RESEARCH ARTICLE

Relation between Interleukin-6, Interleukin-10 and Interleukin-2 Receptor and mortality in Severely Ill COVID-19 patients

Asmaa Embarak Hassan[1], Nahla Abdelaziz Nosair[2], Mohammed Hussien Ahmed[3], Dalia Elsayed Sherif[4], Eman Mohammad Habib[5], Nahla Farahat[6]

Abstract

Objective: To investigate the relation involving soluble interleukin-2 receptor, interleukin-6 and interleukin-10 in hospitalised patients with severe coronavirus disease-2019 infection.

Methods: This single-centre cohort study was conducted at the Kafrelshiekh University Hospital, Egypt, from January to June 2022, and included all patients of either gender who were hospitalised with severe infection with the coronavirus disease-2019 isolation ward. Chemiluminescence immunoassay method was used to measure levels of procalcitonin, ferritin, soluble interleukin-2 receptor, interleukin-6 and interleukin-10. Data was analysed using SPSS version. 25

Results: Of the 250 patients with median age 57.5 years (interquartile range: 45.8-66.0 years), 147(59%) were males and 103(41%) were females. Of them, 102(40.8%) patients died; 68(66.7%) males, 34(33.3%) females, median age 60.0 years (interquartile range: 48.8-70.0). Among the 148(59.2%) survivors, 79(53.4%) were males and 69(46.6%) were females, while the overall median age was 55.0 years (interquartile range: 41.5-65.8 years). The survivors had significantly lower levels of soluble interleukin-2 receptor, interleukin-6 and interleukin-10 ($p<0.001$). Correlation analysis identified significant positive correlation between IL-2R, IL-6 and IL-10 levels and almost all the inflammatory and coagulation parameters, including C-reactive protein, lactate dehydrogenase, procalcitonin, ferritin, D-dimer and fibrinogen ($p<0.05$).

Conclusion: Elevated levels of soluble interleukin-2 receptor, interleukin-6 and interleukin-10 were found to be associated with greater risk of mortality in coronavirus disease-2019 patients.

Keywords: Interleukin-6, Interleukin-10, Procalcitonin, Fibrin fragment D, COVID-19, Luminescence, Interleukin-2, Immunoassay, Fibrinogen, Ferritins. **DOI: 10.47391/JPMA.EGY-S4-36**

Introduction

In coronavirus disease-2019 (COVID-19) disease, the nature of immune response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral infection is the key determinant of the clinical course of the condition. Balanced response is associated with benign and self-liming disease, while exaggerated response is linked with severe disease forms.[1]

The exaggerated immune response in severely ill COVID-19 patients entails the so-called cytokine storm (CS),[2] which is characterised by excessive production of pro-inflammatory cytokines, and is reportedly linked with rapid deterioration and high mortality of severe cases[3]**.** These cytokines include interleukins-1 (IL-1), IL-2, IL-6 and IL-10 in addition to tumour-necrosis factor (TNF) alpha, interferon gamma and others.[4]

[1,2,4,5]Department of Clinical Pathology, Kafrelsheikh University, Egypt.

[3] Department of Internal Medicine and Gastroenterology, Kafrelsheikh University, Egypt.

[6] Department of Clinical Pathology, Alexandria University, Alexandria, Egypt

Corrospondience: Asmaa Embarak Hassan

email: Asmaa_embarak@med.kfs.edu.eg

Soluble IL-2 receptor (sIL-2R) is secreted in response to T-cell activation.[5] In COVID-19 cases, IL-2R was fund to be associated with disease severity[6] and mortality[7]**.** IL-6 is a major cytokine with dual anti- and pro-inflammatory effects[8]**.** Moreover, it affects vascular endothelial cells, producing multiple mediators, and activate the coagulation pathways[9]**.** CS is particularly characterised by the early dramatic elevation of IL-10, especially in severe cases independent of patient age.[10, 11]

The current study was planned to investigate the relationship of sIL-2R, IL-6 and IL-10 with various parameters in severe COVID-19 patients.

Patients and Methods

This single-centre cohort study was conducted at the Kafrelshiekh University Hospital, Egypt, from January to June 2022 after approval from the institutional ethics review committee and informed consent from the participants. The study adopted a convenience sampling by including all patients of either gender who were admitted with severe infection to the coronavirus disease-2019 isolation ward. All patients diagnosed with COVID-19, confirmed using polymerase chain reaction (PCR) assays on nasopharyngeal swab samples by local health authority,

were included, and the patients were classified based on the World Health Organisation (WHO) criteria.[12] Patients who received any treatment, except antipyretic, before admission, patients with any documented co-infection (viral or bacterial) at admission, patients who died less than two days after admission. and patients with documented previous COVID-19 infection, immunological disorders or malignancies were excluded.

Severity of the disease was assessed when sampling was performed and the clinical outcome was defined as death or complete recovery and discharge from the hospital with oxygen saturation (SpO2) >95% on room temperature. Intensive care unit (ICU) admission was dependent on clinical condition of the patient and oxygen requirement. All ICU patients were on ventilatory support (non-invasive continuous positive airway pressure [CPAP] or invasive ventilation) at the time of lab investigations, while non-ICU patients were on no more than 5 litters supplemental oxygen (nasal oxygen, face mask or non-rebreathing mask).

According to the WHO criteria for severity assessment and management of COVID-19[12], the clinical classification of severe patients includes those with one of the three conditions: shortness of breath, respiratory rate ≥30 breaths/min; **o**xygen saturation ≤93%; and arterial oxygen partial pressure (PaO2) to fractional inspired oxygen (FIO2) ratio (**P**aO2/FIO2) ≤300mmHg. Critically ill patients were defined as patients with respiratory failure, shock or organ failure requiring ICU monitoring.

Samples were collected from all COVID-19 patients using ethylenediaminetetraacetic acid (EDTA), 0.129 M trisodium citrate tubes and plain tubes. Routine chemistry parameters, including C-reactive protein (CRP), lactate dehydrogenase (LDH), creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin, were determined using automated chemistry analyser (Cobas C311, Roche Diagnostics, Germany).

All haemostasis tests were measured on platelet-poor plasma after double centrifugation at 2000g for 15 minutes. Chemiluminescence immunoassay (CLIA) method on a fully automated analyser (Immulite 1000, Siemens, or Cobas e411, Roche Diagnostics, Germany) was used to measure levels of procalcitonin, ferritin, sIL-2R, IL6 and IL10.

Data was analysed using SPSS version 25. Data was presented as frequencies and percentages, or as median and interquartile range (IQR), as appropriate. Categorical data was compared using Fisher's exact test or Chi-square test, as appropriate, while numerical data was compared using Mann-Whitney U test. Spearman's correlation coefficient was used to detect correlations among numerical variables. Receiver operating characteristic (ROC) curve analysis was used to measure sensitivity, specificity and cut-off values for sIL-2R, IL6 and IL10. P<0.05 was considered statistically significant.

Results

Of the 250 patients with median age 57.5 years (IQR: 45.8-66.0 years), 147(59%) were males and 103(41%) were females. Of them, 102(40.8%) patients died; 68(66.7%) males, 34(33.3%) females, median age 60.0 years (IQR: 48.8-70.0). Among the 148(59.2%) survivors, 79(53.4%) were males and 69(46.6%) were females, while the overall median age was 55.0 years (IQR: 41.5-65.8 years). The survivors had significantly lower levels of sIL-2R, IL-6 and IL-10 (p<0.001). The survivors and non-survivors had a number of significant differences (Table 1).

Table-1: Comparison between survivors and non-survivors with respect to clinical and laboratory parameters.

	All patients N=250	**Survivors n=148**	**Non-survivors n=102**	***p-value***
Age (yeas) median (IQR)	57.5 (45.8-66.0)	55.0 (41.5-65.8)	60.0 (48.8-70.0)	0.016
Male/Female n	147/103	79/69	68/34	0.036
Comorbidities n (%)				
Obesity	52 (20.8)	16 (10.8)	36 (35.3)	<0.001
Hypertension	96 (38.4)	38 (25.7)	58 (56.9)	<0.001
Diabetes Mellitus	109 (43.6)	65 (43.9)	44 (43.1)	0.9
Ischaemic heart disease	37 (14.8)	19 (12.8)	18 (17.7)	0.29
Liver cirrhosis	27 (10.8)	12 (8.1)	15 (14.7)	0.099
Venous thromboembolism	51 (20.4)	14 (9.5)	37 (36.3)	<0.001
Complete blood count median (IQR)				
Hb (gm/dL)	13.0 (12.0-14.4)	13.0 (12.0-14.5)	13.0 (11.5-14.1)	0.32
WBCs (× 103/mL)	6.9 (4.5-10.0)	7.0 (4.7-10.0)	6.8 (4.0-12.0)	0.93

Continued on next page

Table-1: *continued from previous page*

	All patients N=250	Survivors n=148	Non-survivors n=102	*p-value*
Platelets (× 103/mL)	212.0 (171.0-256.0)	219.5 (183.3-259.0)	189.0 (125.0-220.0)	<0.001
Inflammatory markers median (IQR)				
CRP (mg/dL)	86.0 (44.0-128.0)	50.0 (33.0-78.0)	128.5 (98.0-170.8)	<0.001
Procalcitonin (µg/L)	0.2 (0.09-0.3)	0.1 (0.08-0.2)	0.2 (0.1-0.5)	0.011
LDH (U/L)	614.5 (409.0-907.5)	449.5 (371.8-604.8)	911.5 (728.5-1229.5)	<0.001
Ferritin (µg/L)	676.5 (422.5-1434.8)	545.5 (359.3-673.0)	1456.5 (921.0-2053.8)	<0.001
Coagulation profile median (IQR)				
PT (sec.)	14.3 (13.3-16.0)	13.4 (13.0-14.5)	16.0 (14.9-18.0)	<0.001
APTT (sec.)	35.0 (31.0-44.3)	32.0 (30.0-35.8)	43.0 (36.8-50.0)	<0.001
D-dimer (ng/mL)	1040.5 (740.0-2004.3)	791.5 (690.0-995.0)	2151.0 (1418.0-3200.0)	<0.001
Fibrinogen (gm/dL)	3.7 (3.1-4.8)	3.2 (3.0-3.7)	5.0 (4.5-5.4)	<0.001
Interleukins median (IQR)				
IL-2R U/ml	413.0 (240.8-764.3)	257.0 (219.3-373.0)	813.0 (647.5-990.0)	<0.001
IL-6 µg/L	48.0 (25.0-112.0)	27.0 (19.0-41.0)	134.0 (75.0-235.5)	<0.001
IL-10 µg/L	34.0 (15.0-91.0)	18.0 (13.0-30.0)	106.5 (58.8-180.8)	<0.001
Other laboratory data median (IQR)				
Creatinine (mg/dL)	1.0 (0.8-1.3)	0.9 (0.8-1.1)	1.2 (0.9-1.6)	<0.001
Urea (mg/dL)	48.5 (38.0-73.3)	42.0 (35.0-54.5)	69.0 (50.0-93.0)	<0.001
Albumin (gm/dL)	3.5 (3.1-3.8)	3.7 (3.4-4.0)	3.2 (2.9-3.5)	<0.001
AST (U/L)	45.0 (31.0-74.0)	39.5 (27.0-54.3)	69.0 (38.0-94.0)	<0.001
ALT (U/L)	54.0 (35.0-81.0)	45.0 (31.0-59.78)	73.5 (47.5-106.5)	<0.001
O_2 saturation (%) median (IQR)	81.0 (70.8-85.0)	84.0 (80.0-86.0)	70.0 (60.0-80.0)	<0.001
O_2 support n (%)				
Mask reservoir	120 (48.0)	118 (79.7)	2 (2.0)	<0.001
Face mask	27 (10.8)	27 (18.2)	-	
Invasive	103 (41.2)	3 (2.0)	100 (98.0)	
Hospital stay (days) median (IQR)	10.0 (7.0-13.0)	9.0 (7.0-12.0)	10.5 (7.8-14.0)	0.049
ICU admission n (%)	103 (41.2)	3 (2.0)	100 (98.0)	<0.001

BMUI: Body mass index, PTH: Parathyroid hormone, a: statistically significant difference in comparison to 6m, b: statistically significant difference in comparison to 12m

Table-2: Correlation of pro-inflammatory interleukins (ILs) with clinical and laboratory parameters.

	IL-6		IL-10		IL-2R	
	r-value	*p-value*	*r-value*	*p-value*	*r-value*	*p-value*
Age	0.15	0.019	0.17	0.006	0.17	0.007
Hb	-0.05	0.45	-0.05	0.43	-0.08	0.21
WBCs	0.05	0.43	-0.04	0.6	-0.07	0.38
Platelets	-.25	<0.001	-0.31	<0.001	-0.24	<0.001
CRP	0.61	<0.001	0.67	<0.001	0.54	<0.001
Procalcitonin	0.19	0.003	0.19	0.003	0.03	0.6
LDH	0.51	<0.001	0.5	<0.001	0.54	<0.001
Ferritin	0.48	<0.001	0.48	<0.001	0.5	<0.001
PT	0.45	<0.001	0.48	<0.001	0.46	<0.001
APTT	0.52	<0.001	0.56	<0.001	0.5	<0.001
D-dimer	0.53	<0.001	0.54	<0.001	0.6	<0.001
Fibrinogen	0.66	<0.001	0.68	<0.001	0.56	<0.001

Continued on next page

Table-2: *continued from previous page*

	IL-6		IL-10		IL-2R	
	r-value	***p-value***	***r-value***	***p-value***	***r-value***	***p-value***
IL-6	-	-	0.92	<0.001	0.57	<0.001
IL-10	0.92	<0.001	-	-	0.62	<0.001
IL-2R	0.57	<0.001	0.62	<0.001	-	-
Creatinine	0.36	<0.001	0.4	<0.001	0.28	<0.001
Urea	0.39	<0.001	0.4	<0.001	0.41	<0.001
Albumin	-0.43	<0.001	-0.44	<0.001	-0.36	<0.001
AST	0.31	<0.001	0.34	<0.001	0.24	<0.001
ALT	0.3	<0.001	0.34	<0.001	0.26	<0.001
02 saturation	-0.49	<0.001	-0.54	<0.001	-0.56	<0.001
Hospital stay	0.28	<0.001	0.25	<0.001	0.09	0.15

APTT: Activated partial thromboplastin time, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, Hb: Haemoglobin, ICU: Intensive care unit, LDH: Lactate dehydrogenase, PT: Prothrombin time, WBCs: White blood cells.

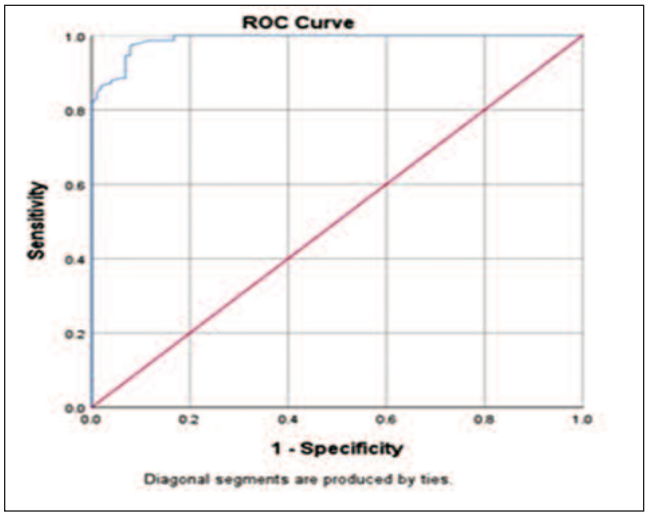

Figure 1: Figure 1: Receiver operator characteristic (ROC) curve of interleukin-2 receptor (IL-2R) for in-hospital mortality in severe coronavirus disease-2019 (COVID-19) patients.

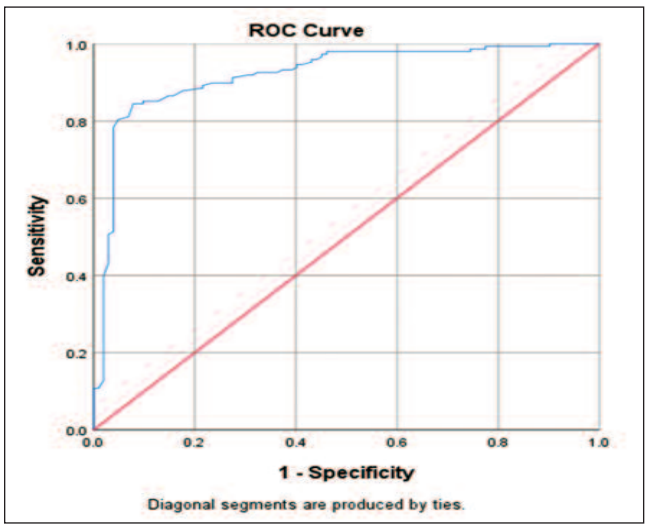

Figure 2: Receiver operator characteristic (ROC) curve of interleukin-6 (IL-6) for in-hospital mortality in severe coronavirus disease-2019 (COVID-19) patients.

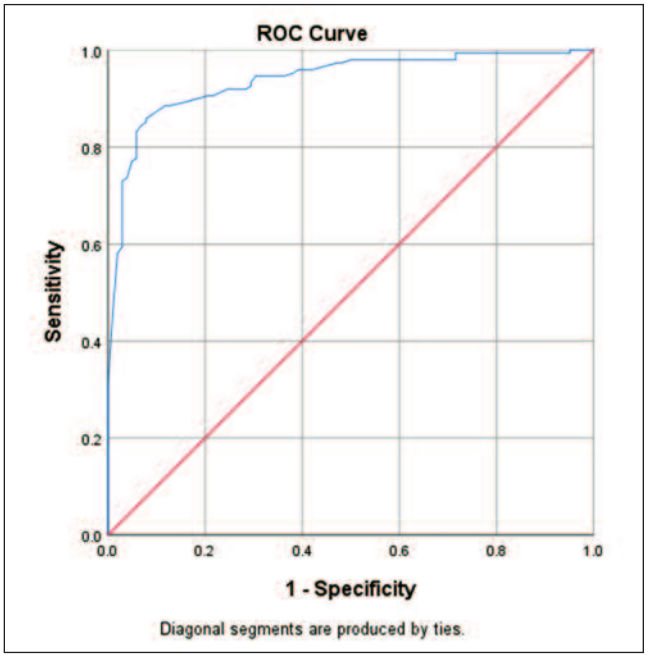

Figure 3: Receiver operator characteristic (ROC) curve of interleukin-10 (IL-10) for in-hospital mortality in severe coronavirus disease-2019 (COVID-19) patients.

Correlation analysis identified significant positive correlation between IL-2R, IL-6 and IL-10 levels and almost all the inflammatory and coagulation parameters, including CRP, LDH, procalcitonin, ferritin, D-dimer and fibrinogen (Table 2).

ROC curve analysis showed excellent performance of sIL-2R (Figure 1), IL-6 (Figure 2) and IL-10 (Figure 3) in the identification of non-survivors.

Discussion

The present study investigated a possible relation between selected pro-inflammatory interleukins and mortality risk in patients with severe COVID-19. The study focussed on

this particular group of patients because they carry the major brunt of the disease mortality burden. The study found a significant association of elevated levels of IL-2R, IL-6 and IL-10 with mortality. Moreover, these markers revealed significant correlation with other inflammatory and coagulopathy parameters characterising COVID-19 infection. The findings are in accordance with multiple reports.[6,7,13-18]

Elevated IL-10 levels have been reported to have an association with COVID-19 infection[19], severe illness, need for mechanical ventilation and mortality[20]**.** Notably, a recent meta-analysis showed a correlation between elevated IL-6 levels and cluster of differentiation 4 (CD4)/CD8 T-cells subsets in COVID-19 patients.[21]

The limitation of the current study is that it did not calculate sample size which could have influenced the power of the study.

Conclusion: Elevated levels of sIL-2R, IL-6 and IL-10 were found to be associated with greater risk of mortality in COVID-19 patients.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Brandão SCS, Godoi ETAM, Ramos JOX, de Melo LMMP, Sarinho ESC. Severe COVID-19: understanding the role of immunity, endothelium, and coagulation in clinical practice. J Vasc Bras 2020;19:e20200131. Doi: 10.1590/1677-5449.200131.
2. Mehta P, Fajgenbaum DC. Is severe COVID-19 a cytokine storm syndrome: a hyperinflammatory debate. Curr Opin Rheumatol 2021;33:419-30. Doi: 10.1097/BOR.0000000000000822.
3. Jiang Y, Rubin L, Peng T, Liu L, Xing X, Lazarovici P, et al. Cytokine storm in COVID-19: from viral infection to immune responses, diagnosis and therapy. Int J Biol Sci 2022;18:459-72. Doi: 10.7150/ijbs.59272.
4. Zanza C, Romenskaya T, Manetti AC, Franceschi F, La Russa R, Bertozzi G, et al. Cytokine Storm in COVID-19: Immunopathogenesis and Therapy. Medicina (Kaunas) 2022;58:144. Doi: 10.3390/medicina58020144.
5. Dik WA, Heron M. Clinical significance of soluble interleukin-2 receptor measurement in immune-mediated diseases. Neth J Med 2020;78:220-31.
6. Gatselis NK, Lygoura V, Lyberopoulou A, Giannoulis G, Samakidou A, Vaiou A, et al. Soluble IL-2R Levels at Baseline Predict the Development of Severe Respiratory Failure and Mortality in COVID-19 Patients. Viruses 2022;14:787. Doi: 10.3390/v14040787.
7. Jang HJ, Leem AY, Chung KS, Ahn JY, Jung JY, Kang YA, et al. Soluble IL-2R Levels Predict in-Hospital Mortality in COVID-19 Patients with Respiratory Failure. J Clin Med 2021;10:4242. Doi: 10.3390/jcm10184242.
8. García-Juárez M, Camacho-Morales A. Defining the Role of Anti- and Pro-inflammatory Outcomes of Interleukin-6 in Mental Health. Neuroscience 2022;492:32-46. Doi: 10.1016/j.neuroscience.2022.03.020.
9. Kang S, Kishimoto T. Interplay between interleukin-6 signaling and the vascular endothelium in cytokine storms. Exp Mol Med 2021;53:1116-23. Doi: 10.1038/s12276-021-00649-0.
10. Lu L, Zhang H, Dauphars DJ, He YW. A Potential Role of Interleukin 10 in COVID-19 Pathogenesis. Trends Immunol 2021;42:3-5. Doi: 10.1016/j.it.2020.10.012.
11. Lindner HA, Velásquez SY, Thiel M, Kirschning T. Lung Protection vs. Infection Resolution: Interleukin 10 Suspected of Double-Dealing in COVID-19. Front Immunol 2021;12:602130. Doi: 10.3389/fimmu.2021.602130.
12. World Health Organization (WHO). Living guidance for clinical management of COVID-19. [Online] 2021 [Cited 2022 March 13]. Available from URL: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2.
13. Ma A, Zhang L, Ye X, Chen J, Yu J, Zhuang L, et al. High Levels of Circulating IL-8 and Soluble IL-2R Are Associated With Prolonged Illness in Patients With Severe COVID-19. Front Immunol 2021;12:e626235. Doi: 10.3389/fimmu.2021.626235.
14. Kaya H, Kaji M, Usuda D. Soluble interleukin-2 receptor levels on admission associated with mortality in coronavirus disease 2019. Int J Infect Dis 2021;105:522-24. Doi: 10.1016/j.ijid.2021.03.011.
15. Luo K, Chen Y, Yang J, Tao Q, Luo M. Liver Injury and Elevated Levels of Interleukins, Interleukin-2 Receptor, and Interleukin-6 Predict the Severity in Patients With COVID-19. Front Public Health 2021;9:e778340. Doi: 10.3389/fpubh.2021.778340.
16. Bagnato G, Imbalzano E, Aragona CO, Ioppolo C, Di Micco P, La Rosa D, et al. New-Onset Atrial Fibrillation and Early Mortality Rate in COVID-19 Patients: Association with IL-6 Serum Levels and Respiratory Distress. Medicina (Kaunas) 2022;58:530. Doi: 10.3390/medicina58040530.
17. Chang Y, Bai M, You Q. Associations between Serum Interleukins (IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10) and Disease Severity of COVID-19: A Systematic Review and Meta-Analysis. Biomed Res Int 2022;2022:e2755246. Doi: 10.1155/2022/2755246.
18. Tasoudis PT, Arvaniti CK, Adamou AT, Belios I, Stone JH, Horick N, et al. Interleukin-6 inhibitors reduce mortality in coronavirus disease-2019: An individual patient data meta-analysis from randomized controlled trials. Eur J Intern Med 2022;101:41-8. Doi: 10.1016/j.ejim.2022.04.004.
19. Naghashpour M, Ghiassian H, Mobarak S, Adelipour M, Piri M, Seyedtabib M, et al. Profiling serum levels of glutathione reductase and interleukin-10 in positive and negative-PCR COVID-19 outpatients: A comparative study from southwestern Iran. J Med Virol 2022;94:1457-64. Doi: 10.1002/jmv.27464.
20. Jiménez-Gastélum GR, Espinoza-Ortega AM, Ramos-Payán R, Aguilar-Medina M, López-Gutiérrez J, Villegas-Mercado C, et al. More Evidence of the Link of Interleukin-6 and Interleukin-10 with Critical COVID-19: A Report in Mexican Patients. Iran J Immunol 2021;18:331-7. Doi: 10.22034/IJI.2021.89905.1978.
21. Alshammary AF, Alsughayyir JM, Alharbi KK, Al-Sulaiman AM, Alshammary HF, Alshammary HF, et al. T-Cell Subsets and Interleukin-10 Levels Are Predictors of Severity and Mortality in COVID-19: A Systematic Review and Meta-Analysis. Front Med (Lausanne) 2022;9:e852749. Doi: 10.3389/fmed.2022.852749.