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TNF-α and IL-10 Serum Levels in COVID-19 Patients and their Relation to Disease Severity

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Abstract

Background: During the COVID-19 pandemic, the severity of symptoms have been coined to the over production of inflammatory cytokines with consequent immune hyperactivation. The cytokine storm is considered as a crucial cause of lung damage, multiorgan failure and mortality in COVID-19.

Aim of Study: To evaluate the serum levels of TNF-α and IL-10 in COVID-19 patients and to explore their potential relation with disease severity.

Patients and Methods: The study was conducted during the period from November 2020 to March 2021 on 46 patients confirmed with COVID-19 and admitted to the isolation hospitals or recruited from the COVID-19 clinic of Kasr Al-Ainy Cairo University Hospitals and 45 matched healthy controls. Patients have undergone full history taking, clinical and radiologic examination and laboratory testing. All candidates were subjected to assessment of the serum levels of TNF-α and IL-10 by enzyme-linked immunosorbent assay technique.

Results: The serum levels of TNF-α and IL-10 were significantly increased in COVID-19 patients compared to healthy controls, and their levels were higher with more disease severity. Significant correlations were observed between serum TNF-α with serum ferritin, CRP and IL-6 and between IL-10 with CRP and D-dimer. Both were inversely related to the lymphocytic counts in COVID-19 patients.

Conclusion: The high levels of serum TNF-α and IL-10 in COVID-19 highlights the pivotal role played by these cytokines in the immune pathogenesis of COVID-19. Their association with severe illness suggests they can possibly serve as reliable biomarkers for monitoring disease activity and predicting severity and outcome in COVID-19. Better understanding of the cytokine response pattern in COVID-19 would contribute to the improved development of more effective immunomodulatory therapies for COVID-19 in the future.

Key Words: *TNF-α – IL-10 – Cytokine storm – COVID-19.*

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Introduction

COVID-19, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in December 2019, and the affected countries have grown rapidly leading the WHO to declare it as a pandemic **[1]**. Much of the critical illness associated with SARS-CoV-2 infection is believed to be due to a hyper-inflammatory process referred to as a "cytokine storm" (CS) **[2]**.

Cytokines are small protein molecules aimed for cell-to-cell communications, andplayimportant immunomodulating functions **[3]**. However, in some infectious diseases, excessive inflammation triggers an uncontrolled proinflammatory cytokine production leading to the occurrence of cytokinestorm (CS) **[4]**. The CS is a fast-developing, life-threatening, clinical condition in which the overly produced inflammatory mediators drived by the unchecked immune system lead to complicated medical syndromes, tissue damage, multiorgan failure and even death if treatment is not adequate. Therefore, the timing of diagnosis and treatment of CS could be life-saving **[3]**. There is a wide spectrum of immune-active molecules including interleukins (ILs) and tumor necrosis factor-alpha (TNF-α) proposed as contributors to the development of cytokine storm **[4]**. The Cytokine storm have been implicated in severe influenza **[5]**, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) **[6]**, and evidence has revealed that cytokine storm is a crucial event with deleterious consequences during severe SARS-COV-2 infection **[7]**.

A better understanding of the early prognostic clinical laboratory parameters could save many lives by enabling timely intervention and better

resource allocation since ICU capacity is limited in most countries and will undoubtedly enlighten the optimal management of the growing pandemic **[8]**.

Accordingly, we aimed in the present study to evaluate the serum levels of TNF-α and IL-10 in COVID-19 patients and to explore the relation between both cytokines and the severity of the disease.

Patients and Methods

This observational case-control study was conducted on 46 COVID-19 patients admitted to the Internal Medicine Isolation hospital or recruited from the COVID-19 clinic of Kasr Al-Ainy Cairo University Hospitals during the second wave of COVID-19 from November 2020 to March 2021. Patients were diagnosed by the characteristic clinical picture, laboratory and radiological findings and all were confirmed by positive swab for nucleic acid RT-PCR testing for COVID-19. The study also included 45 healthy subjects age- and sex-matched and with no history of autoimmune diseases, malignancy or chronic disorders as a control group The research was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University (# MS -311-2020). The study adhered to the tenets of the Declaration of Helsinki. Informed consents were obtained from all participants prior to enrolment.

The demographic and clinical data were collectedincluding age, sex, history of exposure to COVID-19 patients, need of hospital stay and presence of comorbidities in addition to clinical examination findings of patients. Laboratory investigations were performed including complete blood counts (CBC), C-reactive protein (CRP), Lactate dehydrogenase (LDH), D-dimer and serum ferritin while Interleukin-6 (IL-6) and procalcitonin (PCT) were analyzed in moderate and severe disease.

Imaging studies mainly CT chest were done to investigate the occurrence of pathologic lesions associated with COVID-19 including the presence of ground glass opacities, consolidation, vascular thickening, and pleural fluid effusion. Patients were classified by the COVID-19 reporting and data System (CO-RADS) into different groups indicating the level of suspicion of pulmonary involvement of COVID-19 as reflected by non-enhanced chest CT findings. The level of suspicion increases from very low (CO-RADS 1) to very high (CO-RADS 5) **[9]**.

COVID-19 disease severity was assessed based on the National institutes of health (NIH) COVID -19 treatment guidelines panel, 2021 **[10]** and accordingly patients were classified into 3 groups of mild, moderate and severe illness. Mild illness was described as individuals who had any of the following signs and symptoms of COVID-19 as fever, sore throat, cough, headache, nausea, vomiting, malaise, myalgia, diarrhea, loss of taste and smell) but do not have shortness of breath, dyspnea, or abnormal chest imaging. Moderate illness included patients who showed evidence of lower respiratory disease during clinical assessment or imaging and had oxygen saturation values (SpO_2) ≥94% on room air at sea level. Finally severe illness was defined as disease with any of the following: SpO_2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired air (PaO_2/FiO_2) <300mm Hg, respiratory frequency >30 breaths/min, or lung infiltrates >50%.

Measurement of serum levels of TNF-α and IL-10: Four mL of venous blood were collected in plain centrifuge tubes, allowed to clot at room temperature (RT) for 30min, then samples were centrifuged at 1000 x g for 15min. Patients' sera were separated and stored at –20 C till testing. Serum levels of TNF-α and IL-10 were assessed by a sandwich enzyme-linked immune-sorbent assay (ELISA) kits used for the determination of human TNF-α (Elabscience Biotechnology, United States, Catalog#E-EL-H0109) and human IL10 ((Elabscience Biotechnology, United States, Catalog# E-ELH0103). Steps were followed according to manufacturer's instructions and finally the concentrations of TNF-α and IL-10 were calculated by comparing the optical densities of samples read at 492nm to the standard curve.

Statistical analysis:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Numerical data were describedas mean ± standard deviation (SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparisons were evaluated using unpaired *t*-test or analysis of variance (ANOVA) with multiple comparisons. For comparing categorical data, Chi square test was performed. Correlations between quantitative variables were done using Spearman correlation coefficient. *p*-values are considered to be significant if <0.05 and highly significant if <0.001. The Receiver Operating Characteristic (ROC) curves wereplotted for prediction of the cut off values of TNF-α and IL-10 as potential markers of disease severity in COVID-19 patients.

Results

The 46 COVID-19 patients enrolled in this study were 28 males and 18 females (M: F 1.6:1) with a mean age of 57.76±12.49 years. The 45 control subjects were of matched sex (26 males and 19 females; M: F 1.4:1) and age (53.38±15.2 years), (p>0.05). According to COVID-19 disease severity,14 patients (30.4%) had mild illness (5 males and 9 females) with a mean age of 51.86 ±13.98 years, 12 patients (26.1%) with moderate illness (11 males and 1 female) with a mean age of 60.58±11.79 years and finally 20 patients (43.5%) had severe disease (12 males and 8 females) with a mean age of 60.20±10.87 years. No statistical difference in age was detected between the 3 groups of patients (p=0.103). However, there was a statistically significant difference in sex distribution with male predominance in the moderate group and severe groups versus female predominance in the mild disease (p=0.015). Thirty-two patients (69.6%) had other disease comorbidities including hypertension (4), diabetes (6), tuberculosis (1) and rheumatic heart (1), yet no significant differences were observed between the 3 groups. As for the radiological classification of patients, 5 patients had CT findings concomitant with CO-RADS 1 (10.9%), 9 patients with CO-RADS 2 (19.6%), 4 patients with CO-RADS 3 (8.7%), 16 patients with CO-RADS 4 (34.8%) and finally 12 patients with CO-RADS 5 (26.1%). A higher prevalence of CO-RADS 1 and 2 was observed in the mild group, CO-RADS 3 in the moderate group, and lastly, a higher prevalence of CO-RADS 5 in the severe group (p-values <0.001). Regarding the disease course and outcome of COVID-19 patients; 20 patients (43.5%) required non-invasive ventilation,5 patients (10.9%) needed invasive ventilation and unfortunately 7 patients (15.2%) died. Again, significant differences were detected between the patients with severe disease versus others regarding the need for invasive ventilation (p<0.001), as well as the outcome of patients (p=0.005). The demographic data and clinical findings of the patients are described in Table (1).

The laboratory findings of patients are summarized in Table (2). The total leucocytic counts (TLC) and D-dimer levels were significantly higher in the severegroup compared to the mild and moderate ones (p-values=0.022 and 0.017, respectively). The lymphocytic counts, serum ferritin and CRP median levels showed statistically significant differences between the three groups. No significant differences were observed among the 3 groups regarding the serum LDH and ALT levels. IL-6 and PCT levels measured in the 26 patients of moderate and severe illnessrevealed significantly higher levels in IL-6 (but not PCT) in severe COVID-19 compared to moderate disease group (p-values=0.028 and 0.330, respectively).

In COVID-19 patients, the median serum levels of TNF-α (77.95pg/mL) and IL-10 (4.75pg/mL) were significantlyhigher compared to controls (0.40 and 0.07pg/mL, respectively) (both p-values <0.001) and were found to be generally higher with more disease severity.

As for TNF-α, it was significantly higher in patients with severe illness compared to the mild group (p-value <0.001), and although levels were still higher in severedisease compared to the moderate group and in the moderate compared to the mild but values were statistically insignificant (p-values=0.299 and 0.062, respectively).

Serum IL-10 was found to be significantly higher in the severe and moderate groups compared to the mild group (p<0.001 and 0.003, respectively) and although higher levels were detected in the severe compared to the moderate group, yet difference was not statistically significant (p=1.00). (Table 3).

The associations of TNF-α and IL-10 levels with the clinical parameters, radiological findings, need for ventilation and outcome in COVID-19 patients were evaluated. Higher levels of both TNF-α and IL-10 were strongly associated with more significant pulmonary involvement and lung affection (p=0.001) as well as with the need for assisted ventilation in COVID-19 patients (p< 0.001).

The potential correlations of TNF-α and IL-10 with the different laboratory parameters in COVID-19 patients are presented in Table (4). Significant positive correlations between TNF-α levels with serum ferritin (p=0.008) and CRP (p=0.009) and inversely with lymphocytic counts (p=0.001) were observed. No significant relations were observed with the following parameters: TLC (p=0.166), D-dimer (p=0.09), LDH (p=0.132), ALT (p=0.827) and PCT (p=0.757). Finally, a statistically significant positive correlation was detected between TNF-α and IL-6 in COVID-19 patients with moderate and severe illness (p<0.001). As for IL-10, significant positive correlations were detected with CRP (p=0.028) and D-dimer (p=0.036) and again inversely with the lymphocytic counts (p= 0.009). No significant relations were observed with other laboratory parameters including IL-6 levels (p=0.633). Finally, a significant positive correlation

was detected between serum TNF-α and IL-10 levels in COVID-19 patients (p<0.001).

The specificity and sensitivity of serum TNF-α and IL-10 as potential predictors of disease severity were evaluated by ROC analysis and are presented in Fig. (1). Serum TNF-α at a cut-off value of 43.2pg/ml, showed a sensitivity of 83.3% and specificity 71.4%, and an accuracy of 77.0% in discriminating patients with mild and moderate illness and at a cut-off of 262.4pg/ml, showed a sensitivity of 70.0%, a specificity of 75.0%, and accuracy 71.9% in discriminating moderate and severe disease. Serum IL-10 at a cut-off value of 4.0pg/ml, showed a sensitivity of 100%, specificity of 71.4% and accuracy of 84.7% in discriminating between mild and moderate disease and at a cut-off of 5.63pg/ml, showed a sensitivity of 65.0%, specificity of 50.0 and an accuracy of 59.4% in discriminating patients with moderate and severe symptoms. Serum TNF-α showed better sensitivity and specificity in discriminating between moderate and severe disease in COVID-19 patients while IL-10 showed higher sensitivity at discriminating between mild and moderate disease activity.

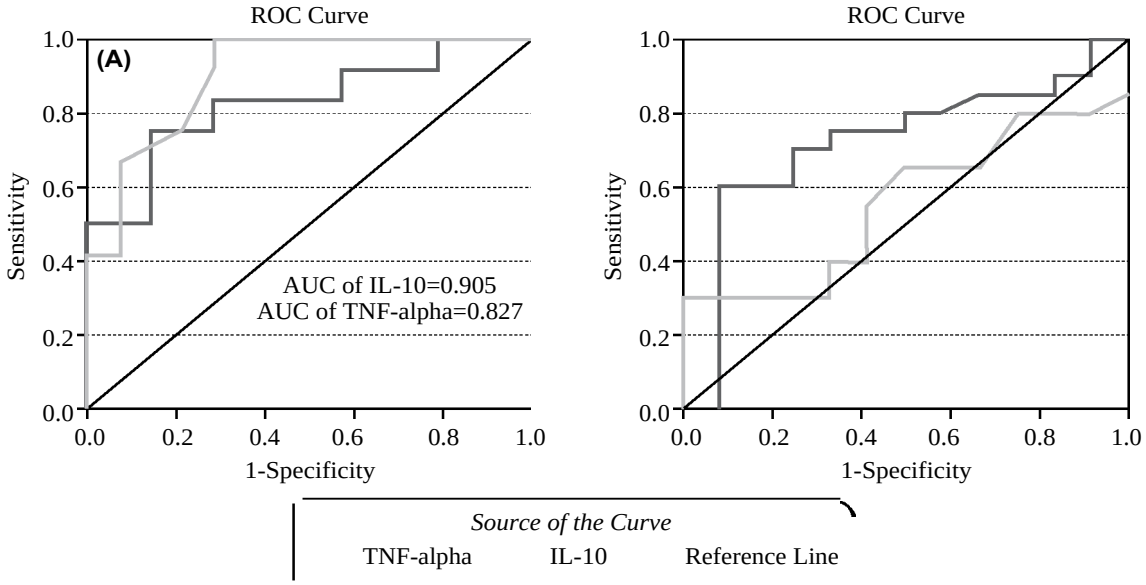

Fig. (1): Receiver operating characteristic (ROC) curve analysis of serum TNF-α and IL-10 in COVID-19 patients as markers of severity. (A) TNF-α and IL-10 levels in patients with mild versus moderate disease. (B) TNF-α and IL-10 levels in patients of moderate versus severe disease.

Table (1): Demographic data and clinical findings in COVID-19 patients.

Parameter	Healthy Controls (n=45)	All COVID-19 patients (n=46)	Mild disease (n=14)	Moderate disease (n=12)	Severe disease (n=20)	p-value
Age (years)	53.38±15.2	57.7±12.49	51.86±13.98	60.58±11.79	60.20±10.87	>0.05
Sex (M:F)	1.4:1	1.6:1	0.6/1[a]	11/1[b]	1.5/1[ab]	0.015
Comorbidities	N.D	32 (69.6%)	7 (50%)	8 (66.7%)	17 (85%)	>0.05
Radiologic classification:						
- CO-RADS 1	N.D	5 (10.9%)	5 (35.7%)	0 (0%)	0 (0%)	
- CO-RADS 2		9 (19.6%)	9 (64.3%)	0 (0%)	0 (0%)	
- CO-RADS 3		4 (8.7%)	0 (0%)	4 (33.3%)	0 (0%)	
- CO-RADS 4		16 (34.8%)	0 (0%)	8 (66.7%)	8 (40%)	
- CO-RADS 5		12 (26.1%)	0 (0%)[a]	0 (0%)[a]	12 (60%)[b]	<0.001
Invasive ventilation	N.D	5 (10.9%)	0a	0[a]	5 (25%)[b]	<0.001
Outcome:						
- Cured	N.D	39 (84.8%)	14 (100%)[a]	12 (100%)[a]	13 (65%)[b]	<0.005
- Died		7 (15.2%)	0[a]	0[a]	7 (35%)[b]	

Data are presented either as Mean ± SD or as the number of patients with the percentage in parentheses.
Sex is represented Male: Female ratio (M: F). Bold values are significant at p<0.05.
Groups bearing different initials are significantly different from each other.

Table (2): Laboratory data of COVID-19 patients.

Parameter	All COVID-19 patients (n=46)	Mild disease (n=14)	Moderate disease (n=12)	Severe disease (n=20)	*p*-value
TLC (10^3/cmm)	6.94 (4.5-8.7)	4.8 (3.6-6.5)**a**	6.9 (4.5-8.84)**a'b**	7.93 (6.44-10.45)**b**	0.026
Lymphocytic count (%)	13.5 (7-31)	39 (30-52)**a**	10.50 (6.50-24.50)**b**	9.50 (4-14.5)**b**	<0.001
s.Ferritin (ng/mL)	499.5 (165-1247)	121 (58-242)**a**	678.9 (488-1438)**b**	606.5 (427.8-1399)**b**	<0.002
CRP (mg/L)	20.5 (6-71)	7.5 (6-12)**a**	55.5 (13.7-90.4)**b**	35 (14.3-109.5)**b**	<0.008
D-Dimer (µg/mL)	0.88 (0.53-2.2)	0.69(0.43-0.78)**a**	0.9 (0.63-2.9)**a,b**	1.94 (0.72-3.6)**b**	<0.02
LDH (U/L)	437 (311-541)	370.5 (306-470)	405 (277-503)	522 (319-656.5)	0.2
ALT (U/L)	42.5 (22-55)	25 (15-47)	42 (26-54)	49 (33.5-105)	0.07
IL-6 (pg/mL)	108.6 (25.8-262)		23.6 (17.8-102.4)**a**	146.8 (38.3-377.8)**b**	<0.028
PCT (ng/mL)	0.13 (0.08-0.4)		0.24 (0.1-0.4)	0.1 (0.06-0.19)	0.33

TLC: Total leucocytic count. IL-6: Interlukin-6. All data are presented as median (1st quartile - 3rd quartile).
CRP: C reactive protein. PCT: Procalcitonin. Bold values are significant at $p<0.05$.
ALT: Alanine transaminase. Groups bearing different initials are significantly different from each other.

Table (3): Serum levels of TNF-α and IL-10 in COVID-19 patients.

Parameter	Healthy Controls (n=45)	All COVID-19 patients (n=46)	*p*-value	Mild disease (n=14)	Moderate disease (n=12)	Severe disease (n=20)	*p*-value
TNF-α pg/mL)	0.4 (0.3-0.62)	77.95 (29.2-513.3)	<0.001	28.6 (25-54.1)**a**	98.8 (54.7-300.3)**a'b**	514.1 (174.2-517)**b**	<0.001
IL-10 (pg/mL)	0.07 (0.06-0.09)	4.75 (3.75-8.5)	<0.001	3.38 (3-4.5)**a**	5.63 (4.63-11.25)**b**	6.75 (4.75-15.25)**b**	0.003 <0.001

All data are presented as median (1st quartile - 3rd quartile). Groups bearing different initials are significantly different from each other.

Table (4): Correlations of serum TNF-α and IL-10 with different laboratory parameters in COVID-19 patients.

Parameter	TNF-α (pg/mL)	IL-10 (pg/mL)
Age (years):		
r	0.093	0.162
p-value	0.54	0.282
TLC:		
r	0.208	0.211
p-value	0.166	0.159
Lymphocytic count:		
r	–0.478	–0.38
p-value	0.001	0.009
s.Ferritin:		
r	0.379	0.268
p-value	0.008	0.072
CRP:		
r	0.38	0.324
p-value	0.009	0.028
D-Dimer:		
r	0.253	0.311
p-value	0.09	0.036
LDH:		
r	0.255	0.119
p-value	0.132	0.43
PCT:		
r	0.064	0.09
p-value	0.757	0.65
IL-6:		
r	0.769	0.103
p-value	<0.001	0.633

TLC : Total leucocytic count. PCT: Procalcitonin,
CRP : C-reactive protein. *r* = Correlation coefficient.
LDH : Lactate dehydrogenase. - Bold values are significant at $p<0.05$.
IL-6 : Interlukin-6.

Discussion

Throughout the COVID-19 pandemic,itbecame obvious that dampening the cytokine storm is a crucial therapeutic goal for COVID-19 patients. In this regard, a large spectrum of immuneactive molecules act as contributors to the development of the cytokine storm asinterleukins, chemokines and TNF **[4]**. Studying these immune players, helps develop new clinical immunomodulatory alternatives to control the gone-wild immune system and calm down the "cytokine release syndrome" characteristic of COVID-19.

In addition, reliable prognostic clinical and laboratory findings that can predict deterioration of COVID-19 patients will also help in evaluating the risk of some patients to develop serious life threatening disease and thus will provide guidance for the best possible management of health resources amid the pandemic **[11]**.

In the current study, the serum levels of TNF-α and IL-10 in COVID-19 patients were significantly higher in patients compared to healthy controls. Our results are consistent with several other studies conducted on COVID-19 patients **[12,13,14]**. The high levels of TNF-α shown in our study as well as others are in harmony with the exaggerated production of pro-inflammatory cytokines in COVID-19 patients. Early research on COVID-19 recognized the pivotal role played by

the abundant immune-active molecules such as IL-1, IL-6 and TNF-α as a keystone event during the disease **[14-18].**

These cytokines including TNF are released by the monocytes/macrophages, activated mast cells, dendritic cells, natural killer cells and lymphocytesin the respiratory tract aggravating the inflammatory state. The inflammatory pool of cytokines associated with overly activated immune cells infiltrate into the pulmonary microvasculature, leading to marked vascular efflux and edema resulting in eminentlung injury, interstitial pneumoniaand eventually Acute Respiratory Distress Syndrome (ARDS) **[19]**. In addition, It was reported that the entry of SARS-CoV-2 into the infected cells could be accelerated by leveraging upregulation of its receptor gene angiotensin-converting enzyme 2 (ACE2) through the induction of cytokine storm involving IFN, TNF-α as well as the transcription factor Nuclear factor kappa B (NFKβ) **[20,21].**

Our results revealed that in COVID -19 patients with moderate and severe illness, TNF-α positively correlated with serum IL6. In the same context , it was previously proposed that the production of IL-6 described as the core player of the CS in severe COVID-19 states, is increased by TNF-α as a pyrogen cytokine released from immune cells in response to inflammation suggesting that IL-6 is a downstream effector of TNF-α **[22]**. In turn, IL-6 augments the production of both TNF-α and IL-8 by enhancing the differentiation of T follicular helper cells; suppressing the antiviral helper Tcell 1 (Th1) axis while driving the differentiation of helper T cell 2 (Th2) cells by IL-4 and interferon γ (IFN-γ) **[23]**. Since TNF-α seems to play a determining role in the pathogenesis of COVID-19, targeting TNF-α might be a promising strategy in attenuating organ damage in severe COVID-19 **[24].** Actually ongoing clinical trials are being conducted to identify the efficacy of the anti-TNF therapeutics in COVID-19 **[25,26]**. It is interesting to mention that patients with any inflammatory disease managed by TNF inhibitors were reported to have lower risk of hospitalization or the development of severe COVID-19 compared with others diagnosed with an inflammatory disease but on other therapies **[27].**

In the present work, IL-10, an anti-inflammatory cytokine, was found to be dramatically elevated in COVID-19 patients compared to healthy controls and higher levels strongly correlated with greater disease severity. This finding is consistent with the results of other studies **[28,29,30]**. Lu et al., stated that the significant increase of IL-10 is a distinctive feature of the cytokine storm in severe Covid-19 **[31]**. Thus far, the exact mechanism for this elevation is currently unclear. The clinical significance of the increased IL-10 levels in COVID-19 has been interpretedas an immune-inhibitory negative feedback tool triggered by the rapidly increasing proinflammatory mediators **[32]**. However, the simultaneous rise in IL-10 and TNF and the unique positive correlation between both of them observed in our study and with various proinflammatory cytokines in other studies, in addition to the observed correlation with disease severity suggest that IL-10 might be failing to efficiently tamper inflammation. IL-10 “resistance was described as the possible escape of effector immune cells from IL-10's inhibitory action leading to an overwhelming inflammatory cytokine counterstrike **[33].** Another tempting scenario is that IL-10 might have deviated from its conventional immunosuppressive function and rather plays a hazardous immunostimulatory role in the pathogenesis ofCOVID -19 **[31]**. In COVID-19, exhausted PD-$1^{+}TIM3^{+}CD8^{+}$ T cells in the circulation of patients correlated with high serum IL-10 levels, denoting a role of IL-10 in T cell exhaustion, probably through overactivation and proliferation **[34]**. Several data have been published supporting the potential pro-inflammatory behavior of IL-10 in severe COVID-19. First, many of the same cytokines that are elevated with high-dose IL-10 administration (e.g. IL-4, IL-7, IL-18, IFNγ, TNFα) are also elevated in severe COVID-19 cases in conjunction with elevated IL-10 levels **[17,29].** Second, high levels of IL-10 were previously found to have the potential to augment the proinflammatory reactions to bacterial lipopolysaccharides (LPS) in human endotoxemia **[35]**. This points to the possibility that the combination of elevated IL-10 and bacterial products (which are abundant in severe COVID-19 cases) could empower the inflammatory machinery in COVID-19 **[33]**. Actually, IL10 might play both seemingly paradoxical roles throughout the course of COVID-19 illness. The initialrise in IL-10 in early COVID infection might indeed indicate a negative control measure to restrain theaction of proinflammatory cytokines. But, as IL-10 yield increases with disease progress, it presumably acts as a proinflammatory mediator that amplifies the viral sepsis-related hyper-inflammation and fuelsthe cytokine storm in severe COVID-19 illness **[31,36]**. Unlike our findings, Taghiloo et al., (2020) did not report significant increases in IL-10 levels in their study between COVID-19 patients compared to healthy controls **[37].**

In conformity with our results showing higher TNF-α and IL-10 levels with more severe illness, TNF-α and IL-10 were proposed in several studies to predict poor outcomes in patients with COVID-19 **[17,37,38]**. In our cohort of patients, TNF-α and IL-10 levels correlated positively with higher CO-RADS scores and more significant pulmonary involvement. In addition, patients who needed invasive ventilation were found to have higher levels of both cytokines. Regarding correlations with various laboratory findings in the present study, TNF-α positively correlated with CRP, serum ferritinand IL-6 in COVID-19 patients. As for IL-10, significant positive correlations were detected with CRP and D-dimer. Both TNF-α and IL-10 were inversely related with the lymphocytic counts in our patients.In agreement with our findings, Han H et al., (2020) **[29]** showed that CRP was significantly positively correlated with IL-10 while Taghiloo et al., (2020) **[37]** reported that CRP levels positively correlated with the levels of TNF-α but not IL-10. They also reported inverse correlation of TNF-α with the lymphocyte counts in COVID-19 patients. It is noteworthy that no significant correlations were detected with either TNF-α or IL-10 with the TLC, LDH and PCT levels in COVID-19 patients in the present study.

In accordance, the strong correlations of TNF-α and IL-10 with some of the established measures of disease severity in COVID-19 in the present studyfurther supports the utility of both cytokines as potential biomarkers for monitoring disease activity and predicting severity in COVID-19 patients. Moreover, ROC analysis performed to evaluate the usefulness of serum TNF-α and IL-10 as predictors of disease severity in our patients affirmed that both of the studied cytokines displayed high sensitivity and specificity at discriminating patients in different stages of disease severity.

Among the study limitations in this work are the small sample size, the lack of asymptomatic and convalescent cases in our patient cohort and the lack of serial measurements of cytokines during the different stages of the disease course in order to follow and detect fluctuations in their levels as disease progresses.

Conclusion:

Serum TNF-α and IL-10 are significantly higher in COVID-19 patients compared to controls. Their association with disease severity suggests they can possibly serve as reliable biomarkers for monitoring disease activity and predicting severity and outcome in COVID-19. The current study highlights the role played by TNF-α and IL-10 in the immune pathogenesis of COVID-19 and the development of the COVID-19 associated cytokine storm. Better understanding of the cytokine response pattern in COVID-19 would contribute to the improved development of more effective immunomodulatory therapies for COVID-19 in the future.

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مستويات TNF-α و LI-10 فى مصل الدم فى مرضى وعلاقتها بشدة المرض

الخلفية : خلال جائحة COVID-19، تم ربط شدة الأعراض مع الإفراط فى إنتاج السيتو كينات الالتهابية مع ما يترتب على ذلك من فرط نشاط المناعة. تعتبر عاصفة السيتوكين سبباً حاسماً لتلف الرئة وفشل العديد من الأعضاء والوفيات فى COVID-19.

هدف العمل : تقييم مستويات مصل TNF-α وLI-10 فى مرضى COVID-19 واستكشاف علاقتهما لمحتملة مع شدة المرض.

المرضى والطرق : أجريت الدراسة خلال الفترة من نوفمبر ٢٠٢٠ إلى مارس ٢٠٢١ على ٤٦ مريضاً تم تأكيد إصابتهم بـ COVID-19 وتم قبولهم فى مستشفيات العزل أو تم تجنيدهم من عيادة COVID-19 فى مستشفيات جامعة الأزهر القصر العينى بالقاهرة و٤٥ من الضوابط الصحية المتطابقة. خضع المرضى للتاريخ الكامل للفحص السريرى والإشعاعى والاختبارات المعملية. خضع جميع المرشحين لتقييم مستويات مصل TNF-α وLI-10 بواسطة تقنية مقايسة الممتز المناعى المرتبط بالإنزيم.

النتائج : وجد زيادة مستويات TNF-α وLI-10 فى المصل بشكل ملحوظ فى مرضى COVID-19 مقارنة بالضوابط الصحية، وكانت مستوياتهم أعلى مع زيادة شدة المرض. لوحظت ارتباطات كبيرة بين TNF-α فى الدم مع الفيريتين ferritin، CRP وIL-6 وبين IL-10 مع CRP وD-dimer. كما وجد أن كلاهما مرتبط انعكسياً بعدد الخلايا الليمفاوية فى مرضى COVID-19.

الخلاصة : إن المستويات العالية من TNF-α وLI-10 فى مصل COVID-19 تسلط الضوء على الدور المحورى الذى تلعبه هذه السيتوكينات فى التسبب المناعى لـ COVID-19. يشير ارتباطهم بالمرض الشديد إلى أنه يمكن أن يكونوا بمثابة مؤشرات حيوية موثوقة لمراقبة نشاط المرض والتنبؤ بشدته ونتائجه فى COVID-19. من شأن الفهم الأفضل لنمط استجابة السيتوكينات فى COVID-19 أن يسهم فى تسحين تطوير علاجات تعديل مناعياً كثر فعالية لـ COVID-19 فى المستقبل.