Interleukin-10 levels in relation to COVID-19 vaccine

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Abstract

A total of 120 cases were incorporated in the current study, who were divided into 4 groups: (Covid-19, non-vaccinated); (Covid-19, vaccinated); (non-Covid-19, non-vaccinated); (non-Covid-19, vaccinated). Each group involved 30 cases. The study was conducted in Cihan University-Erbil campus in November 2021. All subjects were asked questions regarding their history, using a questionnaire sheet. Blood was investigated for the Interleukin-10 level, Lactate Dehydrogenase (LDH) and hematological parameters. The mean serum level of IL-10 was significantly higher in Covid-19, non-vaccinated than Covid-19, vaccinated individuals. Moreover, the level was also equally significant and higher in non-Covid-19, vaccinated than non-Covid-19, non-vaccinated. Non-significant differences were found between Covid-19, vaccinated and non-Covid-19, non-vaccinated individuals. And between Covid-19, non-vaccinated and non-Covid-19, vaccinated individuals. The mean serum levels of LDH were found to be significantly lower in non-Covid-19, non-vaccinated, indicating a significant rise of LDH in all other groups. Despite the rise in all three groups a significant difference was not found between them. The total WBC count shows a significant increase in both non-vaccinated groups. While the mean level of lymphocytes counts reveals non-significant decrease in non-Covid-19, vaccinated group in comparison to the other groups. However, the mean monocyte counts of Covid-19, vaccinated group revealed a significant rise in comparison to other groups. In addition, the mean counts of granulocyte showed a significant increase in both non-vaccinated groups in comparison with vaccinated groups. Platelet mean count presented a non-significant difference when comparing all groups. Our current study may indicate the potency of the vaccines. However, the rise in the LDH needs further investigations to show the reason and the effect. Larger sample size is also required for our current study to verify the ability of the vaccine to change the levels of IL-10, LDH and WBC counts.

Introduction

Countries along with communities strive in an expeditive manner to establish methods of effectiveness to minimize the coronavirus transmission. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has shown a pattern of pandemic spread in just a few months. SARSCoV-2 is the virus's name, and the disorder it produces is called "coronavirus disease 2019" (COVID-19) [1, 2]. In December 2019, China reported to the World Health Organization (WHO) multiple cases of a human related respiratory infections corelated to a large open market specialized in seafood and poultry in Wuhan, the place where the chrysanthemum bats suspected in initiating the pandemic [1, 3]. Cases have been recorded all around the world, with over 700 cases reported across one cruise only, illustrating the numerous possibilities of transmission [1, 4]. The infection can cause serious respiratory illness and death because of these consequences (especially in the elderly as well as those with chronic illnesses), or it can spread asymptomatically [1, 5, 6]. With severe acute respiratory CoV-2's transmission from one person to another, in asymptomatic patients, provides an exclusive perplexing public health issue [1]. The serious disease appears to occur in around 14 per cent to 16 per cent of patients [2, 6]. Comprehensively, the COVID-19 case fatality in patients who appear for medical examination is 2.3

per cent, that can vary from 8-15% in larger communities [1, 6, 7].

The anti-inflammatory cytokine Interleukin-10 (IL-10) is a significant immune response regulator. In macrophages, IL-10 inhibits the metabolic program alteration which is triggered by inflammatory stimuli. IL-10 suppresses both the glycolysis and the absorption of lipopolysaccharide-induced glucose while promoting oxidative phosphorylation. IL-10 stimulates mitophagy, which removes damaged mitochondria with insignificant membrane potentiality along with significant levels of oxygen species. The animal model of colitis and chronic gastrointestinal disease patients, macrophages accumulate damaged mitochondria while signaling the absence of IL-10, resulting in abnormally regulated activation of the NLRP3 inflammasome alongside the rise of IL-1b [8].

The lactate dehydrogenase (LDH) enzyme is associated with the production of energy and it can be detected in almost every body cell. Evaluation of tissue damage due to different causes can be conducted by measuring the LDH levels in the blood, including lung and liver diseases. The increase of LDH is an indicator to the damage of either the tissue or the cell. Viral infection or the damage of the lungs, i.e., pneumonia, is SARS-CoV-2 induced. The serum LDH is always recognized as an essential biological marker to indicate the idiopathic pulmonary fibrosis activity and severity. Hence, patients with severe pulmonary interstitial disease, the LDH increment is substantial and regarded as a prime lung injury prognostic marker [9].

The establishment of the vaccine regime has aided in the reduction of cases of COVID-19, yet the exact immunological changes after the disease and vaccination are not yet very clear. Therefore, this preliminary study was conducted to determine the levels of interleukin-10 in patients previously infected with COVID-19, in relation to vaccination and to explore the complete blood count and differential WBC count. In addition, determining the levels of LDH in the patients previously infected with COVID-19, in relation to vaccination was also investigated.

Materials and Methods

Equipment and instruments

Equipment and instruments	Company / Country
BENCH TOP centrifuge	Kokusan (H-19 F)/ Japan
Disposable Syringe	Morningside Pharmaceuticals Ltd/ UK
Eppendorf tubes	Beckman / USA
Gel Tube	VACUTEST/ Italy
EDTA Tube	VACUTEST/ Italy
Gloves, Tourniquet and Tip	New pharma/ Malaysia
Micropipette (Automatic pipette)	BRAND/ Germany
Hematological Analyzer (3 Parameters)	Convergys x3 / Sweden
ELISA Reader	Biotek (ELx800)/ USA
Accent 200	Cormay / Switzerland and Poland
Auto vortex Mixer	Fisher Scientific/ UK

Reagents and kits

Reagents and kits	Company/ Country
IL-10 (ELISA Kit)	China
LDH (Accent 200) Kit and Control kit	Switzerland and Poland
CBC Analyzer Reagents	Germany

Individuals and Controls

A total of 120 cases were incorporated in the current study, who were divided into 4 groups:

(Covid-19, non-vaccinated = CN); (Covid-19, vaccinated = CV); (Non-Covid-19, non-vaccinated = NN); (Non-Covid-19, vaccinated = NV). Each group involved 30 cases. The study was conducted in Cihan University-Erbil campus in November 2021.

All subjects were asked questions regarding their history, as shown in the questionnaire sheet (Figure 3.1.). Blood was investigated for the serum Interleukin-10 level, serum Lactate Dehydrogenase (LDH) and hematological parameters.

No.:

Phone No.:	**Name:**	
Gender:	**Male:** ☐	**Female:** ☐
Age:		**Years**
Occupation:		
Residence:		
Past infection with COVID-19 before vaccine?	**Yes:** ☐	**No:** ☐
Past infection with COVID-19 after vaccine?	**Yes:** ☐	**No:** ☐
Method of diagnosis, if yes:		**Result:**
Date of infection:		
Duration of infection:		
Source of infection:		
Smoker:	**Yes:** ☐	**No:** ☐
Other diseases:		
Any medications:		
Vaccinated?	**Yes:** ☐	**No:** ☐

Astra Zeneca ☐	**Pfizer/BioNtech** ☐	**Moderna** ☐
Sinopharm ☐	**Janssen** ☐	**Others** ☐
One shot ☐	**Two shots** ☐	
Date of vaccine		
Blood group		
Anti-COVID-19 IgG		
Anti-COVID-19 IgM		

Figure 3.1.: Questionnaire on COVID-19 Individuals in Relation to Vaccine

Blood samples

A total of 120 blood samples were collected aseptically. 7ml of blood were withdrawn from each individual who participated in this study by sterile and disposable syringes. Then, the samples of blood were placed into Gel tube (5ml) and centrifuged at 2500 rpm for 15 minutes to collect the serum (for IL-10 and LDH tests), and EDTA tube (2ml) for performing hematological parameters (CBC test) by Hematological analyzer. The serum of each individual was stored in 4 separate Eppendorf tubes at -20°C until use, for performing Interleukin-10 level by ELISA and LDH by biochemical analyzer.

Interleukin-10 ELISA Test

The Interleukin-10 test was performed for each sample after thawing the serum sample and bringing it to room temperature, using the ELISA test. The procedure was conducted according to kit instructions.

LDH Tests

The concentration of LDH was determined in the serum using a full automated chemical analyzer (Accent 200).

Hematological Tests

The complete blood count (CBC) was conducted by the automated three parameter Convergys x3 Hematology Analyzer for each case and control group within one hour of sample collection.

Statistical Analysis

Data were organized in tables, evaluated and analyzed statistically. The Statistical Package for Social Sciences (SPSS) version 20 computer program software was used. ANOVA (F-test) was used in comparing the quantitative parameters. Obtained results were recognized significant and highly significant if ρ value >0.05 and 0.01, respectively.

Results

Serum Interleukin-10

Table (1) shows the mean levels of IL-10 in the four groups of the study. The mean serum level of IL-10 was significantly higher in Covid-19, non-vaccinated than Covid-19, vaccinated individuals. Moreover, the level was also equally significant and higher in non-Covid-19, vaccinated than non-Covid-19, non-vaccinated.

Non-significant differences were found between Covid-19, vaccinated and non-Covid-19, non-vaccinated individuals and between Covid-19, non-vaccinated and non-Covid-19, vaccinated individuals.

Table (1): Mean serum levels of IL-10

Immunological Parameter	Mean ± SE				F-Test P Value Probability
	CN (No. =30)	CV (No. =30)	NN (No. =30)	NV (No. =30)	
IL-10 (ρg/ml)	78.61 ± 2.21 b	58.52 ± 1.22 a	44.53 ± 2.63 a	73.35 ± 13.73 b	0.004 H.S **
P value ≥0.05: Non-significant	*P value ≤0.05: Significant		*P value ≤0.05: Significant		
[a, b] Different letters: There is Significant difference between them (Duncan)					

(Covid-19, non-vaccinated = CN); (Covid-19, vaccinated = CV); (Non-Covid-19, non-vaccinated = NN); (Non-Covid-19, vaccinated = NV)

Serum Lactate Dehydrogenase

The mean serum levels of LDH were found to be significantly lower in non-Covid-19, non-vaccinated, indicating a significant rise of LDH in all other groups. Despite the rise in all three groups a significant difference was not found between them (Table 2).

Table (2): Mean serum levels of LDH

Parameter	Mean ± SE				F-Test P Value Probability
	CN (No. =30)	CV (No. =30)	NN (No. =30)	NV (No. =30)	
LDH (ng/ml)	413.83 ± 10.08 [b]	412.86 ± 10.72 [b]	385.06 ± 17.56 [a]	411.23 ± 7.51 [b]	0.012 S*
P value ≥0.05: Non-significant	*P value ≤0.05: Significant	*P value ≤0.05: Significant			
[a, b] Different letters: There is Significant difference between them (Duncan)					

(Covid-19, non-vaccinated = CN); (Covid-19, vaccinated = CV); (Non-Covid-19, non-vaccinated = NN); (Non-Covid-19, vaccinated = NV)

Hematological parameters

Table (3) shows all means hematological parameters. The mean of total WBC count shows a significant increase in both non-vaccinated groups ($\rho \leq 0.05$). While the mean level of lymphocytes counts reveals non-significant decrease in non-Covid-19, vaccinated group in comparison to the other groups ($\rho \geq 0.05$). However, the mean monocyte counts of Covid-19, vaccinated group revealed a significant rise in comparison to other groups ($\rho \leq 0.05$). In addition, the mean counts of granulocyte showed a significant increase in both non-vaccinated groups in comparison with vaccinated groups ($\rho \leq 0.05$).

Platelet mean count presented a non-significant difference at ($\rho \geq 0.05$) when comparing all groups.

Table (3): Mean levels of hematological parameters among Covid-19 individuals and healthy controls

Hematological Parameters	Mean ± SE				F-Test P Value Probability
	CN (No.= 30)	CV (No.= 30)	NN (No.= 30)	NV (No.= 30)	
WBC ($10^3/\mu L$)	7.43 ± 0.49 [b]	6.41 ± 0.22 [a]	7.39 ± 0.26 [b]	6.38 ± 0.31 [a]	0.029 S*
Lymphocyte ($10^3/\mu L$)	2.06 ± 0.10 [a]	2.02 ± 0.10 [a]	2.15 ± 0.10 [a]	1.94 ± 0.13 [a]	0.202 N.S.
Monocyte ($10^3/\mu L$)	1.63 ± 1.11 [a]	0.54 ± 0.04 [b]	0.51 ± 0.04 [b]	0.46 ± 0.04 [b]	0.017 S*

Granulocyte (10^3/µL)	4.85 ± 0.47 [b]	3.83 ± 0.19 [a]	4.52 ± 0.20 [b]	3.97 ± 0.22 [a]	0.046 S*
Plt (10^3/µL)	266.63 ± 14.01 [a]	288.64 ± 9.91 [a]	271.67 ± 8.45 [a]	270.30 ± 9.79 [a]	0.236 N. S.
P value ≥0.05: Non-significant		***P value ≤0.05: Significant**		***P value ≤0.05: Significant**	
a,b Different letters: There is a Significant difference between them (Duncan)					

(Covid-19, non-vaccinated = CN); (Covid-19, vaccinated = CV); (Non-Covid-19, non-vaccinated = NN); (Non-Covid-19, vaccinated = NV)

Discussion

The novel coronavirus disease (COVID-19), originally is SARS-CoV-2 Disease 2019, because of its relevance to SARS-CoV, leading to a mortality risk of 3-7 %, and efficient treatments are clearly essential in confronting it [10]. The WHO has identified the novel coronavirus as the pathogen that causes acute respiratory syndrome (ARS) [11]. Because the condition manifests differently in various individuals, there has been no effective treatment for COVID-19 up till now. The main clinical aspects of COVID-19 are that it causes acute lung damage, which in the majority of severe ARS patients develops to acute respiratory distress syndrome (ARDS). Acute lung damage may be complicated due to a certain number of variables. Inflammatory, immunological cells, cytokines, and adhesion molecules have been identified by the WHO as the pathogen that causes ARS. Acute pancreatitis, sepsis and acute lung injury have all been linked to pro-inflammatory cytokines [12, 13]. Furthermore, viral infection induced the overexpression of cytokines such as Tumor Necrosis Factor alpha (TNF-α), a key mediator of inflammation [14, 15].

The cytokines storm was considered to be the cause of hyperinflammation in SARS-CoV-2 immunocompromised patients. Screening and regulating these cytokines may help to reduce mortality [16]. Antibiotics, intravenous immunoglobulin, and targeted cytokine inhibition also could be used to treat SARS-CoV-2 [17, 18]. Cytokines i.e., tumor growth factor-beta (TGF-$^{\beta}$), gamma interferon (IFN-γ), IL-1, IL-8, IL-6, and TNF-α increase inflammatory reactions. Other cytokines, i.e. IL-4 and IL-10, help to reduce inflammation [19]. In the initial phases of SARS, T-lymphocyte numbers are low, specifically shown in cases with severity. This could signal that immunomodulatory has a change in its functionality [20]. As a result, cytokine profile may become an important factor in COVID-19 prognosis, and in concern with our current study, a reflection of the vaccine potency.

A study conducted in Erbil City found the serum concentration of IL-10 to be substantially elevated in cured patient group when compared with the cases of COVID-19 in the control, moderate and severe groups[21] . Our current study found elevated levels of IL-10 in cured patients who have not been vaccinated, which is similar to the mentioned finding of the previous study. This may indicate the ability of the vaccine to change the cytokine profile because those who had recovered and were vaccinated had significant low level of IL-10 when comparison was made with the non-vaccinated. Non the less, future

studies are obliged to demonstrate such mechanisms accurately.

In acute lung injury, IL-10 was illustrated in inhibiting both TNF-α and neutrophil activation [22]. IL-10 increased values in the cured patients are possibly due to IL-10 protective mechanisms. SARS-CoV-2 is now widely identified as a major cause of acute lung damage. The subsequent inflammatory and immunological pathways generate cytokines as a result (13, 14). Another research revealed that IL-10 is linked to age and illness severity independently [23].

The elevation of IL-10 is thought to be a constituent of the cytokine storm in COVID-19. This was interpreted as an adverse response mechanism in suppressing the inflammation. But for all that, evidence of clinical origin suggests that initiated IL-10 escalation may contribute to the pathological changes and the severity of COVID-19 [24]. In fact, it has been documented that IL-6 and IL-10 are likely be employed as severity predictors of COVID-19 disease [25]. The levels of IL-10 in our current study may also be used as a predictor for the efficiency of the vaccine.

The increased levels of LDH in all groups except the non-infected, non-vaccinated may show the virus's potential to damage cells and induce a rise in LDH, whether during infection or as a vaccine. LDH is a critical enzyme in anaerobic metabolism in cells [26]. Serum LDH was increased in patients with severe COVID-19 as per several studies [27, 28]. Furthermore, SARS-CoV-2 patients with increased LDH, when admitted, have a high probability in developing ARDS. The pulmonary tissue status is complicated by inflammation and cell damage [29]. When compared to individuals with SARS-CoV-2 negative confirmed pneumonia, COVID-19 patients had greater LDH levels [30, 31]. The clearance ration of COVID-19 mRNA was also found to be strongly linked to LDH levels [32].

In this current study, the mean of total WBC count demonstrated a significant ascent in both groups (non-vaccinated groups). While the mean level of lymphocytes counts revealed a non-significant decrease in non-Covid-19, vaccinated group in comparison to the other groups. However, the mean monocyte counts of Covid-19, vaccinated group revealed a significant rise in comparison to other groups. In addition, the mean counts of granulocyte showed a significant increase in both non-vaccinated groups in comparison with vaccinated groups.

Furthermore, COVID-19 has been connected to the modifications made in peripheral immune activity [33, 34], such as the augmented levels of acute phase reactants and pro-inflammatory cytokines [35-37], neutrophilia and the advent of neutrophils which were immature and low-density [38, 39]. Additionally, the ratio of neutrophil-to-lymphocyte and lymphopenia was increased [40], myeloid inflammation [41], besides reducing the human's expression of leukocyte antigen DR isotype (HLA-DR). The use of immunological parameters, including cytokines, has long been used as a predictor of many infections, not just viral, including parasitic [42, 43] and bacterial infections [44]. Many other studies have been conducted regarding the immunological changes in diseases [45, 46, 47].

Our current study may indicate the potency of the vaccines. However, the rise in the LDH needs further investigations to show the reason and the effect. Larger sample size is also required for our current study to verify the ability of the vaccine to change the levels of IL-10, LDH and WBC counts.

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