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Comparison between levels of Homocysteine, Interleukin-6 and Interleukin-10 in severe cases of Egyptian COVID-19 patients

Wafaa A. Abdalwahed[1], Eman F. Mohammed[1], Walaa M. Shipl[2], and Fatma M. Mabrouk[1]

[1]Department of Internal Medicine, Faculty of Medicine (Girls), Al-Azhar University, Cairo, Egypt.

[2]Department of Biochemistry, Faculty of Medicine (Girls), Al-Azhar University, Al-Azhar University, Cairo, Egypt.

Corresponding author: Wafaa A. Abdalwahed, Department of Internal Medicine, Faculty of Medicine (Girls), Al-Azhar University, Cairo, Egypt.
Email: wafaaabdalmagied@gmail.com.

Abstract

Coronavirus disease 2019 (COVID-19) is the reason of an outbreak of respiratory illnesses ranging from typical cold to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome. We intended to compare levels of IL-6, IL-10, and homocysteine in the sera of severe COVID-19 Egyptian patients and connect them with the extent of the illness. This cross-sectional study included 90 COVID-19 Egyptian patients. They included 45 non severe cases (group 1) and 45 severe cases (group 2). There was statistically significantly increase in IL-6 in group 2 (median = 7.05, IQR = 6.2 - 7.9; p<0.001) in comparison to group 1 (median = 4.96, IQR = 4.5 - 5.8) and statistically significantly increase in IL-10 in group 2 (154.1 ± 73.3) in comparison to group 1 (47.04 ± 23.8; p<0.001). There were no variations in the examined groups' homocysteine levels (p= 0.318). Furthermore, there was statistically substantial positive connection (r=0.30) between IL-6 and AST (p=0.046) and between IL-10 and HCT (r = 0.37, p=0.012). In addition, data of other studied parameters are presented. In conclusion, IL-6 and IL-10 could be proposed for follow up of COVID-19 patients and to detect cases before progressing to a severe disease stage.

Keywords: COVID-19, IL-6, IL-10, and homocysteine.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic has led to a dramatic loss of human lives worldwide. A frightening number of people have died because of the COVID-19 outbreak, which also presents an unprecedented danger to the food supply, public health, and the workplace. Tragically, the epidemic has caused enormous economic and social disruption.[1] As of 26 August 2022, the World Health Organization (WHO) has received reports of 596,873,121 verified infections with COVID-19 worldwide, including 6,459,684 fatalities.[2] In Egypt, from 3 January 2020 to 26 August 2022, there were 515,264 verified infections with COVID-19 with 24,791 deaths, reported to WHO.[2] The causative COVID-19 virus, the respiratory condition that started the COVID-19 outbreak, is known as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-

2). The coronavirus is often used.[3] One of the beta corona viruses is the SARS CoV-2 virus. They are zoonotic enveloped positive-sense single-stranded RNA viruses. They are spherical to pleomorphic particles that range in size from 80 to 160 nm. Four structural proteins make up SARS CoV-2: the membrane (M), nucleocapsid (N), spike (S), and envelope (E).[4] The viral invasion via its intended host cell receptors is the initial stage of COVID-19 pathogenesis. The S protein is required for viral binding and entrance into host cells, while the M, E, and N proteins of SARS-CoV-2 are essential for viral particle assembly and release.[4].

Several studies have identified the human angiotensin-converting enzyme 2 (ACE2) as the SARS-CoV-2 entry receptor. The SARS-CoV-2 virus directly infects cells lining the upper and lower respiratory passages, most notably nasal ciliated and alveolar epithelial cells. The presence of ACE2 in human tissues other than the lungs shows that when viremia exists, the virus may immediately infect cells in these organs. These tissues include the small intestine, kidneys, thyroid, heart, testicles, and adipose tissue.[5]

Due to the excellent concordance among observers and its discriminating value, the COVID-19 Reporting and Data System (CO-RAD) is a categorical assessment tool for chest computed tomography (CT) in patients believed to have COVID-19. It represents the degree of pulmonary involvement suspected.[4,6]

According to either typical CT readings or CT results with a clear noninfectious etiology, CO-RADS 1 suggests a very low degree of suspicions for pulmonary invasion by COVID-19.[7] According to CT abnormalities in the lungs characteristic of infectious etiologies that are thought to be incompatible with COVID-19, CO-RADS 2 suggests a low degree of suspicion for pulmonary involvement by COVID-19. [7] According to CT characteristics that are also present in other viral pneumonias or noninfectious causes, CO-RADS 3 suggests ambiguous results for COVID-19's pulmonary involvement. The absence of other typical CT abnormalities may reveal perihilar ground-glass opacity, homogeneous widespread ground-glass opacity with or without sparing of specific secondary pulmonary lobules, or ground-glass opacity combined with smooth interlobular septal thickening with or without pleural effusion.[7] CO-RADS 4 shows a great level of suspicion for respiratory participation by COVID-19 depending on CT outcomes that are common for COVID-19 but also show some similarities with other (viral) pneumonias. [7] Based on usual CT results, CO-RADS 5 indicates a substantial level of suspicion for pulmonary involvement by COVID-19. Ground-glass opacities near to visceral pleural surfaces, including the fissures, with or without consolidations, are required criteria, as is a multifocal bilateral distribution. As the illness progresses over time, CO-RADS 5 needs the existence of at least one confirming pattern. [7] Similar to the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) category 6, CO-RADS category 6 was created to designate COVID-19 that has been confirmed, as shown by positive reverse transcription polymerase chain reaction (RT-PCR) test findings for virus-specific nucleic acid. [7]

More than half of COVID-19 patients showed elevated levels of interleukin-6 (IL-6), a critical mediator of the inflammatory and immunological response triggered by infections or injury. Inflammatory response, respiratory failure, the need for mechanical ventilation and/or intubation, and mortality were all connected to IL-6 values in COVID-19 patients. COVID-19 complications were linked to considerably higher mean IL-6 levels. Furthermore, IL-6 levels were connected to an elevated mortality risk when compared to those who did not encounter problems.[6]

The significant increase in IL-10 in diseased individuals is a hallmark of the COVID-19 cytokine storm. Patients with COVID-19 in the intensive care units (ICUs) had considerably greater peripheral IL-10 concentrations than those who did not spend time in the ICU. Furthermore, IL-6 and other inflammatory indicators, such as C-reactive protein (CRP), were highly associated to IL-10 concentrations.[8] The high rise of IL-10 in very sick patients during the COVID-19 cytokine storm is a distinctive characteristic of this condition. ICU COVID-19 patients had considerably greater peripheral IL-10 concentrations than non-ICU patients.

Additionally, there was a notable connection between the levels of IL-10, IL-6, and other inflammatory indicators including C-reactive protein. [8]

The endothelium in capillaries may be injured and the thromboprotective function of the endothelial cells which may be impaired by leukocytes that have been activated during the inflammatory storm caused by IL-6. The ACE2 receptor, which is mostly expressed on endothelial cells, is another route by which the virus might infect cells and damage blood vessels.[9] This micro thrombotic condition, which primarily affects the kidneys and lungs, may be brought on by endotheliitis.[10]

Homocysteine is an amino acid produced by the body as a byproduct of methionine demethylation; it is not essential for living. As a result of its activities, homocysteine may play a role in either beginning or facilitating the management of severe COVID-19 disease. For example, SARS-CoV2 employs spike proteins to enter cells by attaching to ACE2 cellular receptors and producing a tunnel. Homocysteine may aid viral penetration into cells by binding to the enzyme and blocking it from connecting to its receptors. The overproduction of inflammatory cytokines described as cytokine release syndrome or "cytokine storm" causes thrombus formation, endothelial dysfunction, and the suppression of nitric oxide synthesis, all of which are exacerbated by high homocysteine levels. All these disorders are symptoms of severe SARS-CoV2 infection.[11]

Additionally, new research studies indicated that homocysteine may be connected to both the COVID-19 severity and microvascular thrombosis. High plasma homocysteine levels considerably enhance the risk of vascular damage in both small and large arteries and are linked to pulmonary and venous thrombosis. Concentrations over the 90th percentile are linked to a higher risk of coronary, brain, and peripheral circulatory degenerative and atherosclerotic processes. Even though homocysteine is a valuable biomarker for risk for cardiovascular disease and cardiovascular issues. It is crucial in admitted COVID-19 patients, however, there are not many comprehensive studies on this parameter.[11] Consequently, the goal of this research was to determine whether COVID-19-related acute sickness in Egyptian patience influence serum values of IL-6, IL-10, and homocysteine.

Patients and Methods

This was a cross sectional investigation, included 90 Egyptian COVID-19 patients, they were 45 non severe cases and 45 severe cases. They were selected from Al Zahraa University hospital during the period from April 2021 to September 2021. The medical history of study patients (including hypertension, ischemic heart disease, and diabetes mellitus), was obtained from hospital records.

An ABL-90 blood gas analyzer was used to do an instantaneous analysis of a 0.5–1.0 ml arterial blood sample that was collected from the radial artery. To gauge the severity of the COVID infection, O2 saturation was evaluated. Patients were identified to be severe cases according to peripheral capillary oxygen saturation (SpO2) below 94% in room air at sea level, having a partial pressure of oxygen/fraction of inspired oxygen (PaO2/FiO2) ratio below 300 mm Hg, breathing >30 breaths per minute, or having lung infiltrates over 50%.

The carried-out investigations included: complete blood counts (CBC), PCR-COV 2 and CT scan of the chest, such data were collected from patients' medical records. In addition, CRP titer, D dimer, prothrombin time, International Normalized Ratio (INR), liver and kidney function tests, IL-6, IL-10, and homocysteine were assessed.

CT scan data were obtained from patients' medical records to assess CO-RADs score of patients. For laboratory assessment, venous blood samples (7 ml) were drawn on admission. Blood samples were permitted to coagulate for 10–20 minutes at room temperature, then centrifuged for 20 minutes at 1000–2000 x g and the serum was extracted and stored at -80°C until used. A portion of serum was utilized to measure random blood sugar (RBS), blood urea, serum creatinine, ALT, aspartate aminotransferase (AST), serum albumin, and

bilirubin utilizing an automated clinical analyzer (BIOLIS 24i, Carolina Chemistries, North Carolina, USA), according to the manufacturer's instructions. A second serum portion was used for assessment of CRP and D dimer using an immunoassay equipment (Immulite 1000, Siemens, USA), according to the manufacturer's instructions. The third serum portion was utilized to measure IL-6, IL-10, and homocysteine using enzyme-linked immunosorbent assay (ELISA) kits (catalog numbers D6050, DPSE00, and 201-12-8014, respectively, supplied by Bio-Techne Ltd., Minneapolis, USA, and Sun red bio in Shanghai, China), according to the manufacturer's instructions.

Ethical consideration

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine (for Girls), Al-Azhar University (approval number: 815, dated April 2021). Verbal informed consent was obtained from each patient before being enrolled in the study.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 15 was used for analysis statistical analysis. For quantitative variables, both the mean value (to determine central tendency) and a standard deviation (to determine variability) were calculated. Quantitative variables were provided as a frequency distribution and a percentage breakdown. To statistically evaluate variations in qualitative variables, the Chi-Square test was performed. By computing the area under the Receiver Operating Characteristic (ROC) curve, the prediction accuracy was evaluated. A *p*-value less than 0.05 was considered statistically significant.

Results

The current cross-sectional study included 90 COVID-19 Egyptian patients, classified into two groups. Group 1 included 45 non severe COVID-19 patients, and Group 2 included 45 severe COVID-19 patients.

The values of IL-6, IL-10 and homocysteine were compared between the two groups. Also, the values of IL-6, IL-10 and homocysteine were compared among the patients of severe group. There was statistically substantial variation between IL-6 and IL-10 in the studied groups ($p< 0.001$ and $p< 0.022$, respectively). However, no variation in homocysteine was observed between the studied groups (Table 1).

Table 1. Comparison between IL-6, IL-10, and homocysteine values in the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
IL-6	Median IQR	4.96 4.5 - 5.8	7.05 6.2 - 7.9	< 0.001
IL-10	Mean±SD	47.04±23.8	154.1±73.3	< 0.001
Homocysteine	Median IQR	6.4 3.2 - 10.2	7.2 3.9 - 10.01	NS

P > 0.05 is not significant (NS).

The demographic data of the studied groups showed that age was significantly superior in group 2 (62.5 ± 11.2 years) as compared with group 1 (46.1 ± 17.1 years) ($p< 0.001$). Also, the duration of COVIC-19 disease before admission was significantly increased in Group 2 (median = 7, IQR = 5 - 10 days) when compared with group 1 (median = 5, IQR = 3 - 7 days) ($p = 0.017$). Meanwhile, no difference was observed in chronic diseases (diabetes, ischemic chronic renal disease, hypertension, and heart disease) between studied groups ($p> 0.05$) (Table 2).

Table 2. Comparison between demographic variables among the studied groups.

		Non-severe (N = 45)		Severe (N = 45)		*p*-value
Age (years) Mean (±SD)		46.1 (±17.1)		62.5 (±11.2)		< 0.001
		No.	%	No.	%	
Chronic diseases	DM	17	37.8%	20	44.4%	NS
	HTN	21	46.7%	22	48.9%	NS
	IHD	7	15.6%	3	6.7%	NS
	CKD	8	17.8%	3	6.7%	NS
Duration before admission (days)	Median	5		7		0.017
	IQR	3 – 7		5 - 10		

DM: Diabetes mellitus. CKD: chronic kidney disease. IHD: ischemic heart disease. HTN: Hypertension.
MW: Mann Whitney U test. T: independent sample T test; *P* > 0.05 is not significant (NS).

Comparison of the vital signs between the studied groups

There was statistically substantial variation in respiratory rate and O2 saturation between the study groups (*p*< 0.001). The respiratory rate in group 2 (median = 31, IQR = 27 - 37 cycle/min) was higher when compared with group 1 (median = 22, IQR = 19 - 25 cycle/min) and decreased O2 saturation in group 2 (median = 85, IQR = 77 - 88 %) when compared with group 1 (median = 97, IQR = 96 - 98 %). In the meanwhile, there was no distinction between the study groups according to diastolic blood pressure (DBP), systolic blood pressure (SBP), temperature, or pulse (*p*-value > 0.05) (Table 3).

Table 3. Comparison between vital signs in the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
SBP (mmHg)	Median	120	130	NS
	IQR	110 – 145	113 - 140	
DBP (mmHg)	Median	80	80	NS
	IQR	80 – 90	70 - 90	
RR (cycle/min)	Median	22	31	< 0.001
	IQR	19 – 25	27 - 37	
Temp (C0)	Median	37.6	37	0.05
	IQR	37 – 38	37 - 37.5	
Pulse (beat/min)	Mean±SD	93.2±14.6	96.7±15.6	NS
O2 sat. (%)	Median	97	85	< 0.001
	IQR	96 – 98	77 – 88	

Temp: Temperature; O2 sat.: O2 saturation. *P* > 0.05 is not significant (NS).

The random blood sugar (RBS) was increased in group 2 (median = 193, IQR = 139 - 290 mg/dl) when compared with group 1 (median = 154, IQR = 124 - 230 mg/dl) (*p*=0.033). While no variation was observed in levels of creatinine, urea, Na and K between the studied groups (*p* > 0.05) (Table 4).

The present study showed statistically substantial variation in ALT (*p*=0.012), AST (*p*=0.001), INR (*p*=0.022) and Albumin (ALB) (*p*=0.001) between the two studied groups (Table 5).

Table 4. Comparison between random blood sugar (RBS), kidney functions and electrolytes among the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
RBS (mg/dl)	Median	154	193	0.033
	IQR	124 – 230	139 - 290	
Creatinine (mg/dl)	Median	0.9	0.9	NS
	IQR	0.7 - 1.35	0.7 - 1.4	
Urea (mg/dl)	Median	43	43	NS
	IQR	26.5 – 75	32 - 82	
Na (mmol/l)	Median	138	138	NS
	IQR	135.5 - 142.5	136 - 141	
K (mmol/l)	Median	4	4.1	NS
	IQR	3.7 - 4.5	3.7 - 4.5	

RBS: Random Blood Sugar. *P* > 0.05 is not significant (NS).

Table 5. Comparison between liver function tests among the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
ALT (U/L)	Median	22	26	0.012
	IQR	14.5 – 26	18 – 49	
AST (U/L)	Median	22	33	0.001
	IQR	17 – 37	22 – 59	
ALB (g/dl)	Median	3.9	3.3	0.001
	IQR	3.45 – 4	3.1 - 3.6	
T. Bilirubin (mg/dl)	Median	0.6	0.5	NS
	IQR	0.35 - 0.8	0.3 - 0.8	
INR	Median	1.06	1.11	0.022
	IQR	1 - 1.19	1.03 - 1.3	

T. Bilirubin: Total Bilirubin; ALB: Albumen; INR: International Normalized Ratio. *P* > 0.05 is not significant (NS).

Between the two groups under study, there were statistically substantial variations in pH (*p* <0.001), oxygen pressure (PO2) (*p* <0.001), and O2 saturation (SO2) (*p* <0.001). However, there was no change in PCO2 across the groups investigated (*p*> 0.05) as regard, (Table 6).

Table 6. Comparison between arterial blood gases (ABG) in the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
pH	Median	7.38	7.44	< 0.001
	IQR	7.37 - 7.4	7.41 - 7.48	
PCO2	Median	35	33	NS
	IQR	31 – 37	29 - 39	
PO2	Median	72	56	< 0.001
	IQR	70 – 78	48 - 63	
HCO3	Median	22	25.2	0.003
	IQR	19 – 25	22.3 - 28	
SO2.	Median	96	90	< 0.001
	IQR	93.5 – 97	84 – 93	

P > 0.05 is not significant (NS).

There were statistically substantial variations in lymphocytes and hemoglobin (Hb) between the studied groups (p = 0.016 and p= 0.041, respectively) (Table 7).

Moreover, there was statistically substantial variations in ferritin and CRP values between the two studied groups (p< 0.001 each) (Table 8).

CT scan of the chest finding indicated a statistically substantial variations in CO-RAD 5 between the studied groups (p< 0.001) (Table 9).

Table 7. Comparison between complete blood count (CBC) parameters in the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
RBCs (million/μl)	Median	4.2	4.5	NS
	IQR	3.9 - 4.8	4.1 - 4.8	
Hb (g/dl)	Median	11.2	11.9	0.041
	IQR	1.9	1.5	
HCT (%)	Median	33	36	NS
	IQR	29 – 37	30 - 38.5	
MCV (fl/cell)	Median	84	84	NS
	IQR	78.5 – 87	78 - 88	
MCH (pg/cell)	Median	26.3	26.9	NS
	IQR	2.5	2.5	
PLTs (x 10^3/μl)	Median	234.9	217.1	NS
	IQR	115.8	89.0	
WBCs (x 10^3/μl)	Median	7.3	7.1	NS
	IQR	6.25 – 11	6.1 - 10.6	
Lymph (x 10^3/μl)	Median	1.4	1	0.016
	IQR	1 – 2	0.8 - 1.5	
Neut. (x 10^3/μl)	Median	6	6	NS
	IQR	4.2 - 8.4	3.2 - 8.7	

P > 0.05 is not significant (NS).

Table 8. Comparison between ferritin, CRP, and D-Dimer in the studied groups.

		Non-severe (N = 45)	Severe (N = 45)	*p*-value
Ferritin (ng/ml)	Median	85	354	< 0.001
	IQR	43.5 - 163.5	289 - 431	
CRP (mg/L)	Median	5	63	< 0.001
	IQR	4 – 10	27 - 112	
D-Dimer (mg/L)	Median	0.4	0.6	0.022
	IQR	0.3 - 0.49	0.3 - 0.9	

**P* ≤ 0.05 is significant.

Table 9. Comparison in computed tomography (CT) chest scan findings between the studied groups.

		Non-severe (N = 45)		Severe (N = 45)		*p*-value
CT chest	Normal	15	33.3%	2	4.4%	< 0.001
	CO-RAD 3	14	31.1%	2	4.4%	
	CO-RAD 4	14	31.1%	15	33.3%	
	CO-RAD 5	1	2.2%	23	51.1%	

**P* ≤ 0.05 is significant.

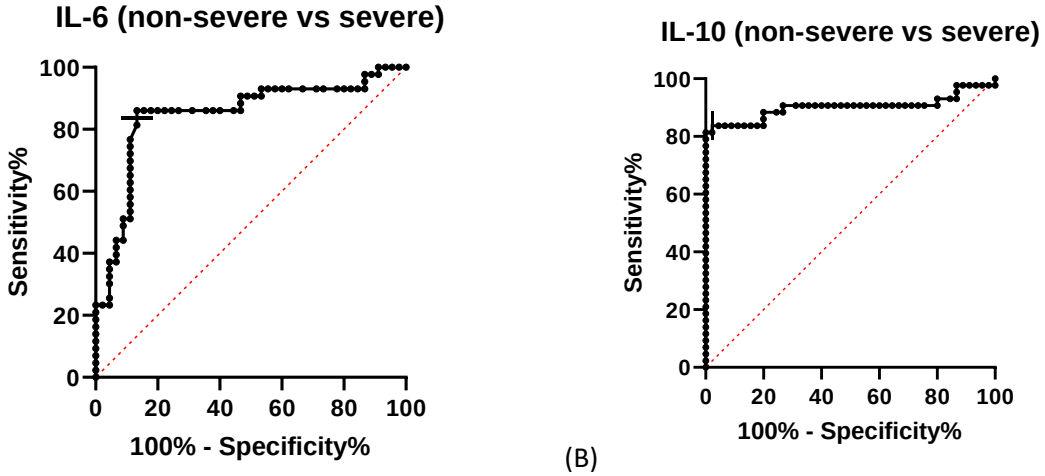
Receiver Operating Characteristic (ROC) curve analysis

The ROC curve analysis indicated that serum IL-6 can be used to discriminate between group 1 and group 2 at a cutoff level of > 6.05, with 81.4% sensitivity, 86.7% specificity, 86% positive predictive value (PPV) and 82.3% negative predictive value (NPV) (AUC = 0.84 and *p*< 0.001). Also, serum IL-10 can be used to discriminate between group 1 and group 2 at a cutoff level of > 93.4, with 81.4% sensitivity, 97.8% specificity, 97.4% PPV and 84% NPV (AUC = 0.9 and *p*< 0.001). (Table 10, Figure 1).

Table 10. Diagnostic performance of serum IL-6 and IL-10 in discrimination between non-severe (groups 1) and severe (group 2).

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	*p*-value
IL-6	> 6.05	0.84	81.4%	86.7%	86%	82.3%	< 0.001
IL-10	> 93.4	0.9	81.4%	97.8%	97.4%	84%	< 0.001

AUC: Area under curve; PPV: positive predictive value; NPV: negative predictive value. *$P \leq 0.05$ is significant.



(A) (B)

Figure 1. Receiver Operating Characteristic (ROC) curve analysis for use of serum IL-6 (A) and IL-10 (B) to differentiate between group 1 and group 2.

Correlation analysis of IL-6, IL-10, and homocysteine with other studied parameters in group 1

There was statistically substantial positive connection between IL-6 and AST (r = 0.30, *p*= 0.046), and between IL-10 and HCT (r = 0.37, *p*= 0.012). However, there was a statistically substantial negative connection (r = -0.3, *p*= 0.045) between IL-6 and IL-10 with some studied parameters as shown in Tables 11 and 12. In addition, correlation analysis of IL-6, IL-10 and homocysteine with other parameters studied in group 2 are shown in Tables 13 and 14.

Table 11. Correlation of IL-6, IL-10 and homocysteine with other parameters studied in group 1 (non-severe disease).

Studied parameters	IL-6		IL-10		Homocysteine	
	r	*p*-value	r	*p*-value	r	*p*-value
Age	-00.18	NS	-00.12	NS	-00.12	NS
Duration	0.08	NS	0.07	NS	-0.06	NS
SBP	0.14	NS	-0.28	NS	-0.12	NS
DBP	0.19	NS	-0.20	NS	-0.13	NS
RR	-0.06	NS	-0.01	NS	-0.04	NS
Temp	0.03	NS	-0.28	NS	-0.11	NS
Pulse	-0.11	NS	-0.30	0.045	-0.15	NS
O2 Sat. (clinical)	0.14	NS	0.10	NS	0.14	NS
RBS	-0.05	NS	0.00	NS	-0.03	NS
ALT	-0.09	NS	-0.05	NS	0.21	NS
AST	0.30	0.046	-0.07	NS	0.06	NS
ALB	-0.01	NS	0.25	NS	0.08	NS
T. Bilirubin	0.04	NS	-0.20	NS	0.12	NS
INR	0.00	NS	-0.15	NS	-0.21	NS
Creatinine	0.01	NS	-0.22	NS	-0.07	NS
Urea	0.04	NS	-0.36	0.015	-0.20	NS
Na	0.02	NS	-0.29	0.052	-0.09	NS
K	-0.17	NS	-0.17	NS	-0.15	NS
pH	0.14	NS	0.02	NS	0.10	NS

r: Pearson correlation coefficient. *P* > 0.05 is not significant (NS).

Table 12. Correlation analysis of IL-6, IL-10, and homocysteine with other studied parameters in group 1.

Studied parameters	IL-6		IL-10		Homocysteine	
	r	*p*-value	r	*p*-value	r	*p*-value
PCO2	00.22	NS	00.20	NS	-00.09	NS
PO2	-0.38	0.009	-0.21	NS	-0.19	NS
HCO3	0.22	NS	0.21	NS	0.16	NS
O2 Sat. (ABG)	-0.34	0.021	0.03	NS	-0.06	NS
RBCs	0.06	NS	0.09	NS	-0.23	NS
Hb	0.18	NS	0.15	NS	-0.11	NS
HCT	0.25	NS	0.37	0.012	0.05	NS
MCV	0.00	NS	0.05	NS	0.11	NS
MCH	0.00	NS	0.00	NS	0.03	NS
PLT	0.18	NS	0.16	NS	0.07	NS
WBCs	0.07	NS	-0.01	NS	-0.02	NS
Lymph	-0.01	NS	0.09	NS	-0.03	NS
Neutrophil	0.07	NS	-0.14	NS	-0.04	NS
Ferritin	-0.01	NS	-0.17	NS	0.13	NS
CRP	-0.01	NS	-0.21	NS	-0.01	NS
D. Dimer	0.00	NS	-0.24	NS	-0.15	NS
IL-6	----	-----	-0.01	NS	-0.07	NS
IL-10	-0.01	NS	----	-----	0.26	NS
Homocysteine	-0.07	NS	0.26	NS	----	-----

ABG: arterial blood gases. *P* > 0.05 is not significant (NS).

Table 13. Correlation analysis of IL-6, IL-10, and homocysteine with studied parameters in group 2 (Sever disease).

Studied parameters	IL-6		IL-10		Homocysteine	
	r	*p*-value	r	*p*-value	r	*p*-value
Age	0.22	NS	0.23	NS	-0.09	NS
Duration	00.05	NS	-00.03	NS	00.25	NS
SBP	00.03	NS	-00.11	NS	-00.12	NS
DBP	-0.03	NS	0.06	NS	-0.12	NS
RR	-0.24	NS	0.08	NS	0.26	NS
Temp	-0.15	NS	0.06	0.69	-0.33	0.029
Pulse	-0.31	0.042	0.22	NS	-0.27	NS
O2 Sat. (clinical)	0.05	NS	-0.15	NS	0.11	NS
RBS	0.25	NS	0.03	NS	-0.20	NS
ALT	-0.06	NS	0.16	NS	-0.08	NS
AST	-0.02	NS	0.24	NS	-0.08	NS
ALB	-0.08	NS	-0.01	NS	0.03	NS
T. Bilirubin	-0.01	NS	-0.06	NS	-0.03	NS
INR	0.06	NS	0.37	0.016	-0.03	NS
Creatinine	0.01	NS	0.05	NS	-0.08	NS
Urea	0.02	NS	0.23	NS	-0.17	NS
Na	0.05	NS	-0.10	NS	0.06	NS
K	0.14	NS	-0.10	NS	-0.04	NS
pH	-0.06	NS	-0.28	NS	0.02	NS

r: Pearson correlation coefficient. *P* > 0.05 is not significant (NS).

Table 14. Correlation study between studied markers, IL-6, IL-10, and homocysteine with other studied data in group 2.

Studied parameters	IL-6		IL-10		Homocysteine	
	r	*p*-value	r	*p*-value	r	*p*-value
PCO2	-0.27	NS	0.10	NS	-0.12	NS
PO2	0.23	NS	0.13	NS	-0.04	NS
HCO3	-0.06	NS	-0.21	NS	-0.12	NS
O2 Sat. (ABG)	0.08	NS	0.05	NS	0.02	NS
RBCs	0.11	NS	-0.23	NS	-0.11	NS
Hb	0.02	NS	-0.13	NS	0.00	NS
HCT	-0.04	NS	-0.01	NS	-0.07	NS
MCV	0.00	NS	0.00	NS	-0.15	NS
MCH	-0.06	NS	-0.13	NS	0.01	NS
PLT	-0.34	0.027	0.02	NS	0.04	NS
WBCs	0.04	NS	0.06	NS	0.02	NS
Lymph	0.10	NS	-0.35	0.023	0.00	NS
Neutrophil	0.04	NS	0.06	NS	0.05	NS
Ferritin	-0.11	NS	0.10	NS	0.02	NS
CRP	0.03	NS	0.18	NS	-0.05	NS
D. Dimer	-0.17	NS	0.16	NS	-0.12	NS
IL-6	-----	-----	-0.11	NS	-0.05	NS
IL-10	-0.11	NS	-----	-----	0.11	NS
Homocysteine	-0.05	NS	0.11	0.5	-----	-----

ABG: arterial blood gases. *P* > 0.05 is not significant (NS).

Discussion

In our cross-sectional research, we aimed to measure the values of IL-6, IL-10, and homocysteine in the sera of COVID-19 Egyptian patients and to compare their levels in severe and non-severe COVID-19 patients and correlate them with the degree of the severity of the illness.

In the current research we detected increased values of IL-6 and IL-10 in the group with severe disease when compared with non-severe group. Patients with COVID-19 who had severe respiratory failure had several forms of immunological dysregulation that were mediated by upregulated IL-6.[24] The inflammation associated with COVID-19 progresses as a result of this dysregulation, which is demonstrated by an elevated production of pro-inflammatory cytokines by macrophages and monocytes as well as CD4 lymphocyte deficiency.[24] However no difference in serum homocysteine level was observed between the two studied groups.

Data of a study by Elshazli et al., 2020[21] agreed with our findings as there were high values of IL-6 and IL-10 in the severe group when compared with a non-severe group. Furthermore, findings of a study by Shafiek et al., 2021[24] also agreed with our data as when they compared patients with intermediate COVID-19 pneumonia and the control group, individuals with severe COVID-19 pneumonia had substantially greater median IL-6 and IL-10 levels.

Severe cases were defined as those with a SpO2 on room air of lower than 94%, a PaO2/FiO2 ratio lower than 300 mm Hg, a respiratory rate >30 breaths per minute, or lung infiltrates >50%.[6]

There was a significant increase in age and duration of infection before admission to hospital in the study groups. Meanwhile there was no difference between the studied groups as regard associated comorbid diseases. As age advances, it is accompanied with more severe disease and associated co-morbidities or on a general lack of resistance in aging.[14] Our results agreed with those of a study by Ghweil et al., 2020[15] who`s study included 30 patients with severe/critical infection and 36 individuals with mild to moderate COVID-19. They reported that those with severe infections tended to be older than those with mild to moderate infections. Also, they observed no differences as regard chronic disease between their studied groups.

Also, in a study by Zayed et al*.,* 2022,[16] the real-time PCR was utilized to determine the occurrence of COVID-19 in 202 individuals. These patients were divided into two groups, group A had mild to moderate COVID-19 and group B had severe to critical COVID-19. Their findings supported ours as being severe. In contrast to COVID-19 patients, severe patients had higher prevalence of diabetes mellitus, hypertension, ischemic heart disease (IHD), bronchial asthma, chronic obstructive pulmonary disease, and hyperlipidemia. COVID-19 patients also had older ages and longer periods of symptom duration prior to admission.

Also, Omran et al., 2021[17] who`s retrospective investigation included 2724 COVID-19 patients, 423 (15.52%) were critically ill. They agreed with ours regarding age, which increased in the group with severe disease. However, their findings disagreed with ours regarding chronic disease as patients with serious illnesses had a greater incidence of diabetes, cancer, hypertension, coronary artery diseases, chronic renal insufficiency, and asthma.

In our current study, as compared to the non-severe group, a considerably higher respiratory rate and lower SO2 were seen in the severe group. Meanwhile there was no variation between the studied groups as regard blood pressure (SBP and DBP), temperature and pulse. It was suggested that these could be due to pathology in lung and air ways caused by COVID-19 virus[18].

Our findings concurred with those of Omran et al. 2021[17] as there was an increase in respiratory rate, decrease in O2 saturation in critically ill patients, but no variation between both groups regarding SBP and DBP. On the contrary, there was an increase in pulse and temperature in critically ill patients in comparison to the non-critical patients.

Our results showed no difference between the studied groups as regards creatinine, urea, Na and K. On the other hand, in a study by Ramadan et al., 2020[19] serum creatinine levels were observed to be substantially different amongst all groups of the 260 COVID-19 patients, with the severe group having the highest levels. Furthermore Omran et al., 2021[17] found increase in serum potassium and creatinine in critically ill patients. Also Zayed et al., 2022[16] noted that severe COVID-19 patients had higher serum creatinine, but lower levels of Na, and K than non-severe patients.

ALT and AST levels were higher among all patients which could be due to direct hepatic injury, in response to inflammation, hepatic ischemia or drug induced liver injury as stated by Moon and Barritt, 2021.[20] Also, higher INR could be due to oral anticoagulants as a part of the regimen of the treatment of the disease and decrease albumin in severe group as compared to non-severe group. While no variation in total bilirubin was noted between the studied groups. Our results agreed with those of Ramadan et al., 2020[19] who reported that when compared to the moderate group, the ALT and AST levels in the severe group were much greater. Also, Ghweil et al., 2020[15] stated that compared to individuals with mild to moderate infection, those with severe COVID-19 infection had considerably lower blood levels of albumin and substantially greater median serum values of both ALT and AST.

Findings of a study by *Omran et al., 2021*[17] agreed with ours as regards serum values of INR, ALT and AST were higher in critically ill patients. While there was an increase in serum albumin and total bilirubin in critically ill patients. Moreover, Zayed et al., 2022[16] documented that higher ALT and AST values were in severe group as compared to non-severe group. Also, Elshazli et al., 2020[21] agreed with ours that there was prolonged prothrombin time in severe COVID-19.

In the current research, we revealed a decrease in PO_2 and in O_2 saturation in the severe group in comparison to the non-severe group. These could be due to the lung damage caused by COVID-19 disease as suggested by *Miller et al., 2020*[18]. Also, the pH and HCO^3 in the severe group were higher in comparison to the non-severe group.

However, findings of a study by Zayed et al., 2022[16] disagreed with ours as the severe group had more hypoxemia than the non-severe group, but there was lower PCO2 in the severe group than the non-severe group.

In contrast to the non-severe group, our findings indicated a decline in lymphocyte counts in the severe group. It was proposed that the host immune system make a significant effort to fight the infection with all available cells and cytokines. The natural killer cells and T cells get worn down because of the prolonged SARS-CoV-2 infection, and a decrease in their number causes lymphopenia.[22] In addition, there was a decrease in Hb level in the non-severe group as compared to the severe group, while there was no variation between studied groups regarding RBCs, HCT, MCV, MCH, PLTs, WBCs and neutrophils. Data of a study by Zayed et al., 2022,[16] agreed with ours as the severe group had more lymphopenia than the non-severe group. Also, findings of the study by Ramadan et al., 2020[19] agreed with ours despite that the median lymphocytic count was below the normal range, the severe group had a substantially lower percentage of lymphocytes than non-severe groups.

Also, findings of a study by Ghweil et al., 2020,[15] agreed with ours as when compared to those with mild to moderate COVID-19 infection, those with severe infections showed considerably lower mean values for lymphocytic count. However, their data disagreed with our findings, since they found that individuals who were badly infected had a considerably lower WBC count than those who had mild to moderate COVID-19. Elshazli et al., 2020,[21] study findings disagreed with ours as they observed elevated levels of white blood cells and neutrophil count in their severe COVID-19 patients.

Our study revealed increased ferritin, CRP and D-Dimer levels in the severe group as compared to non-severe group probably because COVID-19 predisposes patients to complication with hypercoagulability and thrombosis. Data of the study by Zayed et al., 2022,[16] agreed with ours as there was higher

CRP, higher serum ferritin and more positive D-dimer in severe group as compared to the non-severe group. Likewise, the study by Elshazli et al., 2020,[21] agreed with ours as compared to the non-severe group, the severe group had a greater D-dimer level. Also, our outcomes in line with those of Omran et al., 2021,[17] as patients who were seriously unwell had higher blood levels of CRP and ferritin.

In the current study contrasted with the non-severe group, the proportion of CO-RAD-5 increased in the severe group. Ground-glass opacities, with or without consolidations, multifocal bilateral distribution, and at least one confirming pattern, such as many ground-glass regions, crazy paving pattern, consolidations occur inside the ground-glass areas, were necessary characteristics in CT chest finding. Finally, opacities that resembling organized pneumonia start to appear.[25]

Our results indicated that a significant positive correlation was detected in the non-severe group between IL-6, AST, and IL-10 with HCT. But a negative connection between IL-10, pulse and blood urea was noticed. And negative connection between IL-6, PO_2 and O_2 saturation. Moreover, in the severe group there was substantial positive connection between IL-10 and INR. Meanwhile, our results revealed a negative connection between homocysteine and temperature, negative connection between IL-6, PLTs and pulse, and negative connection between IL-10 and lymphocyte counts.

In conclusion, estimation of the values of IL-6, IL-10 and homocysteine in COVID-19 patients is a noninvasive, and readily available tool. Values of IL-6 and IL-10 were substantially greater in the severe group when compared with non-severe group. While no variation in homocysteine was noted between studied groups. Therefore, IL-6 and IL-10 could be proposed to follow up COVID-19 patients and detect cases before progressing to the severe stage.

Author Contributions

WAA wrote the manuscript. FMM, and EFM, revised it. All authors contributed to the work and approved it for publication.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine (for Girls), Al-Azhar University (approval number: 815, dated April 2021).

Informed consent

Verbal informed consent was obtained from each patient before being enrolled in the study.

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