolerance. RDW, red dimeter width; PDW, platelet diameter width; PCT, plateletcrit; GLR, granulocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PHR, platelet-to-hemoglobin ratio; SIRI, systemic inflammatory response index; AIRI, aggregate inflammatory response index; TNF-α, tumor necrosis factor-alpha. The bold values indicates statistically significant results ($p < 0.01$).

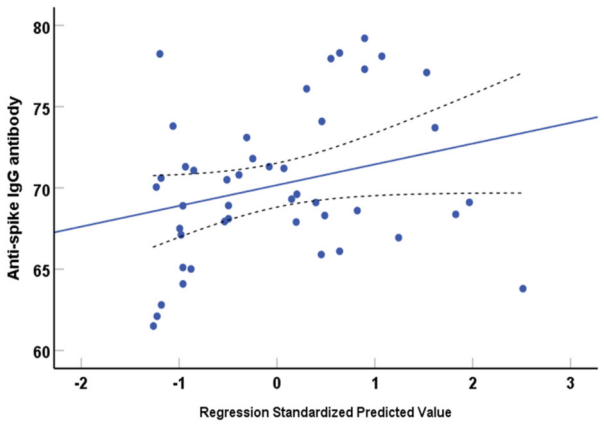

FIGURE 1
Multivariate linear regression showed a non-significant relationship between the inflammatory indices (SIRI, and GIRI) and the anti-spike IgG antibody level (r = 0.276, F = 1.782, p = 0.190). SIRI, systemic inflammatory response index; AIRI, Aggregate inflammatory response index.

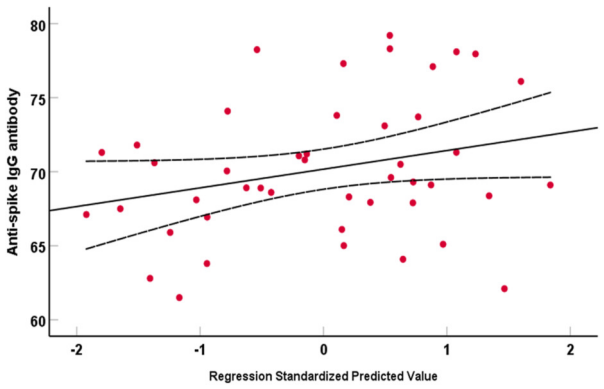

FIGURE 2
Multivariate linear regression showed a non-significant relationship between the hematological indices (PLR, MLR, PHR) and the anti-spike IgG antibody level (r = 0.272, F = 1.093, p = 0.363). PLR: platelet-to-lymphocyte ratio, MLR, monocyte-to-lymphocyte ratio; and PHR, platelet-to-hemoglobin ratio.

the occurrence of short-term alterations in CBC due to the immune reaction. Our current study's findings on CBC parameters before and after vaccination with Pfizer-BNT162b2 mRNA revealed physiological changes that could be considered normal. These changes are likely associated with the vaccine's efficacy and the immune response it triggers. Red cell parameters showed non-significant changes in hemoglobin level, unlike

those observed in COVID-19 infections, while a significantly higher ferritin level is similar to COVID-19 infection (Terpos et al., 2020; Abu-Ismail et al., 2023). One month after vaccination, there is a significant increase in red blood cells despite a non-significant difference in the hemoglobin level, which is explained by a significant reduction in MCV. This observation agrees with other studies that reported a reduction in the MCV in pediatric patients with COVID-19 (Alshalani et al., 2023). Moreover, a decrease in MCV may reflect the early release of new red blood cells due to an inflammatory response (Suresh et al., 2020). There is evidence that vaccine-induced inflammation could be involved in the stimulation of red blood cell production temporary infection (Greinacher et al., 2022), while other studies have suggested that baseline hemoglobin levels can be negatively affected during COVID-19 infection (Kakavandi et al., 2024).

A significant increase in hematocrit levels could be linked to a rise in red blood cells while plasma volume remains stable. After vaccination, only minor changes in hematocrit were observed, suggesting that the immune response to the vaccine may slightly influence blood cell levels without significantly affecting plasma volume. It is expressed as a good indicator, comparing with other studies that show reduced vaccine efficacy in the patients with anemia and iron deficiency who were vaccinated (Stoffel et al., 2020). The marked drooping in RDW-CV from a mean value of 14.5%–13.5% after vaccination indicates an improvement in erythrocyte size uniformity, suggesting a reduction of the inflammatory state compared to acute COVID-19 infection. Increased RDW-CV has been consistently associated with adverse clinical outcomes and higher mortality in COVID-19 patients because of systemic inflammation and impaired erythropoiesis (Connors et al., 2020). Therefore, the reduction in RDW-CV post-vaccination highlights a distinct hematological profile, differentiating the immune response to vaccination from that of active infection and potentially indicating a more favorable clinical outlook.

Following the vaccination, the mean value GLR is elevated from 1.8 to 2.0, which is within normal levels, while in COVID-19, the mean value neutrophil-to-lymphocyte ratio in non-severe conditions is 4.8 and 20.7 in severe conditions (Yang et al., 2020). Therefore, immunization will not induce changes in the proportions of granulocytes and lymphocytes as COVID-19 did. Similar findings are observed with PLR, which is the mean value in non-severe COVID-19 is 176.7, which is higher than the corresponding value in immunized people (Yang et al., 2020). It was reported that mean NLR (11.81) and PLR (307.67) levels were significantly higher in severe COVID-19 patients. Similarly, studies have shown that RDW-CV (14.35) is a statistically significant predictor of disease severity and poor prognosis for clinical outcomes in COVID-19 patients, with higher levels being associated with increased mortality and serious complications (Henry et al., 2020). It an interesting that the median LMR was 4.1 in non-severe cases and 2.1 in severe COVID-19 cases, whereas it reduced from 4.2 to 4.1 in vaccinated persons, which may indicate that the LMR is a suitable discriminatory indicator (Yang et al., 2020).

The reduction of LMR in severe COVID-19 cases is referred to as an elevated proportion of monocytes and a low proportion of lymphocytes, reflecting the excessive inflammatory response associated with acute infection. On the other hand, vaccinated subjects exhibit stable lymphocyte and monocyte ratios, with a slight decrease in the LMR, which may be due to a targeted and non-pathological redistribution of immune cells by the vaccine.

This suggests that the decreased LMR after vaccination could reflect targeted immune activation, contributing to enhanced immune protection without triggering an excessive inflammatory response, unlike what occurs in acute infection. Therefore, LMR can be considered a useful indicator for differentiating between an infection-induced inflammatory response and a vaccine-induced immune response, although larger future studies are needed to support this observation.

This observation supports previous reports that have shown a decreased LMR is associated with increased disease severity and poor clinical prognosis in COVID-19 patients (Yang et al., 2020; Erdogan et al., 2021). There are obvious disproportions between the significant increase in the inflammatory markers (ferritin, TNF-α, and CD256 APRIL) and non-significant changes in the SIRI and AIRI, which were derived from neutrophil, lymphocyte, and monocyte counts after immunization. This observation indicates that immunization induces a redistribution of the leucocyte differential counts. There is evidence that SIRI and AIRI are non-significant predictors for complications of COVID-19 compared with NLR or LMR (Citu et al., 2022).

The systemic inflammation markers SIRI and AIRI, which show significant associations with LMR and PLR, are important indicators for studying lymphocytes, monocytes, and platelets in evaluating the general immune status after vaccination. It should be noted that these markers cannot be considered precise quantitative indicators for predicting anti-spike IgG antibody levels. The production of these antibodies primarily depends on B-cell and long-term immunological memory (Salman et al., 2021). Therefore, they are not directly reflected in blood-based markers such as SIRI and AIRI. Consequently, there is no strong significant correlation between these inflammatory markers and IgG levels, which may indicate a divergence in humoral and cellular immune responses following vaccination compared to COVID-19 infection. Thus, SIRI and AIRI can be regarded as supportive indicators for assessing overall immune response, but cannot be used as direct quantitative measures of antibody levels.

TNF-α usually increases in the acute phase of immune activation, especially during periods of response to vaccines or viral infections. It can be said that TNF-α is a significant indicator of the activity of immune cells, as it is secreted by

several types of immune cells, including macrophages, dendritic cells, and T cells (Al-Sammarraie et al., 2024). Pfizer-BNT162b2 mRNA vaccine produces a significant elevation of CD256 APRIL which is related to its effect on the B cells and the adaptive response. In a study related to COVID-19 patients, researchers found that APRIL levels were elevated in ICU patients with COVID-19. They attributed this increase in APRIL levels to its involvement in activating B-cells, antibody production, and its contribution to the dysregulated immune response (Alturaiki et al., 2023). The strength of this study is that new hematological indices as indicators of immune response to vaccination are added to the anti-spike IgG antibodies. It is important to realize the changes in these indices, i.e., a decrease in RDW, and an increase in the LMR with non-significant changes in lymphocyte and monocyte count. Platelet indices, NLR, SIRI, AIRI, and inflammatory markers (TNF-α, CD256, ferritin) are increased in people infected with COVID-19 or vaccinated.

One of the primary limitations that contributed to the small sample size was the difficulty in locating healthy participants who were uninfected and had not previously been immunized. Furthermore, some participants were excluded from the study because they had concurrent infections that could have an impact on the results, such as urinary tract infections, seasonal influenza, and other related symptoms, indeed, the multi-collinearity diagnostic test and Bootstrap testing using a sample size of 500 showed that the impact of sample size is minimal and affects a few variables.

Long-term follow-up of vaccinated, non-infected COVID-19 people is another limitation of the study to clarify the importance of RDW and LMR as indicators for immune response.

The Pfizer-BNT162b2 vaccine induces minor, temporary changes in blood and immune markers, reflecting a normal immune response without harmful effects. Changes in RDW and LMR suggest a controlled immune activation distinct from actual infection. While these markers may help assess vaccine response, they are not reliable indicators of antibody levels and require further study.

Conclusion

The significant changes in the hematological indices and ratios showed similarity with the COVID-19 infection. The lymphocyte-to-monocyte ratio, derived from the redistribution of lymphocyte and monocyte counts, and red cell width-coefficient variation are additive markers to the anti-spike IgG antibodies of immunization with Pfizer-BNT162b2 mRNA. Although minor changes were observed in some haematological and inflammatory markers, they differed significantly from the alterations observed in COVID-19 patients. RDW-CV may serve as a differentiating marker between COVID-19 infection and Pfizer-BNT162b2 mRNA vaccination, as it has been associated with poor clinical outcomes in COVID-19 patients, in contrast to vaccinated individuals. Furthermore, LMR, NLR, and PLR may have potential utility in evaluating the immune response to the vaccine when combined with anti-spike IgG, unlike SIRI and AIRI. TNF-α is an important indicator for some immune cell activity, as well as CD-256 (APRIL), which may be an indicator for regulating and activating B-cells. Pfizer-BNT162b2 mRNA vaccine had a significant impact on the organized redistribution of immune cells. Moreover, the present study may have contributed to enhancing confidence in the use of the Pfizer-BNT162b2 mRNA vaccine as a safe immunization tool.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: All analyzed and resulting data are available in the manuscript.

Ethics statement

The studies involving humans were approved by the institutional ethical and scientific committee of the College of Medicine at the University of Diyala approved this study (code 2022 MAS260 in 2022, number 158, dated April 4, 2024). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MS, TJ, AA, and ARA conceived and performed the experiments and contributed to the analysis. MS and TJ were involved in processing the experimental data, performing the analysis, and drafting the manuscript. MS and TJ aided in interpreting the results. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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