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Role of Biochemical and Inflammatory Markers in Monitoring of Covid-19 Patients Vaccinated by Pfizer-BioNTech in Kirkuk City/Iraq

Ahmed Mater Sailh Wasme[1], Yildiz Hasan Tahseen[1], Mohammed Khaleel Turab[2*], and Kasim Sakran Abass[3]

[1]Department of Biochemistry, College of Medicine, University of Kirkuk, Kirkuk, Iraq

[2]Department of Clinical Pharmacology, College of Medicine, University of Kirkuk, Iraq, Kirkuk, Iraq

[3]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University Kirkuk, Kirkuk, Iraq

*Corresponding author:

dr.turab.pharma@uokirkuk.edu.iq

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has killed millions of people and infected millions more. Thus, there was an urgent worldwide need for the scientific community to create vaccines. A retrospective observational study was performed in Azadi teaching hospital from the period of June 2020 to December 2021 on 250 patients (106 female and 144 male patients). The cases were followed up at two different time points, firstly before receiving the vaccine, then after receiving two doses of the vaccine. Lactate dehydrogenase (LDH) enzyme, D-Dimer, CRP, S. Ferritin, S. Creatinine B. Urea and Lymphocyte %, were measured for all patients enrolled in the study before and after vaccination. The concentration levels of B. Urea, S. Creatinine, LDH, D-Dimer, S. Ferritin, and CRP were lower post vaccination. Lymphocyte percentage exhibited the highest concentration levels post vaccination. There were significant differences ($P< 0.05$) between the concentration levels of all the biomarkers pre and post vaccination with the exception of the concentration levels of S. Creatinine, and B. Urea that showed non-significant variances after vaccination. These results were true for both male and female patients. In conclusions Pfizer-BioNTech vaccines have a significant role in down regulating of some inflammatory biomarkers such as: LDH, D-Dimer, S. Ferritin, and CRP and upregulating of lymphocyte percentage. Generally, the vaccine has an insignificant impact on renal function tests in both male and female patients. The Pfizer- BioNTech COVID-19 vaccine might prompt mild side effects after the first and/or second shot.

Keywords: Covid-19, Pfizer-BioNTech vaccine, biomarkers, Kirkuk

INTRODUCTION

Corona virus disease 2019 (COVID-19) pandemic has killed millions of people and infected millions more [1]. Most Covid-19 patients were asymptomatic. However, some of patients experienced respiratory distress syndrome (RDS) which necessitated hospitalization [2]. Thus, there was an urgent

worldwide need for the scientific community to create vaccines [3]. Many studies have shown that when a new vaccine is introduced, a range of factors affect the vaccine's social acceptance. These worries included the new vaccines' safety and efficacy, for instance the acceptance proportion ranged between 8% and 67% when the vaccine was first released during a previous pandemic of H1N1 influenza A [4].

The Pfizer-BioNTech (BNT162b2) COVID-19 vaccine received an emergency use authorization from the Food and Drug Administration (FDA) on December 11th, 2020. The vaccine is a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine that was designed to prevent COVID-19 [5]. One huge randomized, double-blind, placebo-controlled Phase II/III clinical trial with almost 43,000 participants (median age = 52 years, range = 16-91 years) provided the majority of the data supporting the effectiveness of Pfizer-BioNTech COVID-19 vaccine. According to the preliminary results from this clinical trial, the Pfizer-BioNTech COVID-19 vaccine was 95.0% effective in preventing symptomatic laboratory-confirmed COVID-19 in people without evidence of prior SARS-CoV-2 infection [6]. Messenger RNA (mRNA) is the key component of the Pfizer-BioNTech and Moderna COVID-19 vaccination technologies. The COVID-19 mRNA vaccines provide instructions on how to make a harmless fragment of a S protein, which is a spike-shaped surface feature present on the coronavirus surface. Following vaccination, cells begin producing more of the protein fragments and exposing them to the surfaces of the coronavirus. The immune system will recognize that the protein does not belong there and began to produce antibodies and immune response [6]. The process of developing immunity following a vaccination might occasionally result in undesirable side effects. Following the first and/or second shot, the Pfizer-BioNTech COVID-19 vaccine may cause mild side effects, such as soreness, redness, or swelling at the injection site, fatigue, headaches, chills, muscle and joint discomfort, and fever. These signs can be a symptom that the body is building the required defense immunity [7].

The severity of COVID-19 assessed using a variety of biomarkers. These biomarkers could be used to manage patient treatment processes and predict their prognosis. The COVID-19 routinely employs some biomarkers such as white blood cells (WBCs) counts, neutrophil, lymphocyte, platelet, D-dimer, CRP, Procalcitonin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Urea, Creatinine, Ferritin, Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), and International Normalized Rate (INR). Vaccination to COVID-19 might effect these biomarkers levels [8]. Concerns about adverse reactions prompted the necessity to conduct a study on adverse reactions following COVID-19 vaccination from the various vaccine manufacturers that are accessible in Iraq. The main aim of the present study was to evaluate the biochemical marker's importance in assessing the effectiveness of the Pfizer-BioNTech vaccine among Covid-19 patients from Kirkuk city-Iraq.

MATERIALS AND METHODS

Study design and sample collections

A retrospective observational study was performed in Azadi Teaching Hospital/ Kirkuk from the period of June 2020 to December

2021 on 250 patients involved (106 female and 144 male patients). The cases were followed up at two different time points, firstly before receiving the vaccine, then after receiving two doses of the vaccine. A questionnaire form was utilized to gather basic information from patients (age, sex, history of chronic diseases). A 5 ml of blood sample was withdrawn from each patient and separated into two amounts; 2.5 ml of whole blood put in EDTA tube for complete blood count (CBC), and the rest placed in a gel tube to obtain a clear serum for the biochemical assays.

Biochemical, and Hematological assays

Lactate dehydrogenase (LDH) test measures the level of Lactate Dehydrogenase in blood; steps were followed in accordance with the manufacturer's instructions (From Fujfilm, Lot No. 324712, Japan) [9]. D-Dimer test is performed through the iChroma quantitative test; steps were followed in accordance with the manufacturer's instructions (From iChroma^{TM}, Lot: DDRHN06, Korea) [10]. Serum Ferritin (S. Ferritin) levels were evaluated using the Ferritin CLIA test (From Mindary, Lot No. 2022090111, Thailand) [11]. Blood urea (B. Urea) concentrations were estimated through the enzymatic colorimetric test (From Biomaghreb, Ref:20141, Spain) [12]. Serum Creatinine levels were measured through the Creatinine Jaffé Gen.2 test [13]. C - reactive protein (CRP) levels were measured through the latex agglutination test (From Angstrom Biotech PVT. LTD) [14]. Lymphocyte percentage was measured through the latest fully automated hematology autoanalyzer from GENEX Labs, USA.

Exclusion criteria

Patients who passed away were excluded from the study. Pregnant women and children were also excluded from the study. Patients who lost connection were excluded.

Statistical Analysis

Statistical Package for Scientific Services (SPSS) version 26 was used to conduct computerized statistical analysis, using the paired sample T-test, one-way ANOVA (for continuous variables), and probability to compare data (P value). Findings were considered significant when the P value was less than 0.05, statistically significant, and highly significant when the P value was less than 0.01 (HS). The Bland-Altman method was used to compare the two methods [14]. All statistical analysis was carried out using MiniTab statistical software version 15.

RESULTS

The means levels of B. Urea, S. Creatinine, LDH, D-Dimer, S. Ferritin and CRP were lower after taking the vaccine compared to before administration of the vaccine, with the exception of lymphocyte percentage which showed highest mean levels after taking the vaccine. There were highly significant differences in regards to the mean levels of the all biomarkers before and after taking the vaccine with the exception for the mean level of B. Urea that exhibited non-significant differences (P>0.5). Paired sample t-test was used to analyze these findings as shown in Table 1.

However, in female patients there was a decrease in the means levels of (S. Creatinine, LDH, D-Dimer, S. Ferritin, and CRP) after taking the vaccine in comparison to levels before having the vaccine, while the lymphocyte percentage displayed higher levels after taking the vaccine. With the exception of the mean level of B. Urea, which found non-significant differences, there were extremely significant differences between the mean levels of all the biomarkers before and after receiving immunization as shown in Table 2.

Male patient's mean levels of (B. Urea, S. Creatinine, LDH, D-Dimer, S. Ferritin, and CRP) reduced, but the lymphocyte percentage showed greater levels following vaccination. There were extremely substantial variations between the mean levels of all biomarkers before and after receiving the vaccination, with the exception of the mean level of B. Urea, and S. Creatinine which revealed non-significant differences (P> 0.5) as shown in Table 3. The linear regression of means biochemical and inflammatory markers levels of female and male patients is shown in (Figure 1 and 2). The R^2 values was tended to be very high in both cases of before and after vaccination (R^2 = 0.96, P < 0.0001 in female Figure 1, wheras R^2 = 0.95, P = 0.0002 in male Figure 2).

The Bland and Altman plot of the ratio of two vaccinations before and after showed mean differences between two vaccinations to be 70.6 and SD was 143.8 and -6.2 for upper and lower limits, respectively for female patients (Figure 3), while for male patients the mean differences between two vaccinations were 75.33 and SD was 148.27 and -0.48 for upper and lower limits, respectively (Figure 3).

Table 1. Biochemical and inflammatory markers levels before and after taking Pfizer-BioNTech vaccines.

Variables	Before Vaccination	After Vaccination	P. Value
B. Urea	39.12±1.14	38.68±1.57	0.828
S. Creatinine	1.05±0.03	0.92±0.04	0.027*
LDH	367.90±14.03	161.06±4.65	0.000**
D-Dimer	458.86±18.21	185.80±6.35	0.000**
S. Ferritin	229.68±12.02	88.76±4.49	0.000**
CRP	26.93±2.65	7.96±0.54	0.000**
Lymphocyte %	13.16 ±0.76	28.24±0.48	0.000**
Values in the table are the mean± SE *P < 0.05: Significant in the same column, **P <0.01: Highly significant in the same column. P value greater than 0.05: Non-significant in the same column.			

Table 2. Biochemical and inflammatory markers levels before and after vaccination with Pfizer-BioNTech vaccines in female patients.

Variables	Before Vaccination	After Vaccination	P. Value
B. Urea	40.17±1.45	40.38±2.11	0.940
S. Creatinine	1.16±0.07	0.92±0.07	0.018*
LDH	439.35±21.84	168.00±8.20	0.000**
D-Dimer	410.23±27.46	183.97±10.30	0.000**
S. Ferritin	163.50±11.04	66.41±3.14	0.000**
CRP	26.94±4.41	5.86±0.53	0.000**
Lymphocyte%	8.32±0.59	28.85±0.75	0.000**
Values in the table are the mean± SE Pfizer-BioNTech vaccines in female patients. *P< 0.05: Significant in the same column, **P <0.01: Highly significant in the same column. P value greater than 0.05: Non-significant in the same column.			

Table 3. Biochemical and inflammatory markers levels before and after vaccination with Pfizer-BioNTech vaccines in male patients

Variables	Before Vaccination	After Vaccination	P. Value
B. Urea	38.20±1.72	37.20±2.29	0.737
S. Creatinine	0.95±0.02	0.92±0.05	0.580
LDH	305.61±10.88	155.02±4.89	0.000**
D-Dimer	497.51±22.61	187.41±7.92	0.000**
S. Ferritin	287.38±15.25	108.25±6.54	0.000**
CRP	26.92±3.20	9.79±0.79	0.000**
Lymphocyte %	17.38 ±0.87	27.71 ±0.63	0.000**
Values in the table are the mean± SE Pfizer-BioNTech vaccines in male patients. *P < 0.05: Significant in the same column, **P<0.01: Highly significant in the same column. P value greater than 0.05: Non-significant in the same column.			

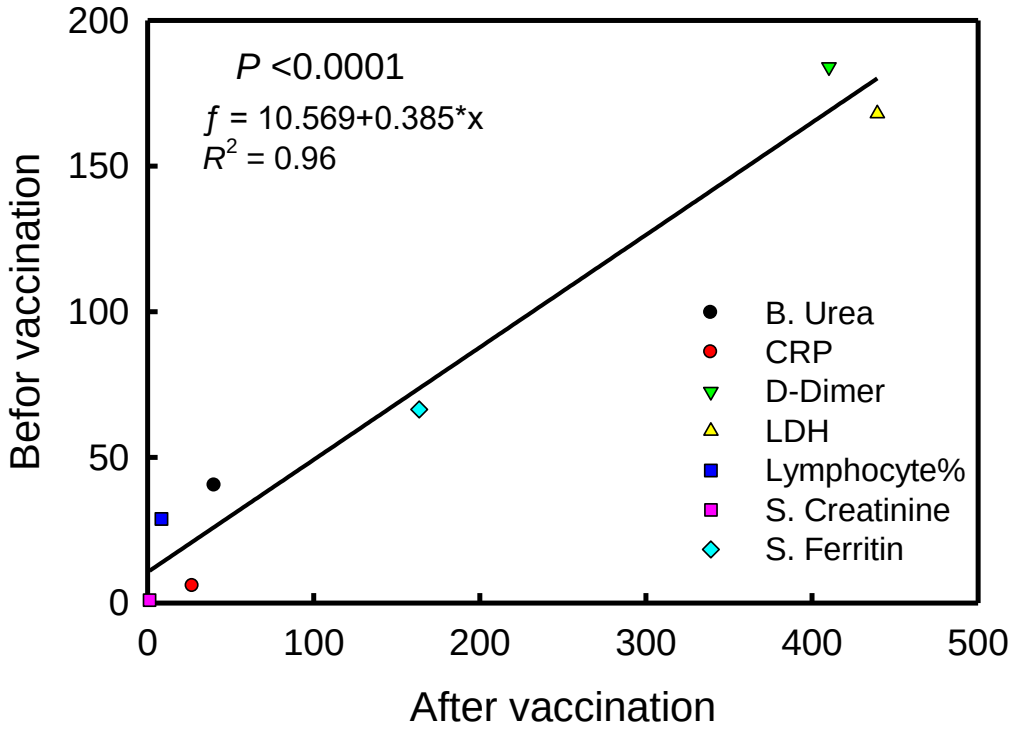


Figure 1. Regression analysis of the biochemical and inflammatory markers levels before and after vaccination with Pfizer-BioNTech vaccines in female patients.

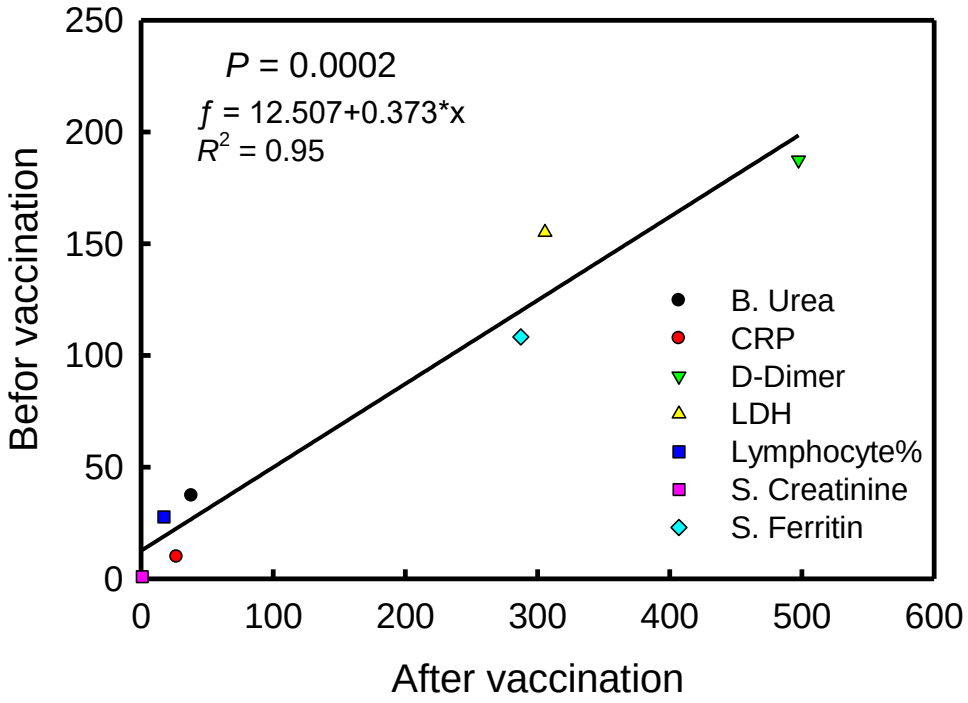
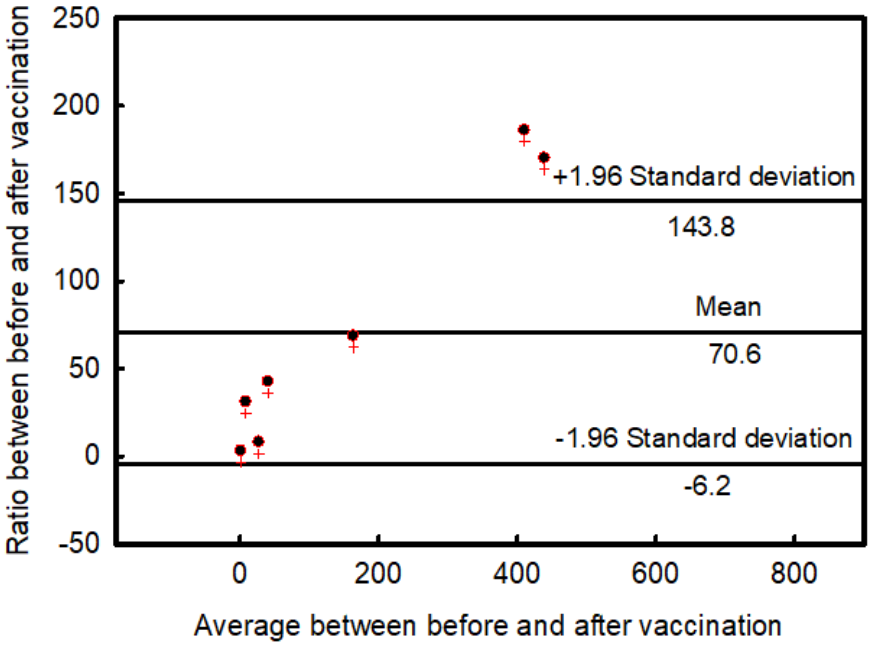


Figure 2. Regression analysis of the biochemical and inflammatory markers levels before and after vaccination with Pfizer-BioNTech vaccines in male patients.



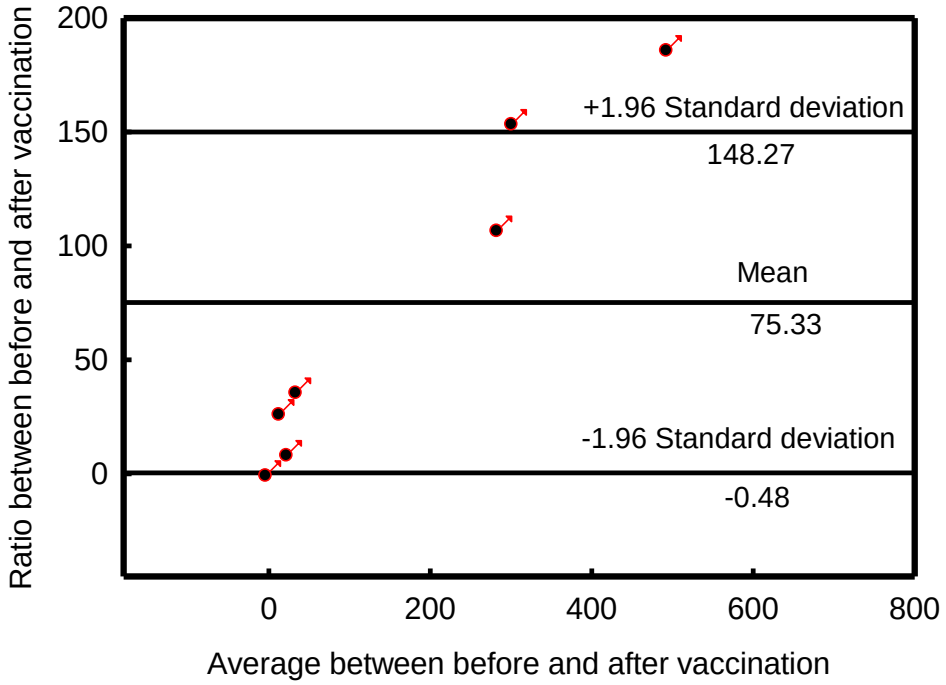


Figure 3. Bland and Altman plot of the ratio of the before and after vaccination (plotted on the y-axis) versus the average of the two methods (x-axis) for the biochemical and inflammatory markers levels in female and male patients. Horizontal lines are drawn at the mean difference, and at the mean difference ± 1.96 SD of the differences (dashed line). If the differences within mean ± 1.96 SD are clinically not important, the two methods cannot be used interchangeably.

DISCUSSION

The COVID-19 pandemic caused heavy global mortality and morbidities that are linked to the illness. The COVID-19 vaccinations were deployed in a preventative strategy to reduce and manage these outcomes. However, certain peculiar side effects were observed in addition to these findings. Vaccine side effects are rather typical. They show that the immune system is working and that vaccinations are successful. In order to evaluate the vaccine's safety, observation of adverse reactions and side effects is essential [15]. The COVID-19 routinely employs biomarkers: white blood cell, neutrophil, lymphocyte, platelet, D-dimer, CRP, AST, ALT, LDH, Urea, Creatinine and Ferritin [8]. [8]

Overall, both male and female patients showed that the concentration levels of LDH, D-Dimer, S. Ferritin, and CRP were lower following vaccination, with the exception of lymphocyte percentage, which exhibited the greatest concentration levels post vaccination. Apart from the concentration levels of B. Urea, which showed non-significant differences ($P > 0.5$), there were extremely significant differences between the concentration levels of all the biomarkers pre and post vaccination. These results were true for both male and female patients as shown in table 2 and 3 respectively; these findings were in accordance with the results reported by

AlKhafaji et al., who found highly significant differences in the levels of LDH, D-Dimer, S. Ferritin, and CRP, and Lymphocyte % before and non-significant differences in regards to S. Creatinine and B. Urea concentration levels before and after getting vaccination. These results showed disagreement with our findings in regards to the S. Ferritin levels, where they revealed non-significant differences in S. Ferritin levels post vaccination, which might be due the differences in the kinetic responses of S. Ferritin to vaccination in these areas [16]. These findings were also near to results found by Di Bella et al., who did not report significant inflammation-related symptoms and noticed that following vaccination, seven patients had increased D-dimers. C-reactive concentration level exhibited some elevation post vaccination and in terms of blood count and LDH levels; there were no notable changes in their levels post vaccination. D-dimer revealed elevation after vaccination which disagreed with our results, but only seven patients that showed this type of elevation, so the investigators reported this as a non-significant change. Furthermore, the types of patients are different from our study in that they might have a history of cardiac diseases. Additionally, these reported differences might be attributed to the fact that the researcher conducted their research on patients who were pancreas transplant recipients [17]. On the other hand our results showed some differences with a study conducted in Samarra, Iraq, that disclosed when compared to their pre-vaccine levels, LDH and D-dimer levels were significantly higher after receiving the first and second doses of the Pfizer vaccine. This may be due the differences in the patients group, number of patients, the variation of the reaction to the vaccine. Moreover these patients might suffer from comorbid conditions such as hypertension and cardiac diseases that led to differences in their D-Dimer and LDH levels [18].

In terms of measurement of S. creatinine levels, male patients had higher levels after vaccination, but female patients had slightly lower levels. The concentration levels of the various biomarkers varied greatly amongst the research groups. This was in accordance with findings of a study conducted by Lebedev et al., who observed acute kidney injury and minimal changes in renal function tests in a male patients which developed quickly after the initial injection of the BNT162b2 COVID-19 vaccine [19]. This agreed with an earlier study conducted in Kirkuk city, Iraq, by Kamal BJ and Khalaf MA, who stated that the majority of those who received vaccines only experienced moderate symptoms, through checking of LDH, S. Ferritin, D-Dimer, and CRP levels [20]. The present study moreover demonstrated that unvaccinated female patients revealed higher concentration levels of S. Creatinine compared to unvaccinated male patients, this was also true for LDH and CRP levels, which was in line with a study conducted in Duhok city, Iraq, which found that females who were not vaccinated had greater infection rates [21]. This was also accordance with findings of Maamar et al [22]. On the other hand, the study performed by Khazaal and Çanlı, detected that lymphocytes considerably increased in unvaccinated female patients in comparison to male patients which was different to our results, and they detected that D. Dimer greatly increased in female patients in comparison to male patients which was also different from our findings [23]. These differences can be attributed to the fact that hormonal changes among female patients

might have a role in these differences. Linear regression of mean biochemical and inflammatory markers levels was observed in all mean samples before and after vaccinations (Figure 1 and 2). The mean differences in the inflammatory and biochemical markers between two vaccinations are plotted by Bland and Altman plot and showed clinically important in male, due to the mean was found lesser than ± 1.96 (Figure 3).

CONCLUSIONS

Pfizer-BioNTech vaccines have an important role in down regulating some inflammatory biomarkers such as; LDH, D-Dimer, S. Ferritin, and CRP and upregulating Lymphocyte percentage. Generally the vaccine has insignificant impact on the renal function tests in both male and female patients. The Pfizer- BioNTech COVID-19 vaccine might prompt mild side effects after the first and/or the second shot.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval: On 31 May 2023, an ethical approval was given by the ethical committee of Kirkuk Medical College-University of Kirkuk. The head of ethics committee (Prof. Dr. Ayla Kh. Ghalib). Ethic reference No.: 37.

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دور المؤشرات البيوكيميائية والالتهابية في مراقبة مرضى كوفيد-19 الذين تم تطعيمهم بشركة فايزر-بيونتيك في مدينة كركوك/العراق

أحمد مطر صالح وسمي[1]، يلدز حسن تحسين[1]، محمد خليل تراب[2*]، قاسم سكران عباس[3]

[1] فرع الكيمياء الحياتية، كلية الطب، جامعة كركوك، كركوك، العراق

[2] فرع الادوية السريرية، كلية الطب، جامعة كركوك، العراق، كركوك، العراق

[3] فرع الفسلجة والكيمياء الحياتية والادوية، كلية الطب البيطري، جامعة كركوك، كركوك، العراق

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الخلفية والأهداف : أودت جائحة مرض فيروس كورونا 2019، والمعروفة أيضًا باسم كوفيد-19، بحياة الملايين وأصابت ملايين آخرين. ونتيجة لذلك، أصبحت لدى المؤسسات البحثية العلمية في المجتمع حاجة عالمية ماسة وعاجلة لتطوير اللقاحات.

منهجية الدراسة : تم إجراء دراسة رصدية بأثر رجعي شملت 250 مريضًا (106 أنثى و144 ذكرًا) في مستشفى آزادي التعليمي في محافظة كركوك-العراق بين يونيو 2020 وديسمبر 2021. تم رصد الحالات مرتين؛ قبل وبعد إعطاء التطعيم، في فترات زمنية منفصلة. بالنسبة لكل مريض مشمول في هذه التجربة، تم إجراء قياسات لإنزيم هيدروجين اللاكتات (LDH)، نسبة وجود D-dimer في الدم، اختبار البروتين المتفاعل C-Reactive Protein (CRP)، مستوى الفريتين في الدم S. ferritin. مستوى الكرياتينين في الدم S. creatinine.، مستوى اليوريا الدم B. urea.، ونسبة الخلايا الليمفاوية قبل وبعد التطعيم. بعد التحصين، كان هناك انخفاض في تراكيز اليوريا الدم، مستوى الكرياتينين في الدم، تركيز إنزيم هيدروجين اللاكتات، نسبة D-dimer في الدم، مستوى الفريتين في الدم، وكذلك البروتين المتفاعل CRP. بعد التحصين ظهرت نسبة الخلايا الليمفاوية بتراكيز اعلى.

النتائج : كانت هناك فروق ذات دلالة إحصائية ($P<0.05$) بين مستويات تركيز جميع المؤشرات الحيوية قبل وبعد التطعيم باستثناء مستويات تركيز S. Creatinine وB. Urea التي أظهرت اختلافات غير معنوية بعد التطعيم. وكانت هذه النتائج صحيحة لكل من المرضى من الذكور والإناث. الخلاصة، تلعب لقاحات Pfizer-BioNTech دورًا رئيسيًا في تنظيم نسبة الخلايا الليمفاوية وتقليل تنظيم العديد من مؤشرات الالتهاب، بما في ذلك LDH وD-Dimer وS. Ferritin وCRP.

الاستنتاج : بشكل عام، تظهر اختبارات وظائف الكلى لدى المرضى من الذكور والإناث تأثيرات ضئيلة من التطعيم. وقد تحدث تأثيرات ضارة خفيفة من لقاح Pfizer-BioNTech ضد كوفيد-19 بعد الجرعة الأولى و/أو بعد الجرعة الثانية.

الكلمات الدالة: كوفيد-19، لقاح فايزر-بايونتك، المؤشرات الحيوية، كركوك