Interleukin-6 Levels in Relation to COVID-19 Vaccine

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Abstract-SARS COV-2 is a pulmonary disease known as COVID -19 that has been infecting people since late December 2019 and continues to cause organ failure due to elevated cytokines and chemokines causing cytokine storm syndrome, elevating levels of Interleukin-6 and lactate dehydrogenase enzyme (LDH). In this study, a total of 120 cases were included that were collected in Cihan University-Erbil Campus from October to November 2021. In these 120 samples, categorized into four different groups (NN, CN, NV and CV) N is for non-vaccinated, C is for COVID-19 and V is for vaccinated, each group consisting of 30 cases. The study found that those who had COVID-19 infection and were vaccinated had much greater levels of interleukin 6 than the other groups. COVID-19 causes IL-6 to rise, but immunization doubles the level. Furthermore, the serum samples taken from them for lactate-dehydrogenase LDH test, the study showed that both COVID-19 infection and vaccination will cause a massive increase in LDH level in the body. Lastly, there were hematological parameters for the samples which indicated that those who got COVID-19 infection their WBC and monocyte were increased compared to those who got vaccinated which was significantly increased. The study did a platelet test for the samples, but there was no significant difference between the groups.

Keywords: Interleukin-6, Covid-19 vaccine, COVID-19, LDH

I. INTRODUCTION

COVID-19 is a pulmonary disease caused by infection with SARS-CoV-2, a coronavirus that was first detected in Wuhan, China, in late December 2019 during an epidemic of viral pneumonia [1]-[2]. This virus is spread from person to person by respiratory contact [3] that will cause Gastroenteritis and Diarrhea and mainly pulmonary disease [4]-[5]. The majority of occurrences occur between the ages of 30 and 79. Fever (82.2%) and cough (61.7%) are the most prevalent symptoms, which are comparable to those of other viral respiratory infections. Myalgia, sore throat, nausea, vomiting, and diarrhea, on the other hand, might indicate another illness [6]. Gastrointestinal symptoms are reported in 2-40 percent of patients, and diarrhea might be the first sign of infection. It's unclear whether SARS-CoV-2 causes these symptoms directly by infecting the gastrointestinal system, indirectly through neurological involvement, or indirectly through the generation of cytokines [7].

COVID-19 patients' secretion of cytokines and chemokines, which attract the immune cells to the lungs, Signature cytokines in severely ill COVID-19 patients enhanced expression of IL-6 [8] that plays a key role in a variety of host defense systems, including the immune system such as the immune response, hematopoiesis, and acute-phase reactions , IL-6 has been implicated in the pathology of many diseases including multiple myeloma, rheumatoid arthritis, Castleman's disease, AIDS, mesangial proliferative glomerulonephritis, psoriasis, Kaposi's sarcoma, sepsis and osteoporosis [9]. The term "coronavirus infection 2019 -related cytokine storm syndrome" was coined to describe why some people who were exposed to the virus became extremely sick with acute respiratory failure, mortality and multi-organ failure. The evidence that IL-6 is responsible for immunological dysregulation and respiratory failure in such case is quickly growing [10].

Interleukin-6 (IL-6) is a multitasking cytokine that has a high level of activity in individuals with SARS-

CoV-2 and cancer [11]. Many of these patient populations have a cytokine storm, with increased serum inflammatory cytokines (such as IL-6, IL-1, and IFN-) and vascular endothelium activation markers (such as von Willebrand factor (vWF), factor 8 coagulant (F8), and soluble P-selectin (sP- Lymphopenia) and immune paralysis are caused by high levels of IL-6, which can cause lymphopenia and CRP stands for C-reactive protein, while NET stands for neutrophil extracellular trap [10]. Although vaccinations against SARS-CoV-2 have proven effective it is uncertain if coronavirus vaccines may also protect against additional coronaviruses that may infect people in the future [12].

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II. METHODOLOGY

TABLE 1 - 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

TABLE 2 - REAGENTS AND KITS

Reagents and kits	Company/ Country
IL-6 (ELISA Kit)	China
LDH (Accent 200) Kit and Control kit	Switzerland and Poland
CBC Analyzer Reagents	Germany

This study comprised 120 cases that were gathered on the Cihan University-Erbil campus during October and November 2021. In these 120 samples, 30 of them did not have COVID-19 and were not vaccinated, therefore, they were categorized as a negative control (NN). In this investigation, 30 samples were infected with COVID-19 but were not vaccinated, and they were counted as Positive controls (CN). 30 samples were infected with COVID-19 and vaccinated, and the results were categorized as (CV). The remaining 30 samples were not infected with COVID-19 and were vaccinated, hence they were categorized as (NV). Complete blood count (CBC), Interleukin-6 level, and Lactate Dehydrogenase level were all examined (LDH). All of the patients yielded a total of 120 blood samples, which were taken aseptically. Using sterile disposable syringes, 7 ml of blood was taken from each patient and control group. For serum collection, blood samples were put in a Gel tube (5ml) and centrifuged at 2500 rpm for 15 minutes; for hematological parameters, blood samples were deposited in an EDTA tube (2ml) and spun at 2500 rpm for 15 minutes. Each patient's serum was maintained in Eppendorf tubes at -20°C until used for ELISA testing of Interleukin-6 and Accent 200 testing of Lactate Dehydrogenase (LDH). The data was statistically examined, processed, and summarized in tables. The Statistical Package for Social Sciences (SPSS) version 20 computer application was used. The ANOVA was used to compare quantitative variables (F-Test). If the P-value was less than 0.05 or 0.01, the results were declared significant and highly significant, respectively.

All subjects were asked questions regarding their history, as shown in the questionnaire sheet (Figure 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 1.: Questionnaire on COVID-19 Individuals included in this study in Relation to Vaccine

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III. RESULTS

The laboratory tests were done and the result shown below:

TABLE 3 - MEAN LEVELS OF IL-6

Immunological Parameter	Mean ± SE				F-Test P Value Probability
	CN (No.=30)	CV (No.=30)	NN (No.=30)	NV (No.=30)	
IL-6 (ng/ml)	39.97 ± 1.53 [c]	41.60 ± 11.49 [c]	25.21 ± 1.19 [a]	34.70 ± 5.67 [b]	0.016 S*
P value ≥0.05: Non significant; *P value <0.05: Significant; **P <0.01: Highly Significant					

Table (3): An ELISA test was performed for detection of the levels of IL-6 in the patients. After running the total sample with tests for all the four groups, the results showed a significant difference between the groups presented in table (3). the levels of interleukin 6 in those who got COVID-19 infection and vaccinated (CV) groups were increased highly significant compared to other groups, after this group, those who got the infection and didn't get vaccinated (CN) was also highly significantly increased compared to (NN, NV), as for those who didn't get the infection and got vaccinated (NV) group which is also increased significantly but not as (CV, and CN) and the lowest group is (NN) which were at their normal range.

The Lactate dehydrogenase test performed for the groups showed that the amount of LDH. as in table (4) showed, in these groups that either got COVID-19 infection or got vaccinated (CN, CV, and NV) were significantly increased in comparison with the control group which is (NN). The group that got an infection and didn't get vaccinated (CN) stands at the top among the groups in having increased LDH level, after (CN) group (NV) is significantly high than (NN, CV) group. The next group is (CV), which is significantly high in comparison to

TABLE 4 - 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)					

Table (5) shows all mean of hematological parameters. As the table shows, there was a significant difference of the levels of WBC increased among the (CN and NN) groups in compare with the (CV and NV) groups. As for the lymphocytes, there was non-significant changes among all the groups. However, the Monocyte levels among (CV, NN, NV) groups were significantly decreased in compare with (CN) group which is the highest among the group. For the granulocyte levels, in the (CV, NV) groups there was a significant decrease of Granulocytes compared with (CN, NN) groups. But there weren't any significant changes of platelets PLT between the four groups.

TABLE 5 - 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/µL)	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/µL)	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/µL)	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*

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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)					

IV. DISCUSSION

From 120 samples, our result for the levels of Interleukin 6 which were highly significantly increased in those who got COVID-19 infection and got vaccinated (CV) which stands at the top among the groups. Next to that, those who didn't get COVID-19 infection, but got vaccinated (NV) were also highly increased in comparison with (CV and NN) groups. After these two groups (CV) groups will come which those that got COVID-19 infection and got vaccinated, again they were highly increased, but not much as the first two groups (CV, NV). Lastly, is the control group (NN) which neither got the infection nor vaccinated.

This study, investigated that both COVID-19 infection and Vaccination will increase IL-6 levels, but somehow, the effect each other and interfere IL-6 levels, Vaccination will increase the level of IL-6 but not as much as those who get COVID-19 infection which increases IL-6 more, but if a person has both of them (got COVID-19 infection and vaccinated) IL-6 will dramatically increase.

There was a total of nine investigations that comprised 1426 laboratory-confirmed patients (mean age: 53.0 - 6.4 years, females: 46.6 per cent). All of the trials were conducted in China, and the study period was from January 1 to February 28, 2020. The researchers discovered that patients with COVID-19 infection had considerably higher levels of IL-6 [13].

In 10 cohort studies (n = 1798), the immunological response to SARS-CoV-2 in individuals diagnosed with Covid-19 was established. the mean age was 54.8 14.4, and 42 percent of the patients were female. All of the research were conducted in China, and all of them found greater levels of IL-6 in patients with Covid-19. Some of the studies found higher levels of IL-6 in patients with the more severe (complex) conditions [14].

In this study, Lactate dehydrogenase LDH in these groups that either got COVID-19 infection or got vaccinated or both (CN, CV, and NV) were increased compared to those who didn't get COVID-19 infection and vaccination (NN). This may indicate that COVID-19 infection and its Vaccination will awaken the immune system to respond by elevating Lactate dehydrogenase LDH.

High lactate dehydrogenase (LDH) levels were connected to 6 times greater risk of severe COVID-19 disease in COVID-19 patients, according to Henry et al. [15].

According to studies, early recognition of severe patients with coronavirus disease 2019 (COVID-19) is crucial for personalized treatment. They included 203 COVID-19 patients who were matched by propensity score in their retrospective case-control study. The impacts of serum lactate dehydrogenase (LDH) following entry on COVID-19 patients were examined. Serum LDH levels were shown to have a specificity and sensitivity of 58.7% and 82.0 per cent, respectively. As a result, achieving a strong LDH level at the time of admission is a potential risk for COVID-19 worsening and mortality. Early COVID-19 assessment can be aided by LDH. The admission, clinicians should check the serum LDH concentration in COVID-19 patients [16].

Another research looked at the effects of pentoxifylline on indicators such LDH, lymphocyte count, days in the hospital, mortality, and the proportion of patients who needed intubation in 38 patients with moderate and severe COVID-19. Twenty-six patients were randomly assigned to receive 400 mg of pentoxifylline with standard medication (pentoxifylline group), whereas the remaining patients got normal treatment (control group). For statistically significant factors, linear regression models were created. Treatment with pentoxifylline resulted in a 64.25 per cent rise in lymphocyte count (CI95 per cent 11.83, 116.68) and a 29.61 per cent drop in serum LDH (CI95 percent 15.11, 44.10) [17].

As for the hematological parameters, our study showed that the WBC levels which were increased in (CN and NN) groups in comparison with the (CV and NV) groups with nearly decreased lymphocyte in all groups, decreased or normal monocyte in (CV, NV, NN) compare to (CN) group also indicated low neutrophils and other granulocytes in (CV, NV) group compare to (CN, NN)

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groups which were increased or balanced. This may indicate that those who got COVID-19 infection tend to have more or increased WBC level which it should be, to prevent the body from SARS-CoV-2 by increasing WBC levels and to create immunity. As for those who vaccinated, they have lower amount of WBC because after they got vaccinated, their body immune system deals with the weakened or dead particles of the virus for creating immunity and memory cells, so that means they don't require that much amount of WBC for immunological process after vaccination unlike those who didn't get vaccinated and got COVID-19 infection which is a real deal for the human's immune system.

A study on 233 patients who were admitted to Pamukkale University Hospital's Emergency Department (ED) for two months (March–April 2020) and underwent Sars CoV-2 PCR (Polymerase Chain Reaction), complete blood count (CBC), and CRP tests in order due to COVID-19 complaints found that CRP, LDH, PLR, and NLR levels remained significantly higher in COVID-19 positive patients, while eosinophil, lymphocyte, and platelet levels were [18].

Peripheral blood samples were taken from six healthy controls and 14 COVID-19 samples from three severely/critically unwell and four moderately ill patients, including severe, moderate, and convalescent samples. We discovered that monocytes in COVID-19-infected severely/critically unwell individuals were significantly altered, with an increased percentage of monocytes and significantly decreased diversity [19].

Lastly, for the platelets, our study didn't indicate any significant changes between the groups which may be due to the mild form of the infection or because they previously got the infection so that their platelets into their normal range.

Blood was obtained from 115 individuals with COVID-19 who had non-severe (n=71) and severe (n=44) pulmonary symptoms. Platelets, according to their findings, are at the forefront of COVID-19 pathophysiology, since they produce a variety of chemicals at different phases of the illness. Platelets may thus have the ability to contribute to COVID-19's excessive thrombo-inflammation, and inhibiting platelet activation pathways may enhance COVID-19's results [20]-[21].

CONCLUSION

This study, 120 samples were taken, it shows that the levels of the interleukin 6 of the serum of the samples, who got COVID-19 and got vaccinated are increased highly significant compared to Those who didn't vaccinate and didn't get COVID-19 infection which their IL-6 lever are lower in highly significant. Those who got vaccinated most likely have decreased amount of Monocyte and there were no significant changes between groups for the levels of lymphocytes, the study showed that there aren't any significant changes in platelets levels between all the four groups unless those who got the infection in a severe and complicated form. Furthermore, the study showed that for those who got COVID-19 infection or vaccinated or both, the amount of lactose dehydrogenase LDH were significantly increased.

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