Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 2, 561-567

Interleukin-1 Beta (IL-1β) Persistence in Post SARS-CoV-2 Infection and Vaccination: A Double Case Control Study

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Received: 1/6/2022 Accepted: 17/10/2022 Published: December 20, 2022

Abstract: With the continuous increase in the number of infections with COVID-19 in comparison to the relatively slow increase in the number of vaccinated individuals in our country, the efficiency and longevity of protection against SARS-CoV-2 infection compared to vaccine recipient individuals or even to a combination of previously infected and respective vaccination is yet unclear. The IL-1β levels using an enzyme linked immunosorbent assay (ELISA) assay were investigated in the serum samples of convalescent and vaccinated groups of both sex and different ages after one month of infection and vaccination and compared to samples from apparently healthy individuals, matching other groups in age and sex, as control group to evaluate and compare immune responses between the groups. A total of 75 convalescent patients (mean age 54.1±19.4 years, 54.67% males) and 75 vaccinated participants (mean age 39.8±14.3, 50.67% males) with two doses of Pfizer (62.7%) or Sinopharm (37.3%) vaccine were analyzed and compared to 50 unvaccinated controls. The results revealed an elevated serum level of IL-1β in both the convalescent and vaccinated groups, which indicated a robust natural infection and vaccine elicited immune response, with the presence of long COVID-19 syndrome in the patients who recovered from SARS-CoV-2 infection.

Keywords: SARS-CoV-2, IL-1β**,** Convalescent, Vaccinated, Pfizer, Sinopharm, post COVID-19 syndrome.

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Introduction

The SARS-CoV-2 is a beta-coronavirus with genetic similarity to SARSCoV-1 which is the etiological agent of severe acute respiratory syndrome (SARS). Since its emergence, the disease has been a challenge and a threat both on public health and economical level. The novel coronavirus has impacted negatively on Iraq like other nations. Numerous variations in the most important epidemiological indices have been reported. Major flaws in the health care systems were exposed by the pandemic, and people throughout the globe are still awaiting a solution to control this unforeseen calamity (1).

Thus, scientists and doctors around the world have been investigating the virus epidemiology, genomic variations, clinical characteristics, and preventive and therapeutic interventions (2). One of their main focuses is the relationship of the disease and its severity with the immune system response of the infected and vaccinated individuals against SARS-CoV-2. In a recent local study by Abduallah *et al.* (3), the importance of all biomarkers was proved to be vital to predict SARS-CoV-2 prognosis and severity. While the immune system is constantly functioning and conducting

surveillance, its activity is heightened when an individual becomes sick or vaccinated (4,21).

Among these biomarkers is the interleukin 1 beta, which considered the most studied interleukin in the interleukin 1 family because of its function in mediating autoinflammatory diseases. It is a powerful pro-inflammatory cytokine that plays a major role in the body's immune responses to pathogens. IL-1β is secreted by the cells of the innate immune system (e.g., monocytes and macrophages) in the early stages of SARS-CoV-2 infection and has a critical role in the strong proinflammatory response known as cytokine storm (5). Suppression of pro-inflammatory IL-1 family members, particularly IL-1 β, in SARS-CoV-2 infected patients has been shown to have a therapeutic effect in a variety of inflammatory disorders, including viral infections (6). It is necessary to continuously evaluate and compare these levels in both previously infected and vaccinated individuals, in order to determine when immunity is waning in certain population and whether further vaccination doses are necessary to regain protection (7).

Materials and method

Two hundred samples were collected from participants during the period from December 2021 to June 2022. The samples from convalescent patients were obtained from different individuals, Hospitals (Dar Al- Salaam 1 field hospital and Al Shifa Specialized Center), and private laboratories (Istishari Medical lab, Diqat Al Rawaa, and Al-Safa Private Laboratories). The study included both sex at different age ranged between 20-80 years.

Ethical approval

This study was approved by Institute of Genetic Engineering and Biotechnology for Post Graduate Studies committee, and Research Committee in Health Departments of Iraqi Ministry of Health (Form number 01/2021).

Molecular diagnosis of SARS-CoV-2 infected participants

All enrolled participants must be tested positive for COVID-19 in order to be included in this study and were followed up after one month from the test. A nasopharyngeal swab was obtained for PCR according to the WHO established protocol for the detection of novel coronavirus. Precautions, safety, and appropriate personnel protective equipment PPE were followed during the process. The test was performed on samples taken from patient through nasopharyngeal swab and preserved in disposable viral sampling tube. The RNA was extracted automatedly according to manufacture protocol (Zybio Inc, China, kit #YXB20180096) based on Magnetic Bead Method using nucleic acid isolation system (EXM6000). Then, the SARS-CoV-2 RNA was qualitatively detected in the specimen through One Step RT-PCR method using SARS-CoV-2 Nucleic Acid Detection Kit (cat. # CoV2-96) by reversed transcribed to complementary DNA (cDNA) in the RT-PCR reaction system and combined with specific primers and probes for PCR amplification (FAM, ROX, and VIC). The PCR amplification parameters were set up according to manufacturer recommendation as shown in Table (1). When reaction ended the results were analyzed and clamed positive when the Ct of two targets (FAM, ROX) < 40. Interpretation and

explanation of results were according to criteria provided with the kit (Table 2).

Table (1): PCR amplification parameters setup

Steps		Temperature	Time	Cycle
1	UNG reaction	37 °C	1 min	1
2	Reverse transcription	50 °C	5 min	1
3	Initial denaturation	95 °C	2 min	1
4	Denaturation	95 °C	5 sec	45
5	Amplification and fluorescence detection	60 °C	30 sec	

Table (2): Test results explanation

Results	Criteria
Positive	The amplification curves of FAM and ROX fluorescence channels are typical S-shaped and Ct < 40, suggesting SARS-CoV-2 is positive. FAM indicates N gene, ROX indicated ORF1 ab test result.
Negative	FAM and ROX fluorescence channels are not detected or Ct= 45, and VIC channel Ct <40, suggesting SARS-CoV-2 is negative
Gray zone	FAM or ROX fluorescence channel 40 ≤ Ct values <45, and VIC channel Ct values < 40, indicating that result is in gray zone and need re-test, if the results are same and show typical S-shaped curves, it is judged as positive, otherwise it is negative.
Invalid	Ct values= 45 or no value in FAM and ROX fluorescence channels, and Ct values ≥ 40 or no value in VIC channel, indicating that the result is invalid, and re-test is needed.

Detection of human IL-1β by ELISA test

Three ml of whole blood was collected from 200 of both sex at different age (75 convalescent, 75 vaccinated, and 50 control groups) in gel tubes, and centrifuged for 15 minutes at 3000 rpm to obtain the serum for the detection of IL-1β. Double antibody sandwich ELISA detection method was used for quantitative detection of Human IL-1β using MyBioSource Human Interleukin 1 Beta (IL-1β) ELISA Kit (USA) at 450 nm wave length. The principal of this technique is based on the characteristics of a target analyte with more than two possible epitopes which can be identified by both the pre- coated capture antibody and the detection antibody simultaneously.

Statistical analysis

The statistical analysis was performed by using the IBM Statistical Program for Social Science (SPSS) software version 28.0 (SPSS, Chicago, IL, USA). The quantitative data were expressed as Mean with probability $P \leq 0.05$ considered to be significant and $P > 0.05$ was considered not significant. Additionally, t-test was utilized to compare among two means. The analysis of variance Chi-square test (χ^2) and one- way ANOVA were used to determine the significant differences between demographic variables and multiple comparisons.

Results and discussion

The findings of (SARS - CoV-2) nucleic acid testing who visited public and private labs in Baghdad governorate were tested positive to SARS-CoV-2 antigen by PCR were:

1- Interleukin- 1 beta (IL-1β) level in convalescent patients

The results of the ELISA assay revealed that the level of IL-1β was significantly higher in convalescent patients compared to healthy controls

with a p-value less than 0.00001 as shown in Table (3).

Table (3): Statistics of IL-1β for convalescent patients and control

	Group	N	Mean	Std. Deviation	Std. Error Mean
IL-1β	**Convalescent Patients**	75	895.07	131.94	15.23506
	Control	50	281.45	91.48	12.93763
P-value is < 0.00001					

****** Highly significant at p < 0.00001.**

Comparing the detection range provided by the manufacturer (15.6 pg/ml-1000 pg/ml) to the results range of the convalescent patient titers (1159.94 pg/ml- 282.82 pg/ml), it was found that some individuals sustained high level of circulating IL-1β after one month of infection. From the total number of tested samples, 19% of them found to be above detection range. The excessive pro-inflammatory release post COVID-19 infection especially the elevated levels of IL-1β titers was explained in many literature publications and the results of this study was in line with them. In two separate studies involved longitudinal analysis of elevated levels in proinflammatory signatures, including IL-1β, on individuals' samples who have previously infected with SARS-CoV-2, Ong *et al.* (8) and Acosta- Ampudia (9) results showed deregulation of systemic cytokines in people recovered from SARS-CoV-2. They also pointed out the possibility to drive chronic inflammation if immune dysregulation continued in recovered patients from SARS-CoV-2. Moreover, Merad and Martin (10) found that the probability of the persistent relapsing symptoms was the result of an ongoing excessive cytokine release response in all known patients recovered from the novel coronavirus with long symptoms. The excessive reaction of these individuals to the continuing production of cytokines is essential to comprehending the pathophysiology of their condition. Furthermore, when sex was tested to investigate the relationship with IL-1β level, it was found that despite the higher IL-1β titers in males than in females, and the higher mean value for males (901.18 pg/ml) that the mean value for females (887.71 pg/ml), however, the findings were statistically insignificant (p =.665). Similarly, as what was found with sex, the results of age group relationship to the IL-1β level were also non-significant and no difference was found in the levels of IL-1β across the various age groups (P-value 0.757). This was determined by analyzing the data summarized in Table (4).

Table (4): Relationship of IL-1β levels with age and sex

Sex	Age Group	Mean	Std. Deviation	N
Male Mean = 901.18	**Young**	886.85	165.98	11
	Middle Age	930.61	102.07	5
	Elderly	901.6	101.63	25
	Total	**901.18**	**119.55**	**41**
Female Mean = 887.71	**Young**	869.47	233.98	12
	Middle Age	880.03	107.6	6
	Elderly	904.27	54.34	16
	Total	**887.71**	**147**	**34**
P-value 0.665$^{N.S}$	**P-value 0.757$^{N.S}$**			

N.S Non-significant.

The relationship between IL-1β level with sex and age is intriguing and also based on speculation. For example**,** when Schultheiß *et. al* (11) noticed the IL-1β elevated level in the participants who had previous infection with SARS-CoV-2 and studied the post COVID-19 syndrome correlation to IL-1β level, they discovered that the correlation was independent of age and sex. In contrast to some post COVID-19 syndrome studies related to cytokine elevation level estimation reported that female sex and old age have higher risk to develop post COVID-19 syndrome irrespective the severity of the disease (12,13). The reasons can be ranged from the hyperimmune impacts of estrogen in females contributing to an enhanced cytokine response and autoimmune propensity which is well established, to the fact that females had 43% more olfactory bulb cells and 50% more olfactory neurons than males which may raise the likelihood of viral transmission through the olfactory-to-CNS pathway. In addition, elder people tend to have lower immunity due to the presence of comorbidities and drug intake which inhibit the function of IL-1β and reduce its serum level (14,15).

2- Interleukin- 1 Beta (IL-1β) level in vaccinated participants

As part of the innate immune response, IL-1β levels in the serum of post-vaccinated participants were investigated to assess immune response after one month of vaccination. The results in Table (5) appeared a higher mean value of IL-1β titer for the vaccinated group (726.77 pg/ml) than the control group (281.45pg/ml). The highest IL-1β titer was (939.25 pg/ml) to (424.73 pg/ml) as the lowest titer in the vaccinated group, while the highest IL-1β titer was (398.24 pg/ml) and the lowest titer was (79.76pg/ml) in the control group. These results indicated a considerable difference between the two groups with a P- value ≤ 0.001, which means that result was highly significant. Moreover, the IL-1β titers were tested to see if there was a correlation between the titer and the type of vaccine included in this study and result had revealed a significant correlation with P- value less than 0.003 between them.

The above results proved the stimulation of IL-1β by COVID-19 vaccine and its maintenance at relatively at high level when compared to control group. These results were also proved in many studies assessing immune response to different types of vaccines. As Zhang *et al.* (16) and Mata- Miranda *et al.* (17) reported that both inactivated and mRNA vaccines showed increased cytokine levels in general and in IL-1β level specifically post vaccination. Nevertheless, in this study Sinopharm vaccine showed to have less mean value (675.96pg/ml) than Pfizer vaccine (757.04pg/ml), which may indicate lower protection against the SARS-CoV-2 for individuals vaccinated with the inactivated vaccine. This result was consistent with what Mohe and Al-Thwani (18) reported that Sinopharm vaccine gave insufficient immunity to individuals who were vaccinated with it.

Table (5): Statistics of IL-1β for vaccinated participants and control and the correlation between vaccine type and IL-1β level.

IL-1β		N	Mean	Std. Deviation	Std. Error Mean	P-value
Group	Vaccinated	75	726.77	117.24	13.54	≤ 0.001
	Control	50	281.45	91.48	12.94	
Vaccine type	Pfizer	47	757.04	105.93	15.45	≤0.003
	Sinopharm	28	675.96	119.51	22.59	

**** Highly significant at p ≤ 0.001 and ≤ 0.003.**

The relationship between IL-1β serum level with age and sex have been tested also. The results in Table (6) displayed that there was no significant association between IL-1β and age (P-value 0.509), and sex (P-value 0.691). In a study by Björk *et al.* (19) and Collier *et al.* (20), they didn't find any relation of IL-1β level to age and sex which was confirmed the results presented in this study.

Table (6): Statistics of IL-1β for vaccinated individuals in relation to age and sex

IL-1β		Female	Male	P- value
Sex	Frequency	37	38	$0.689^{N.S}$
	%	49.33%	50.67%	
	Mean	721.23	732.17	
	Std. Deviation	124.29	111.36	
Age	Mean	36.16	43.39	$0.509^{N.S}$
	Std. Deviation	12.95	14.74	

N.S Non-significant.

Conclusion

In contrast to infections with other prominent coronaviruses, our results clearly imply that SARS-CoV-2 infection is accompanied by a persistent inflammatory response. The present understanding of the natural history of post COVID-19 syndrome and its pathophysiological causes is speculative. However, this information is vitally required for the development of sensible therapy solutions. Moreover, Persistent of these immunological signatures in the serum of participants after one month of two dose vaccination is an evidence of good COVID-19 vaccines immunogenicity for their effectiveness in provoking immune response and protection they provide in sample of Iraqi population. Additionally, there is an imminent need for research that pertain to the post COVID-19 syndrome immune response and are more comprehensive, as they might lead to a greater comprehension of the relationship between IL-1β with age and sex.

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