Evaluation of S-Adenosylmethionine (SAM), Folic acid and cobalamin levels in a samples of Iraqi patients with Severe & Critical coronavirus disease 2019 (COVID-19)

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Abstract

Coronavirus disease 2019 (COVID-19), a newly identified respiratory illness caused by the SARS-CoV-2 (severe acute respiratory syndrome coronavirus), has recently gone pandemic. To replicate from the host's S.adenosylmethionine (SAM) and trigger the virally produced T-cell and macrophage-mediated cytokine storm, the coronavirus RNA cap structure is methylated, which appears to be a primary cause of COVID-19 mortality. Folate is required for the metabolism of nucleic acid precursors, certain amino acids, and methylation processes. SARS – COV-2, on the other hand, may disrupt vitamin B12 metabolism, limiting microbial proliferation in the intestine. Vitamin B12 deficient symptoms, such as enhanced oxidative stress and lactate dehydrogenase, as well as renal and pulmonary vasculopathy, are similar to COVID-19 infection symptoms.

Objective

S-adenosylmethionine (SAM), folic acid (B9), and cobalamin (B12) are markers of global transmethylation and may be useful as COVID-19 severity measures.

Method

Serum concentrations SAM, B9 and B12 were measured by ELISA and chemiluminescence method. 90 subjects were taken composed of both genders. With age range (18-75) years, 50 patients admitted with severe and critical COVID-19 respectively were diagnosed according the national guidance for clinical management and treatment of COVID-19 August 2020 from ministry of health in Iraq, and 40 persons as control.

Results

The results showed that blood SAM, B9, and B12 levels were considerably higher in COVID-19 patients when compared to non-COVID-19 controls ($P<0.001$), ($P=0.o5$), respectively, and that there was no significant difference between the severe and critical groups in these biomarkers.

Conclusion

Results of the present study found that the serum levels of SAM and B12 were higher in patients with COVID-19 than those without COVID-19 also serum level of B9 was lower in Patients with COVID-19 than those control subject.

Introduction

Coronavirus disease 2019 (COVID-19), a newly discovered respiratory ailment caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus), has just become pandemic (Huang *et al*., 2020; Xu *et al*., 2020). Within the coronaviridae family, which is part of the nidovirales order, coronavirinae and torovirinae are two subfamilies of coronaviruses.

According to the International Committee for Logical Classification of Infections, the coronavirinae subfamily is divided into four main genera: alpha-coronavirus, beta-coronavirus, gamma-coronavirus, and delta-coronavirus (Pillaiyar *et al.*, 2020). SARS-CoV-2 is a positive-sense, single-stranded RNA virus belonging to the Beta coronavirus genus (Lu *et al*., 2020).

The majority of COVID-19 patients experience mild to moderate symptoms, while 15% develop severe pneumonia, and 5% develop ARDS, septic shock, and/or multiple organ failure(Huang *et al*., 2020 ; Xu *et al.*, 2020).

Immune dysregulation, including a diminished type I interferon response (Blanco-Melo *et al.*, 2020), elevated inflammatory markers, surging IL-6, IL-10, and TNF suggestive of a cytokine storm (Pedersen and Ho, 2020 ; Cao, 2020), growing lymphopenia, and irregular blood coagulation, are all symptoms of severe illness (Meredith Wadman and Jocelyn Kaiser, 2020 ; Wang *et al.*, 2020).

S-adenosylmethionine (SAM) is an endogenous molecule generated from the essential amino acid methionine and adenosine triphosphate. It was discovered in 1952 and is not readily available from food sources

(Cuomo *et al.*, 2020). SAM is found in every live cell and serves as a methyl group donor in more than 100 different methyltransferase enzyme-catalyzed processes (Bottiglieri, 2002). Two viral methyltransferases, which transfer methyl groups from S-adenosylmethionine to the coronavirus RNA cap structure, methylate it (SAM). The amount of methionine in the host determines how well the virus is methylated. The virally produced T-cell and macrophage-mediated cytokine storm, which appears to be a major cause of Covid-19 mortality.

Folic acid is also known as folate, folacin, vitamin B9, vitamin M, Folvite, Acifolic, Folcidin, and pteroylglutamic acid in scientific circles (Liew, 2016). It is a water-soluble vitamin that can be found in fortified foods as well as supplements. Folic acid is required for the formation of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as well as a variety of methylation processes.

Folate is required for the production of DNA and proteins, as well as the adaptive immunological response. Furin is a bacterial and viral infection-related enzyme that could be a promising target for infection treatment. Folic acid has only lately been found to inhibit furin, which prevents the SARS-CoV-2 spike protein from attaching to it, As a result, cell entrance and viral turnover are prevented. Folic acid, as a result, has been recommended as a possible treatment for COVID-19-related respiratory illness in its early phases (Sheybani *et al.*, 2020).

Vitamin B12 is a water-soluble vitamin produced by microorganisms in the form of a crystallisable cobalt-complex known as cobalamin. Vitamin B12 cannot be synthesized by humans, so it must be obtained from food (Romain *et al.*, 2016). Cobalamin is required for red blood cell creation, the maintenance of a healthy neurological system, cell division, myelin

synthesis, cellular development and reproduction, and quick DNA synthesis (Stipp, 2020). COVID 19 virus is thought to interfere with cobalamin metabolism, thereby reducing gut bacteria proliferation and generating cobalamin deficient symptoms. Increased oxidative stress, homocysteine concentration, activation of the coagulation cascade, thrombocytopenia, elevated lactate dehydrogenase (LDH), low reticulocyte count, intravascular coagulation thrombosis, vasoconstriction, and renal and pulmonary vasculopathies are all symptoms of vitamin B12 deficiency (Sabry et al., 2020; Grangé S, et al. 2015).

Method

A case control study performed during the period from January 2021 and April 2021; this study involved 90 individuals composed of both genders. With age range (18-75) years; which were divided into three groups; Included group1 and 2 contained 50 patients with severe and critical respectively were diagnosed according the national guidance for clinical management and treatment of COVID-19 August 2020 from ministry of health in Iraq, and group three consist of 40 persons as control.

All patients' samples were collected from volunteer patients who tested positive for SARS-CoV-2 in respiratory specimens through real-time reverse transcriptase-polymerase chain reaction. The patient's samples collected from isolation ward in Al-Imamain Al Kadhimain Medical City, and control samples collected from relatives and friends.

Blood specimens were collected from these individuals to measure these individuals to measure the study markers: serum of S-

adenosylmethionine (SAM) for all patients and control subjects using enzyme- linked Immune-Sorbent assay (ELISA) method, Folic acid (B_9), Cobalamin (B_{12}) and Ferritin were measured by chemiluminescence method, CRP was measured by immunoturbidimetric method, LDH was measured by enzymatic colorimetric method and plasma EDTA, Sodium citrate for CBC, D-dimer respectively.

Ethics committee

The study was approved by the Al- Nahrain University College of Medicine's ethical committee. Each participant gave their informed consent.

Blood sample preparation

Eight milliliters of blood samples was collected form patients and control as follow:

1. Tow milliliters of blood were taken in EDTA tube for CBC tests.
2. Tow milliliters of blood were taken in sodium citrate 3.8% tube for D.dimer test.
3. Four milliliter of blood were taken in gel tube and left 15 minutes at ambient temperature. After coagulation, sera were separated at 3000 rpm for 5 minutes and were divided into small aliquots for:

   a. Immediate measurements of B9 (Folic acid), B12 and ferritin were done using appropriate chemiluminescence method.
   b. Immediate measurement of CRP was done using appropriate colorimetric method.

c. Immediate measurement of LDH was done using appropriate enzymatic method.

The rest was stored at -20 until assayed for concentration of serum human SAM. It is going to be determining using enzyme-linked immunosorbent assay (ELISA) kit.

Analytical Statistics

SPSS software version 25.0 was used to conduct statistical analysis (SPSS, Chicago). The normality of continuous data was tested (Shapiro Wilk test); data with normally distributed distribution were presented as mean and standard deviation and analyzed using analysis of variance (ANOVA). Non-normal data were provided as median and range and evaluated using the Mann Whitney U test (for two group comparisons) or the Kruskal Wallis test (for three group comparisons).

The Chi-square test was used to examine categorical variables that were expressed as a number and a percentage. The diagnostic usefulness of SAM B9 and B12 in the distinction between patients (severely and critically sick) and controls was assessed using a receiver operating characteristic curve (ROC). The association between SAM, B9, and B12 and demographic and clinical characteristics in each group was investigated using Spearman's correlation test. A statistically significant difference was defined as a p-value of less than 0.05.

Results

The median serum concentration of SAM in critically-ill patients were 29.24 ng/ml (range 11.44- 93.25 ng/ml) which was significantly higher than that of control group (median= 17 ng/ml, range= 6.83-54.41 ng/ml) and non-significantly from severely-ill patients (median= 27.92 ng/ml, range= 12.92-90.4 ng/ml), as shown in table (1) and figure (1).

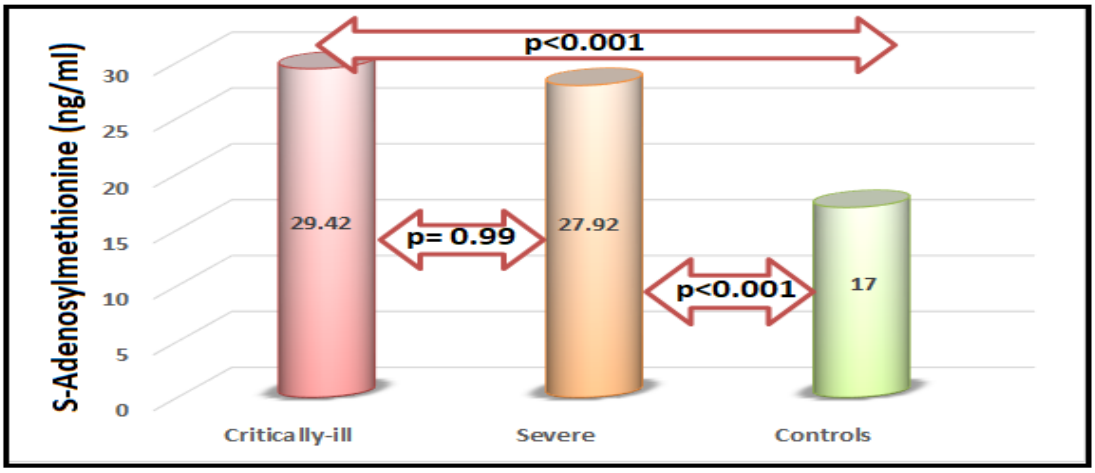

Figure (1): Median serum level of SAM in different groups

Similarly, the median serum level of B12 in severely-ill patients was 502 pg/ml (range 206-2000 pg/ml) which was significantly higher than that of control group (median= 313 pg/ml, range= 193-842 pg/ml) and non-significantly from critically-ill patients (median= 497 pg/ml, range= 111-2000 pg/ml), as shown in table (1) and figure (2).

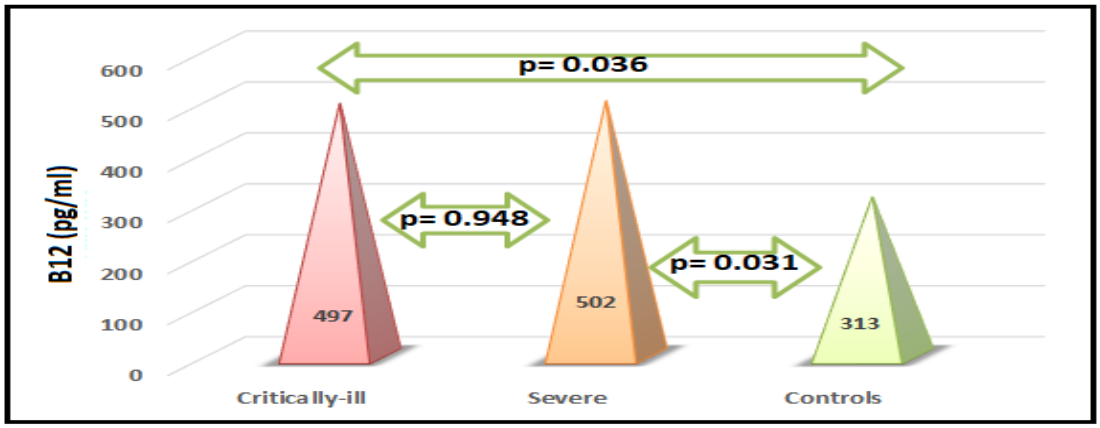

Figure (2): Median serum level of B12 in different groups

In contrast, control group had significantly higher level of B9 (median= 10.1 ng/ml, range= 3.2-17.5 ng/ml) than either severely-ill (median= 6.3 ng/ml, range= 1.8-19.9 ng/ml) or critically-ill patients (median= 6.6 ng/ml, range= 2.4-14 ng/ml), as shown in table (1), figure (3).

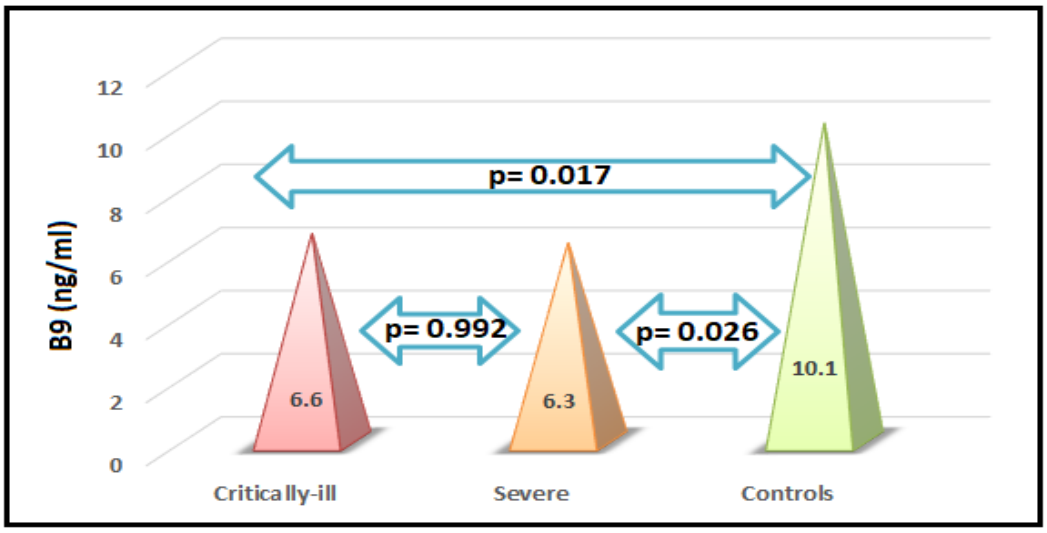

Figure (3): Median serum level of B9 in different groups

Table (1): Serum Levels of SAM, B9 and B12

Variables	Controls (n=40)	Severe COVID-19 (n=25)	Critically-ill COVID-19 (n=25)	p- value*
SAM, ng/ml	19.5±9.87 17.0[a] 6.83-54.41	36.04±20.52 27.92[b] 12.92-90.4	32.63±17.0 29.42[b] 11.44-93.25	**<0.001**
B_9, ng/ml	9.96±3.98 10.1[a] 3.2-17.5	7.56±4.81 6.3[b] 1.8-19.9	7.0±3.28 6.6[b] 2.4-14.0	**<0.001**
B_{12}, pg/ml	377.13±183.15 313[a] 193-842	818.32±710.8 502[b] 206-2000	658.36±564.8 497[b] 111-2000	**0.050**

SAM: S- Adenosylmethionine, Different small letters indicate significant differences

*Kruskal Wallis test

In this study results showed that serum CRP, LDH, Ferritin, and plasma D-dimer, Lymphocyte, Neutrophil, NLR, and MLR in severe and critical groups had a highly significantly increased in COVID-19 patients when compared with non COVID-19 controls group (P<0.001). Results showed that serum CRP, LDH, Ferritin and plasma D-dimer a highly significantly deference results between severe and critical groups in COVID-19 patients (P<0.001) as shown in table (2) and (3) respectively.

Table (2): Inflammatory and coagulopathy markers in different groups

Variables	Controls (n=40)	Severe COVID-19 (n=25)	Critically-ill COVID-19 (n=25)	p- value*
CRP, mg/L Mean±SD Median Range	0.29±0.23 0.21[a] 0.02-1.04	12.61±9.68 12.81[b] 1.61-32.0	16.07±10.71 14.19[b] 1.69-32.0	**<0.001**
LDH, U/L Mean±SD Median Range	186.4±31.47 189[a] 118-242	421.4±174.34 370[b] 223-998	855.52±416.5 773[c] 306-1995	**<0.001**
Ferritin, ng/ml Mean±SD Median Range	71.08±35.34 69.35[a] 7.11-154.1	819.44±640.89 668.31[b] 51.64-2170	1678±1751 1096[c] 267.5-8000	**<0.001**
D-dimer, ng/ml Mean±SD Median Range	87.58±52.79 71.5[a] 25-277	1122.6±1463.2 498[b] 18-6040	3473.6±6063 1454[c] 196-25980	**<0.001**

CRP: C-reactive protein, LDH: lactate dehydrogenase, Different small letters indicate significant differences

*Kruskal Wallis test

Table (3): Hematological indices in different groups

Variables	Controls (n=40)	Severe COVID-19 (n=25)	Critically-ill COVID-19 (n=25)	p- value*
10^3/ml×Neutrophil Mean±SD Median Range	4.05±0.87 4.12[a] 1.78-5.91	10.43±4.85 9.41[b] 1.29-19.9	14.55±9.9 12.3[b] 1.88-44.4	**<0.001**
10^3/ml×Lymphocyte Mean±SD Median Range	2.49±0.5 2.45[a] 1.1-3.84	0.93±0.89 0.62[b] 0.2-4.2	0.93±0.6 0.67[b] 0.2-4.2	**<0.001**
10^3/ml×Monocyte Mean±SD Median Range	0.45±0.1 0.45 0.27-0.71	0.61±0.51 0.48 0.1-2.34	0.53±0.46 0.39 0.1-2.38	0.751
NLR Mean±SD Median Range	1.68±0.45 1.69[a] 0.84-3.24	17.32±15.58 14.66[b] 2.93-68.34	18.23±11.85 14.58[b] 3.7-46.96	**<0.001**
MLR Mean±SD Median Range	0.19±0.07 0.18[a] 0.11-0.47	0.87±0.67 0.78[b] 0.12-2.77	0.64±0.59 0.57[b] 0.1-0.329	**<0.001**

NLR: neutrophil-lymphocyte ratio, MLR: Monocyte-lymphocyte ratio

Different small letters indicate significant differences

*Kruskal Wallis test

In the current a study there is no correlation was detected between the SAM and other continuous variables (age, CRP, LDH, Ferritin, Lymphocyte, Neutrophil, Monocyte, B9, and B12) in critically-ill patients and severely-ill patients table (4), (5) respectively. In critically-ill patients, B9 demonstrated a positive significant correlation with each of CRP (r= o.495, *p*= 0.050) and B12 (r= 0.391, *p*= 0.050). Additionally, D-dimer had a positive significant correlation with LDH (r= 0.564, *p*=0.003) as shown in table (4).

Table (4): correlations between SAM, B9 and B12 with different variables in critically-ill patients

Variables	SAM		B12		B9		Ferritin		D-dimer	
	r	P-value	R	P-value	r	P-value	r	p-value	r	p-value
Age	0.275	0.184	-0.203	0.329	0.112	0.594	0.308	0.134	0.115	0.586
CRP	0.120	0.567	-0.004	0.985	**0.495**	**0.012**	0.009	0.966	0.120	0.585
LDH	0.131	0.532	-0.127	0.544	0.148	0.481	0.289	0.161	**0.564**	**0.003**
Ferritin	0.265	0.201	-0.221	0.288	-0.257	0.215				
D-dimer	-0.035	0.870	-0.076	0.719	-0.052	0.805	0.275	0.184		
Lymphocyte	-0.086	0.682	0.201	0.335	-0.107	0.612	-0.279	0.176	-0.188	0.367
Neutrophil	0.297	0.149	-0.288	0.163	-0.314	0.127	0.052	0.804	0.052	0.804
Monocyte	0.177	0.398	0.139	0.507	-0.100	0.634	0.071	0.737	-0.151	0.472
SAM			-0.046	0.828	0.302	0.143				
B12					**0.391**	**0.050**				

In severely-ill patients, B9 also had a positive significant correlation with each of age (r= 0.461, p =0.020) and B12 (r= 0.391, p= 0.050) as shown in table (5).

Table (5): correlations between SAM, B9 and B12 with different variables in severely-ill patients

Variables	SAM		B12		B9		Ferritin		D-dimer	
	r	P-value	r	P-value	r	P-value	R	P-value	r	P-value
Age	-0.003	0.988	0.179	0.393	**0.461**	**0.020**	0.370	0.068	0.215	0.303
CRP	0.249	0.230	0.289	0.161	0.259	0.211	0.211	0.311	0.145	0.488
LDH	-0.204	0.328	0.069	0.744	0.117	0.576	-0.113	0.590	0.225	0.279
Ferritin	0.275	0.183	0.177	0.398	-0.048	0.819				
D-dimer	-0.298	0.148	0.229	0.272	0.070	0.741	0.026	0.903		
Lymphocyte	-0.308	0.135	-0.005	0.982	0.080	0.704	-0.364	0.074	-0.002	0.993
Neutrophil	0.133	0.526	0.176	0.400	0.117	0.578	0.188	0.260	0.260	0.210
Monocyte	0.267	0.197	0.171	0.415	0.054	0.798	0.115	0.585	-0.050	0.811
SAM			-0.046	0.828	0.302	0.143				
B12					**0.391**	**0.050**				

Discussion

As indicated in table (1), the median serum level of SAM in critically sick patients was considerably higher than that of the control group (ng/ml) and non-significantly higher than that of severely ill patients. The recent study compatible with other studies by increasing the methylation of replication and translation leading to increased serum level of SAM,

however, very few studies concerned the relation of SAM with COVID-19(Kryukov *et al.,* 2021; Bobileva *et al.,* 2021; Hoffman and Han, 2020).

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The median serum level of B9 in control group was significantly higher than severely-ill patients and critically-ill patients respectively, and there is no-significant difference between severely sick patients and critically ill patients, as shown in table (1). The decrease in folic acid results, possibly due to malnutrition or malabsorption as a result of COVID-19 affecting the gut. The recent study agrees with other studies through folic acid effect on SARS COV-2 (Meisel *et al.,* 2021; Zhang *et al.,* 2021). The median serum level of B12 in severely-ill patients and the median serum level of B12 in critically-ill patients were significantly higher than that of control group on the other hand there is non-significant results between severely ill patients and critically sick patients, as shown in table (1). Our findings back up recent studies in both severely and critically ill patients, where high B12 levels have been linked to higher fatality rates. High Vitamin B12 levels in our study participants can be caused by a number of factors, including increased levels of carrier proteins, which reduce Vitamin B12 clearance by the liver and limit uptake by peripheral tissues (Flores-Guerrero et al., 2020). The latest study supports previous research by raising vitamin B12 levels in hospitalized patients (Dalbeni et al., 2021).

Results showed that serum CRP, LDH, Ferritin, and plasma D-dimer, Lymphocyte, Neutrophil, NLR, and MLR in severe and critical groups had a highly significantly elevated in COVID-19 patients when compared with non COVID-19 controls group ($P<0.001$). Results showed that serum CRP, LDH, Ferritin and plasma D-dimer a highly significantly

deference results between severe and critical groups in COVID-19 patients (P<0.001), as shown in table (2, 3) respectively.

In the current a study there is no correlation was detected between the SAM and other continuous variables (age, CRP, LDH, Ferritin, Lymphocyte, Neutrophil, Monocyte, B9 and B12) in critically-ill patients and severely-ill patients table (4, 5) respectively. This is meaning that SAM may be act as independent markers in COVID-19 patients. This finding was agreed with a study by (Kryukov *et al*., 2021) there are very few studies on SAM biomarker and its correlation with COVID-19.

Currently, a study is being conducted that has a positive correlation was detected between two markers B9 and B12 in the severely- ill patients and critically- ill patients have the same (r 0.391, *P* 0.050), as shown in table (4, 5) receptively. Vitamin B9-B12-dependent biochemical pathways that are important for DNA synthesis, cellular control, and bodily repair could be a major modifiable risk factor in COVID-19 morbidity and mortality, according to new research (Shakeri et al., 2021). In the recent study a positive correlation was detected between the B9 and the age in the severely- ill patients (r 0.461, *P* 0.020) table (5), and a negative correlation were detected between the B9 and the age in the critically- ill patients (r 0.112, *P* 0.594) table (4), this finding was agreed with a study by (Dalbeni *et al*., 2021) in critical patients and was not agreed in severe patients. It is possible that this difference and inconsistency in the results is due to the difference in the methods that used in this study. In critically-ill patients, B9 demonstrated a positive significant correlation with CRP (r= o.495, *p*= 0.050). Folate is an important vitamin for adaptive immune response, and it was recently shown that folic acid can inhibit furin, which prevents the SARS-CoV-2

spike protein from attaching to it, preventing cell entry and virus turnover. COVID-19 mediated a cytokine storm by induced T cells and macrophages that also triggered IL6 elevation and triggered a CRP response to IL6 elevation (Sheybani *et al*., 2020; Marnell *et al*., 2005). The recent study was supported by (Beigmohammadi *et al*., 2021). In the current study there is no correlation was detected between B12 with other markers and other variables, may be due to different methods and measurement. This finding agreed with a study by (Dalbeni *et al*., 2021). However, a few data exist on the relationship between B12 with other markers and other variables.

Conclusion

Results of the present study found that the serum levels of SAM and B12 were higher in patients with COVID-19 than those without COVID-19 also serum level of B9 was lower in Patients with COVID-19 than those control subject. It were concluded that assessment serum levels of SAM, B9, and B12 could be a useful diagnostic value biomarkers for patients with severe or critical COVID-19 and there was no significant difference between the severe and critical groups, however, the sample size and time must be taken in consideration.

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