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Evaluation of Interleukin 6 Levels in Severe COVID-19 Patients

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ABSTRACT

Objectives: Interleukin 6 (IL-6) plays a leading role in the proliferation and differentiation of immune cells. Level of IL-6 is manifestly increased under many inflammatory conditions, including cytokine release syndrome. We evaluated the IL-6 levels of patients who were hospitalized with the diagnosis of COVID-19.

Methods: In the study, the test results of 19 cases whose IL-6 levels were measured between March 11, 2020 and May 31, 2020 retrospectively. The inpatients in the covid service (Group 1) and the patients admitted to the Intensive Care Unit (ICU) of our hospital (Group 2) were compared and evaluated. In addition, patients with positive and negative RT-PCR test results were checked for IL-6.

Results: While 8 (Group 1) of 19 patients observed in the clinic were transferred to the ICU, 11 patients (Group 2) were observed in the covid service until their discharge. Group 1 IL-6 levels (median 34 pg/mL) and Group 2 IL-6 levels (median 116 pg/mL) were found to be high in both groups (p = 0,099). However, it was found to be significantly higher in patients with positive COVID-19 RT-PCR test (median 90.60 pg/mL) than in negative patients (median 29.90 pg/mL) (p = 0.018).

Conclusion: No significant difference in IL-6 levels between the patients who were monitored in the clinic and transferred to the ICU was found in this study. The significant difference between IL-6 levels among COVID-19 RT-PCR positive and negative patients reveals the importance of IL-6 level with regard to tocilizumab treatment in COVID-19 patients in cytokine storm.

Keywords: COVID-19, Interleukin 6, Cytokine Storm, Macrophage Activation Syndrome

The clinical spectrum of the new Coronavirus-19 (COVID-19) disease is wide, and in a preliminary study conducted by the World Health Organization in China 18.3% patients developed severe disease requiring oxygen while 6.1% patients developed intensive care. [1] The monitoring of prognostic markers in COVID-19 disease, which has undergone dynamic changes in treatment algorithms since the situation was defined as a pandemic, is important in terms of predicting the clinical course.

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In this epidemic period, where immunological and thromboembolic events are common, it has been demonstrated in studies that proinflammatory cytokines such as tumor necrosis factor (TNF) receptor, interleukin-10 (IL-10), interleukin-8 (IL-8), interleukin-6 (IL-6) and interleukin 2 (IL-2R) are higher in severe patients. [2]

IL-6 is a cytokine that plays a role in the proliferation and differentiation of immune cells. In healthy individuals, the circulating level of IL-6 is very low (1-5 pg/mL), but increases prominently in many inflammatory conditions, including cytokine release syndrome. [3] It is known to be secreted by T cells, macrophages, endothelial cells, fibroblasts and monocytes. The thromboembolic process seen in the course of COVID-19 disease begins with the stimulation of tissue factor receptors involved in the coagulation cascade with the direct effect of IL-6 on monocytes. Another effect is that IL-6 activates the extrinsic coagulation cascade. [4]

In this study, we aimed to evaluate the relationship between the clinical course and other laboratory parameters of the degrees of IL-6 levels measured in severe COVID-19 patients.

METHODS

In this study, the IL-6 levels of 19 patients were evaluated and who were monitored with the diagnosis of COVID-19 in the pandemic service of Acıbadem Atakent Hospital, which operates as a pandemic hospital, were retrospectively analyzed. Results of the patients monitored in our clinic (Group 1) and those transferred to the Intensive Care Unit (ICU) of our hospital (Group 2) were compared and evaluated. Demographic data of the patients, COVID-19 Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) results in nose and throat swab samples, clinical course and laboratory findings were recorded retrospectively. In addition, patients with positive and negative RT-PCR test results were checked for IL-6; hemoglobin (Hb), PT, INR, D-dimer, urea, glomerular filtration rate (GFR), lactate dehydrogenase (LDH), C-Reactive Protein (CRP), Creatine Kinase (CK), troponin, fibrinogen, ferritin, Brain natriuretic peptide (BNP) upon admission- and values were compared between the groups. Additionally, the relationship between IL-6 level and other parameters was evaluated by using correlation analysis.

Statistics

The obtained data were analyzed using SPSS 24 software (IBM Corp, Armonk, NY). Descriptive statistical methods (mean, standard error, ratio) were used to evaluate study variables. In the analysis of data, Student's t test, Mann-Whitney U test, Chi-square test and Fisher exact test were used. A value of $p < 0.05$ was considered statistically significant. Ethics committee approval numbered 2020-08/20 was obtained for the study.

PT, INR, aPTT, D-dimer and fibrinogen tests were performed in Siemens CS 2000i device by coagulometric method from citrated plasma. Urea, LDH, CRP and BNP tests were performed by photometric method, where CK test was performed by enzymatic method from serum samples in Siemens Dimension device. Sodium, potassium and chlorine tests were performed using the ISE method from serum samples in the same device. Troponin, ferritin

Table 1. Data of Service (Group 1) and Intensive Care Unit (Group 2) Patients

	Group 1 (n = 11)		Group 2 (n = 8)		
	Frequency (n)	%	Frequency (n)	%	
Female	2	(18,2%)	3	(37,5%)	
Male	9	(81,8%)	5	(62,5%)	
Positive RT-PCR	4	(46,2%)	6	(75%)	
	Median		Median		*p*
IL-6	34 pg/mL		116 pg/mL		0,099
LDH	**239 U/L**		**365 U/L**		**0,045**
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RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction; IL-6: Interleukin-6; LDH: Lactate Dehydrogenase

and CK-MB mass tests and chemiluminescence (CLIA) method were performed from serum samples in Siemens Advia Centaur device. The measurement of IL-6 was performed in the Siemens Advia Centaur device using the CLIA method from a frozen serum sample.

RESULTS

While the female/male ratio in the clinic (Group 1) was 2/9 (18.2%/81.8%), the female/male ratio in the ICU (Group 2) was 3/5. (37.5/62.5%) No significant difference was found between the groups ($p > 0.05$). However, the number of male patients was higher in all critically sick patients (73.6%). The mean age of female patients (76.8 ± 4.9 years) was significantly higher than male patients (59.9 ± 13.9 years) ($p = 0.018$). The positive/negative PCR rate in the clinic was 4/7 (46.2%/53.8%), while the rate in the ICU was 6/2 (75%/25%). There was no significant difference between the groups in terms of test results. ($p > 0.05$). While the median IL-6 level was found to be high in both groups, 34 pg/mL in the clinic, 116 pg/mL in the ICU, the difference between them was not statistically significant. ($p > 0.05$). When ICU and clinical patients are compared, when the examinations performed upon admission are evaluated, there is no significant difference between leukocytes, neutrophils, lymphocytes, monocytes, hemoglobin, platelets, MPV, D dimer, CRP, troponin, fibrinogen, ferritin, CK, creatinine, calcium, albumin and glucose. However, LDH level was found to be significantly higher in patients who went to the ICU upon admission (365 U/L) compared to patients monitored in the clinic (239 U/L) ($p = 0.045$). Two patients transferred to the ICU have passed away and the RT-PCR results of these patients were positive. (Table 1).

Since there was no significant difference between group 1 and group 2 IL-6 levels, patients with positive and negative RT-PCR tests were divided into 2 groups and examined. A significant difference was found between IL-6, Hb, PT, INR, urea, GFR, LDH and BNP levels between positive and negative test results ($p < 0.05$). When D-dimer, CRP, CK, troponin, fibrinogen and ferritin values were evaluated, no significant difference was found between the groups (Table 2).

When the correlation between IL-6 level and other parameters in all patients was examined, it was noticed that IL-6 level was negatively correlated with monocytes (r = -730; p = 0.005) and positively correlated with CK level (r = 0.518; $p = 0.04$). ($p < 0.05$). (Table 3)

Table 2. Comparison of IL-6 and Other Parameters of Patients According to RT-PCR Test Results

	RT-PCR negative					RT-PCR positive					
	Mean	SD	Min	Max	Median	Mean	SD	Min	Max	Median	p
IL-6 (pg/mL)	37.60	27.40	8.18	93.18	29.90	225.48	366.54	5.70	1231.00	90.60	**0.018**
Hb (g/dL) First day	10.71	2.45	5.6	14.4	10.80	13.08	1.20	11.3	14.9	12.85	**0.010**
PT (second)	19.50	12.65	12.9	50.4	14.65	12.46	1.26	11.6	15.9	12.15	**0.003**
INR	1.62	1.09	1.1	4.5	1.20	1.06	0.13	1.0	1.4	1.00	**0.002**
D-dimer(ng/mL)	5.1067	7.47	0.19	20.00	2.60	14550	1.98	0.49	6.31	0.8750	0.245
Üre (mg/dL)	46.76	27.70	17	104	37	20	10.06	9.00	38.00	17	**0.032**
GFR (mL/Minute)	67.41	20.33	34.9	97.5	66.40	92.90	23.02	47.4	116.0	97	**0.030**
LDH (U/L)	252.00	87.36	174.0	362.0	214.50	349.67	100.18	239.0	568.0	357	**0.045**
CRP (mg/dL) First day	13.36	10.15	3.16	29.87	10.24	10.02	3.46	5.37	16.88	9.50	0.775
CK (U/L)	225.86	342.96	4.0	989.0	106	566.88	496.34	76.0	1156.0	519.50	0.165
Troponin (ng/mL)	0.16217	0.29	0.014	0.753	0.06	0.06	0.07	0.006	0.210	0,05	0.518
Fibrinojen(mg/dL)	489	319.17	257	853	357	514.25	82.65	405.0	645	486	0.414
Ferritin (ml/ng) First day	1356	1419.87	352	2360	1356	644.43	496.53	116.0	1480	450	0.558
BNP (pg/mL)	1925	1566.77	313	4099	1475	433.14	652.31	7.0	1807	90	**0.042**

RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction; IL-6: Interleukin-6; Hb: Hemoglobin; PT: Prothrombin Time; INR: International Correction Rate; GFR:Glomerular Filtration Rate; LDH:Lactate Dehydrogenase; CRP:C-Reactive Protein; CK: Creatine Kinase; BNP: Brain Natriuretic Peptide; SD: Standart Deviation

Table 3. The Relationship of IL-6 with Other Parameters

Interleukin 6 and	r value	p value
Monosit	-,730**	**0,005**
D-dimer	-0,086	0,771
Urea	-,681*	**0,010**
K	-,499*	**0,035**
CRP	0,189	0,439
CK	,518*	**0,048**
Troponin	0,102	0,740
Fibrinogen	0,064	0,853
Ferritin	0,133	0,732
BNP	-0,014	0,966

IL-6: Interleukin-6; K: Potassium; CRP: C-Reactive Protein; CK: Creatine Kinase; BNP:Brain Natriuretic Peptide

DISCUSSION

The condition defined as Macrophage Activation Syndrome (MAS) due to excessive cytokine secretion in infectious sepsis can also be seen in the course of COVID-19. Acute respiratory distress syndrome (ARDS) also occurs as a result of excessive cytokine secretion, and studies have shown that IL-6 level is associated with prognosis in Acute respiratory distress syndrome (ARDS), and its use in clinical practice is recommended. [5] It was stated in a study by Herold et. al that IL-6 level can be associated with prognosis in COVID-19 disease and that the risk of respiratory failure is 92% (22 times higher than normal) in patients with IL-6 level > 80 pg/mL.[6] In this group with high IL-6 levels, the median time for mechanical ventilation was found to be 1.5 days (min0-max4). In our study, while the median IL-6 level was 116 pg/mL in our ICU patients, it was found to be 34 in our clinic patients, but there was no significant difference between them. One of the reasons for this is the low number of patients, while another reason may be that the patients whose IL-6 levels are evaluated in the service receive high oxygen or BIPAP support, that is to say, that they are in the course of severe disease.

When the patients with negative and positive RT-PCR test results were compared, a statistically significant difference was found between IL-6 levels. This may be due to the fact that the viral load is higher in patients with a positive test, thus severe disease burden. In the study of Chen *et al*., COVID-19 patients with higher viral load had a more severe course, and ARDS was more common in this group of patients.[7] If we associate viremia with a positive RT-PCR test, it can be suggested that the burden of disease and thus the risk of MAS may be higher in patients with a positive test result. In the treatment guideline updated by the Turkish Ministry of Health on November 7, 2020, the use of tocilizumab, an anti-IL-6 receptor monoclonal antibody, is recommended in patients who have developed MAS.[8] Tocilizumab (400 mg/day) was administered to 5 patients with positive RT-PCR results in our clinic, and the mean IL-6 level was 347.18 pg/mL in these patients.

When we examine these cases, in which we applied tocilizumab, one by one; The first case is a 32-year-old male patient without any comorbidities, who applied to the emergency department with difficulty in breathing 1 week after the onset of COVID-19 symptoms. The patient, who had completed plaquenil and azithromycin treatments at home, was hospitalized because his room air saturation was 92%. The IL-6 level of the patient, whose saturation decreased to 88 during the follow-up and was given oxygen support with a reservoir mask, was found to be 108 pg/mL on the second day of hospitalization. Tocilizumab was administered for 2 consecutive days, and the patient was monitored in the clinic. 2 days upon application, his IL6 level decreased to 17.3 pg/mL, and the patient was discharged without any complications, as his general condition improved. In the second case; a 70-year-old male patient applied to the emergency department with fever, difficulty of breathing and tachypnea. He had hypertension and type 2 diabetes. Favipiravir and plaquenil treatments were applied. He was transferred to the ICU and intubated due to desaturation while he was being monitored with a non-invasive mechanical ventilator on his 3rd day in the clinic. IL-6, measured on his 6th day of monitoring in the ICU, was 161 pg/mL, and tocilizumab was administered for 2 consecutive days. The patient, who was admitted to the clinic after 29 days of treatment in the ICU, was discharged without complications.

The third case is a 60-year-old male patient who was referred to the hospital from another center and had no comorbidities other than essential hypertension. After completing the favipiravir treatment, he was transferred to the ICU due to desaturation and worsening in his clinical condition despite Bipap support on his 2nd day of monitoring in the clinic. On the 2nd day of his hospitalization, IL-6 was detected

as 1231 pg/mL, the first dose of tocilizumab was administered in the clinic, the treatment continued in the ICU the next day. Immune plasma therapy was applied to the patient whose clinical condition did not improve. The patient, who was monitored with non-invasive mechanical ventilation, was transferred to the clinic one week later and was discharged without complications.

In the fourth case; plaquenil and azithromycin treatments were given to a 60-year-old male patient with coronary artery disease and hypertension. On his 2nd day of hospitalization in the clinic, he was transferred to the ICU and intubated. On the 3rd day of monitoring in the ICU, IL-6 was 163 pg/mL. The patient, who was administered tocilizumab for 2 days, was extubated after 1 week of treatment in the ICU, and was discharged without complications.

In the fifth case, a 49-year-old male patient with a known history of hypertension was transferred to the ICU on his 5th day of monitoring in the clinic because of desaturation despite Bipap support. Plaquenil and favipiravir treatments were administered. The IL-6 level of the patient, who was monitored in the ICU as intubated, was found to be 73.2 pg/mL on his 4th day in the ICU, and tocilizumab was administered for 2 days. The patient, whose treatment continued for 9 days in the ICU, was taken to the clinic after clinical improvement, and was discharged without complications after 5 days of monitoring in the clinic.

One of the most important cytokines triggering MAS is IL-6, and its high levels are known to cause ARDS by damaging the lung vascular endothelium, at the same time, there are studies showing that IL-6 plays a remodeling and anti-inflammatory role in the lung. Due to the two contrasting features, the timing of administration of anti-IL-6 (tocilizumab) therapy is critical. Therefore, successive IL-6 monitoring may be clinically important.[9]

In our study, the frequency of male patients (73.6%) in critically ill patients was higher than female patients (26.4%). Likewise, in the meta-analysis conducted by Barek and his friends on 10,000 patients, it was shown that the prevalence of male gender was significantly higher at 62.8% in critically ill patients.[10] The average age of female patients being 76.8 years, may indicate that the course of COVID-19 is more severe, especially in postmenopausal women. Although mortality is higher in male gender in the general population, it has been shown in the study of Cagnacci and his friends that COVID-19 is more mortal than the premenopausal age group due to decreased estrogen level in postmenopausal women.[11]

Successive monitoring of hematological and biochemical parameters, which are important in the course of critically ill patients, is substantial. However, there is a need for parameters that can give an idea about the course of the patients at the time of application. The fact that only the LDH level was different in our study may be a significant indicator. As a result of the analysis performed by Li and his friends in Wuhan, a significant correlation was shown between LDH and provincial mortality at the time of admission, and the risk of going to the ICU and mortality was calculated to be twice as high in patients with an LDH level higher than 445 U/L.[12] In the light of studies like this, we can deduce that LDH is a cheap and fast-resulting test in many health units, so its use as a prognostic marker in the course of COVID-19 is a cost-effective test. Furthermore, CK can be considered as one of the parameters to be followed in patients with cytokine storm, since IL-6 level is significantly positively correlated with CK.

CONCLUSION

The clinical course of COVID-19 disease varies, and it has a wide spectrum from asymptomatic disease to severe pneumonia and MAS. IL-6 has been shown to occur more likely in severe patients, and treatment strategies for IL-6 have been used in some patients. Anti-cytokine treatment algorithms vary between countries, and more detailed studies on the application time and indications of tocilizumab treatment will contribute to similar applications. Consecutive monitoring of IL-6 level as a prognostic marker is valuable in the pandemic period where the patient burden is accumulated, therefore, the accuracy of triage is substantial.

Authors' Contribution

Study Conception: YO, IK,; Study Design: YO, IK,; Supervision: YO, IK,; Materials: YG, AY, FH,; Data Collection and/or Processing: YG, AY, FH,; Statistical Analysis and/or Data Interpretation: YO, YG,; Literature Review: YO, CG, YG, OO,; Manuscript Preparation: YO, CG, YG, OO and Critical Review: IK, YO.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

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