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Serum Levels of Intercellular Adhesion Molecule-1 and TNF-α in Patients with COVID-19 and Its Relation to Disease Severity

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) is a major health concern and can be devastating, especially for the elderly. It causes a systemic inflammatory response, involving dysregulation and misexpression of many inflammatory cytokines.

Objectives: To assess the cytokine profile in COVID-19 patients and detect its relation with disease severity.

Patients and methods: This study included 50 COVID-19 patients confirmed by reverse transcription–polymerase chain reaction (RT-PCR), of whom 20 with severe pneumonia, 20 with moderate and 10 with mild disease, and 50 healthy control. Quantitative ELISA tests were performed for tumour necrosis factor alfa (TNF-α) and intercellular adhesion molecule1 (ICAM-1).

Results: Level of ICAM-1 is higher in cases of COVID-19 than healthy controls (median for cases = 420 ng/ml). Also TNF-α level is higher in cases with a median of 165 both levels were higher in CORADS 6 and positively correlated with CT findings.

Conclusion: Patients with severe COVID-19 might have a cytokine storm syndrome and increased expression of endothelial cell adhesion molecules as ICAM-1, which is related to COVID-19 and disease severity and may contribute to coagulation dysfunction. Levels of ICAM 1 were associated with more severe CT findings and longer hospital stay, also ICAM 1 and TNF can be used as diagnostic measures as they have a higher sensitivity specificity and predictive values.

Keywords: Covid19, Cytokines, ICAM1, TNF.

INTRODUCTION

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) marks as the third highly pathogenic coronavirus to spill over into the human population. SARS-CoV-2 is highly transmissible with a broad tissue tropism that is likely perpetuating the pandemic. SARS-CoV-2 is a novel virus of the genus and exhibits similarities to SARS-CoV in genome structure, tissue tropism and viral pathogenesis. Yet, SARS-CoV-2 appears to be more transmissible and the diversity of immune responses are poorly understood[(1)].

SARS-CoV-2 infects the host using the angiotensin converting enzyme 2 (ACE2) receptor, which is expressed in several organs; including the lung, heart, kidney and intestine. ACE2 receptors are also expressed by endothelial cells[(2)].

SARS-CoV-2 infection is believed to trigger a cytokine storm that plays a critical role in the pathogenesis of endotheliitis and vascular injury, eventually leading to respiratory and multi-organ failure in COVID-19 patients involving dysregulation and misexpression of many inflammatory cytokines. The recruitment and activation of inflammatory cells depend on the expression of many classes of inflammatory mediators, such as cytokines (interleukin (IL) 1, IL-6, and IL-18), chemokines (fractalkine [FKN]) and adhesion molecules (intercellular adhesion molecule 1 [ICAM-1)] and vascular cell adhesion molecule-1 [VCAM-1]) and growing evidence suggests that the levels of serum soluble endothelial adhesion markers, such as sVCAM-1, sICAM-1, sP-selectin, are associated with disease severity of COVID-19. Pathological evidence of venous thromboembolism, direct viral infection of the endothelial cells and diffuse endothelial inflammations have been reported in recent studies[(3)].

Also COVID-19 patients, especially severe and critical patients, commonly have histological characteristics of endotheliopathy, vascular thrombosis and new vessel growth in the lung, suggesting the presence of endothelial activation and recruitment of immune cells as well as angiogenesis in the lesions caused by SARS-CoV-2 infection[(4-8)].

Cytokine Release Syndrome (CRS), is closely related to development of clinical symptoms; for example, IFN-γ can cause fever, chills, headaches, dizziness and fatigue; TNF-α can cause flu-like symptoms similar to IFN-γ, with fever, general malaise and fatigue, but can also cause vascular leakage, cardiomyopathy, lung injury and acute-phase protein synthesis[(9)].

IL-6, a key target in CRS, can cause vascular leakage, complement activation, and the coagulation cascade to activate, leading to severe CRS symptoms such diffuse intravascular coagulation (DIC) [(10-11)].

It's worth noting that IL-6 may induce cardiomyopathy by encouraging myocardial

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dysfunction, which is common in CRS patients. Furthermore, endothelial cell activation may be one of the markers of severe CRS. Capillary leakage, hypotension, and coagulopathy are all symptoms of endothelial dysfunction. Virus-induced immunopathological processes also play a key role in the deadly pneumonia that follows Human Corona Virus infections[12].

This study aimed to assess the cytokine profile in COVID 19 patients and its relation with disease progression and severity using COVID reporting and data system (CORADS) score.

PATIENTS AND METHODS

Study Participants

This was a prospective case control study done from February to December 2021, 50 COVID19 patients and 50 control participants after taking their written consents were recruited from the Emergency Department and Chest and ENT Outpatient Clinic.

Inclusion criteria:

All patients confirmed by RT-PCR, as positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) who visited the emergency room and the chest and ENT outpatient clinic in the study period. The control group included 50 healthy volunteers. All patients had chest CT scan with reports by radiologist. Mild moderate and severe cases were included.

Mild cases was confirmed by positive (qRT-PCR) tests, with no chest tomography features of viral pneumonia [4], moderate cases had pneumonia in CT chest while severe cases was diagnosed as pneumonia plus 1 of these criteria: (1) respiratory rate ≥ 30 breath per minute with respiratory distress; (2) saturation of oxygen ≤ 92 % at rest; (3) index of oxygenation ≤ 300 mmHg; (4) respiratory failure that require ventilation; (5) shock; and (6) admission to the ICU unit for other organ failure. CORAD score was calculated for all cases and was included in the radiology report. The degree of suspicion ranged from very low to very high (CO-RADS categories 1–5), while category 0 reflects negative infection and category 6 establishes RT-PCR-positive SARS-Cov-2 infection at time of examination[13]. CORADS score was used for assessment of severity[14-15].

Sample Collection:

Blood samples were taken at admission from each patient and repeated during the convalescence period for severe pneumonic cases. Serum C-reactive protein (CRP), D-dimer, lipids and glucose were determined by conventional laboratory methods. Blood samples of the control group were also collected and tested. The obtained blood samples were placed in tubes containing EDTA and blank tubes then immediately centrifuged at 1500 g and stored at −80°C.

Enzyme-Linked Immunosorbent Assay:

Quantitative detection for tumor necrosis factor alfa (TNF-α) and intercellular adhesion molecule1 (ICAM-1) was performed using ELISA kits according to the manufacture instructions.

Ethical consent:

An approval of the study was obtained from Benha University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Data were analysed using SPSS software, version 25.0 (IBM, Armonk, NY, USA) for Windows. Quantitative data were tested for normality using Kolmogorov test assuming normality at P>0.05. They were proved to be non-parametric, so they were presented as median and interquartile range (IQR), and analyzed by Mann-Whitney U test and Kruskal-Wallis (KW) test for independent groups. Qualitative data were presented as frequency and percentage. P ≤0.05 was considered significant.

RESULTS

Characteristics of the patients:

This study involved 100 subjects who were divided into 50 confirmed COVID 19 cases and 50 controls; 21 patients were males, 29 were females, 25 controls were males and 25 were females, 10 cases had mild illness, while twenty cases had severe illness and twenty cases had moderate illness. The median ages in the severe, mild and control groups were 60, 50, and 40 years, respectively. Tables 1 and 2 shows clinical and laboratory data of cases.

Table (1): Clinical and radiological data of the studied COVID 19 cases

Variable			Confirmed cases of COVID-19 (n=50)	
			No.	%
Personal data	**Age**	Median	**55.0**	
		Interquartile range	**40.0-60.8**	
	Gender	Male	**21**	**42.0**
		Female	**29**	**58.0**
	Residence	Urban	**29**	**58.0**
		Rural	**21**	**42.0**
Presenting symptoms	**Fever**	Yes	16	32.0
		No	34	68.0
	Dyspnea	Yes	33	66.0
		No	17	34.0
	Cough	Yes	7	14.0
		No	43	86.0
	Anosmia and loss of taste	Yes	13	36.0
		No	37	74.0
	Others	Shock	15	30.0
		Acute abdomen	2	4.0
		None	33	66.0
Medical history		HTN	11	22.0
		Asthma	2	4.0
		Epilepsy	1	2.0
		Others	1	2.0
		None	35	70.0
Hospital admission and stay		Median	13.5	
		Interquartile range	7.0-20.3	
CT finding		Co-RADS 1	10	20.0
		Co-RADS 2	6	12.0
		Co-RADS 3	7	14.0
		Co-RADS 4	14	28.0
		Co-RADS 5	6	12.0
		Co-RADS 6	4	8.0
		Others e.g. pleural effusion, hemothorax and lung abscess	3	6.0
Treatment and need for oxygen		Room air	**17**	34.0
		Nasal bronge	**10**	20.0
		Simple face mask	**15**	30.0
		High flow nasal cannula	**1**	2.0
		CPAP	**2**	4.0
		Mechanical ventilation	**5**	10.0
Outcome		Discharged from hospital	**19**	38.0
		Ward discharged	**5**	10.0
		ICU	**23**	46.0
		Died	**3**	6.0

Table (2): Laboratory data among the studied cases

Variable		Confirmed cases of COVID-19 (n=50)	
		Median	**IQR**
CBC	HGB	11.3	9.9-12.2
	RBCs	4.2	3.5-5.0
	WBCS	9.2	6.3-11.4
	PLT	190.5	142.8-253.8
	LYMPHOCYTES	1.2	0.8-1.6
	NEUTROPHIL	7.2	4.1-8.6
	Eosinophil	0.1	0.03-0.7
	Basophil	0.01	0.01-0.03
	Monocytes	0.7	0.6-1.1
Ferritin		130.5	38.8-323.0
D-dimer		285.1	60.0-455.8
Kidney functions	S. UREA	60.7	31.3-122.3
	S. Creatinine	1.5	0.9-3.5
	S. Uric acid	3.5	2.6-4.6
Liver function	AST	31.0	17.8-51.8
	ALT	27.7	18.0-39.4
	Total Bilirubin	0.9	0.6-3.5
	Albumin	3.1	1.6-3.6
CRP		24.0	12.0-73.0
ngmlICAM1level		455.0	274.5-614.0
TNF-α		165.0	131.0-256.3

There was statistically significant difference between cases of COVID-19 and healthy controls regarding ICAM-1 level with a higher median for cases. There was also statistically significant difference between cases of COVID-19 and healthy controls as regarding TNF-α level with a higher median for cases (Table 3).

Table (3): Expression of ICAM1 level and TNF-α in COVID-19 patients and control participants

Variable	Normal group (n=50)		Confirmed COVID-19 (n=50)		Mann-Whitney U test	P value
	Mean	**SD**	**Mean**	**SD**		
ICAM-1 level (ng/ml)	156.5	38.11	420.0	45.65	33.0	<0.001
TNF-α	73.0	4.21	165.0	9.11	22.0	<0.001

IQR=interquartile Range (Percentile25-Percentile75)

There was statistically significant difference as regard ICAM-1 level in relation to different CT findings with a higher median for Co-RADS 6 and there was statistically significant difference as regard CT scan finding and TNF-α level with a higher median for others findings (Table 4).

Table (4): ICAM1 level and TNF-α in relation to different CT findings

Variable	ICAM-1 level (ng/ml)		TNF-α	
	Mean	SD	Mean	SD
Co-RADS 1	286.5	8.11	154.5	7.21
Co-RADS 2	273.0	59.23	122.0	29.31
Co-RADS 3	365.0	9.32	142.0	24.12
Co-RADS 4	595.0	47.13	250.0	6.31
Co-RADS 5	526.5	33.36	175.0	4.35
Co-RADS 6	631.0	55.21	162.5	8.31
Others, e.g. pleural effusion, hemothorax and lung abscess	620.0	240.0-620	310.0	165.0-310.0
Control	164.0	131.3-184.0	74.0	68.8-83.3
Kruskal-Wallis test	39.352		31.981	
P value	<0.001		<0.001	

IQR=interquartile Range (Percentile25-Percentile75)

There was positive statistically significant correlation between results of CT scan finding and duration of hospital stay as well as ICAM-1 level. However, the correlation was weak for TNF- α (Table 5).

Table (5): Correlation between CT scan finding and some of the studied variables

Variable	Result of CT finding for confirmed cases of COVID-19 (N=50)	
	rho	P
Age	0.120	0.405 (NS)
Duration of hospital stay	0.483	<0.001
ICAM-1 level (ng/ml)	0.524	<0.001
TNF- α	0.111	0.441

rho: Correlation coefficient

Multivariate analysis showed that ICAM-1 level and TNF- α were significant predictors of COVID-19. While, univariate analysis showed that ICAM-1 level was a significant predictor of COVID-19 (Table 6).

Table (6): Univariate and multivariate logistic regression analysis for the predictors of COVID-19

Variable	Univariate logistic regression (n=100)				Multivariate logistic regression (n=100)			
	β	Crude OR	95%CI	P	β	Adjusted OR	95%CI	P
ICAM-1 level (ng/ml)	-0.039	0.962	0.944-0.979	**<0.001**	-0.025	0.976	0.955-0.997	**0.026**
TNF- α	-0.222	0.801	0.696-0.921	**0.002**	-0.238	0.788	0.613-1.012	**0.062**

Using cut off point for ICAM-1 level (205 ng/ml) showed a sensitivity of 98.0%, specificity of 100.0%, positive predictive value (PPV) of 100.0% and negative predictive value (NPV) of 90.9%. The figure also shows that using cut off point for TNF- α (89.0) showed a sensitivity of 96.0%, specificity of 100.0%, PPV of 100.0% and NPV of 83.3% (Figure 1).

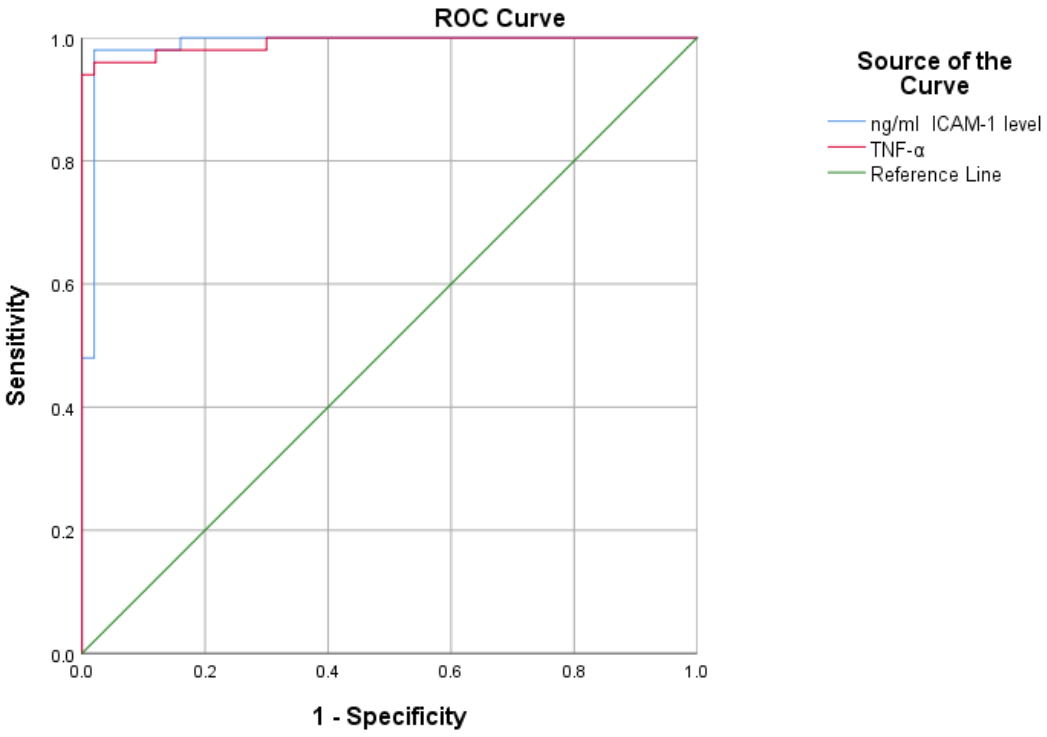

Figure (1): The specificity and sensitivity of ICAM-1 level and TNF- α

DISCUSSION

Since December 2019, the COVID-19 sickness has had a terrible impact on the world. The causative virus has acquired unique features as a result of mutations in its original form. Clinical deterioration is often rapid in serious cases, and systemic hyper-inflammation, also known as the "cytokine storm,"[16] appears to play a role in the pathogenesis of a number of severe manifestations, including acute respiratory distress syndrome, thromboembolic diseases such as acute ischemic strokes caused by large vessel occlusion and myocardial infarction, encephalitis, acute kidney injury, and vasculitis[17].

This study showed that ICAM-1 level had statistically significant difference between cases of COVID-19 and healthy controls with a higher median for cases (420 ng/ml). There was also statistically significant difference between cases of COVID-19 and healthy controls as regard TNF-α level with a higher median for cases (165) which agrees with **Tong *et al.*** [18] who reported that the serum levels of the following were higher in patients than in control participants: FKN (fractalkine), VCAM-1, ICAM-1, CRP and TNF-α. Also **Li *et al.*** [19] found that the serum levels of another endothelial cell adhesion molecule, platelet endothelial cell adhesion molecule 1 (sPECAM-1) was not only significantly higher in COVID-19 patients than in healthy controls but also significantly higher than in asymptomatic carriers.

Kim *et al.* [16] reported blood levels of various cytokines such as IL-1ß, IFN-γ, IFN-γ-induced protein 10 (IP10) and TNF- α were elevated in COVID-19. This study showed that higher ICAM-1 level was associated with more severe CT findings and longer hospital stay, and also multivariate analysis showed that ICAM-1 level and TNF- α were the significant predictor of COVID-19. While, univariate analysis showed that ICAM-1 level was a significant predictor of COVID-19.

Hojyo *et al.* [20] reported that the severity of COVID-19 is associated with an increased level of inflammatory mediators including cytokines and chemokines such as interleukin (IL)-2, IL-7, IL-10 and TNF. **Kim *et al.*** [16] found that patients admitted to the intensive care unit (ICU) have higher cytokine levels, also **Bhaskar *et al.*** [14] declared that patients who were admitted to intensive care unit (ICU) had higher levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP1A) and TNF-α compared to those who were not admitted to ICU.

Ruan *et al.* [21] reported that predictors of a fatal outcome in COVID-19 cases included age, the presence of elevated inflammatory indicators in the blood. **Tong *et al.* [18]** identified the severity of COVID-19 was associated with the serum levels of CRP, IL-18, TNF-α, IFN-γ, FKN, VCAM-1, ICAM-1 and VAP-1. Also, recovery from severe COVID-19 was associated with reductions in these markers.

CONCLUSIONS

Patients with severe COVID-19 might have a cytokine storm syndrome and increased expression of endothelial cell adhesion molecules as ICAM-1, which is related to COVID-19 and disease severity and may contribute to coagulation dysfunction. Levels of ICAM 1 were associated with more severe CT findings and longer hospital stay. ICAM 1 and TNF also can be used as diagnostic measures as they have a higher sensitivity, specificity and predictive values.

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Conflicts of interest:

The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.

This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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