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The importance of serum homocysteine as a biomarker in diabetic and obese COVID-19 patients

Haitham Shawkat Ahmed[1*], Saman Hussein Noori[2]

[1] B.Sc Biotechnology, College of Sciences, Baghdad University, Iraq
[2] Department of Biochemistry, College of Medicine, University of Sulaimani, Kurdistan Region-Iraq

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ABSTRACT

Homocysteine is a possible risk marker in hematological complications of COVID-19 infection. This study aimed to elucidate the significance of homocysteine as a biomarker for COVID-19 infection, and the relation of homocysteine with COVID-19 severity in obese people and diabetic patients. The study groups were 1- COVID-19 patients + Diabetic + Obese (CDO), 2- COVID-19 patients + Diabetic (CD), 3- COVID-19 patients + Obese (CO), 4- Healthy Group (HG). Serum levels of homocysteine, IL-6, D-dimer, vitamin B12, and folate were measured by a fully automated biochemistry device Cobas 6000 analyzer series. The mean serum concentration of homocysteine in the COD, CD, CO and H groups were 32.0114, 23.604, 19.4154, and 9.3206 umol/l respectively. The mean concentration of homocysteine levels between every two groups was statistically significant differences ($P<0.05$) except for the CD and the CO group ($P=0.957$). In the CDO group, the males have higher mean concentrations than females ($P<0.05$). The means of homocysteine concentrations in the CDO group among different age groups were different ($P <0.001$). The serum homocysteine level in the CDO group has a strong positive correlation ($R=0.748$) with D-dimer and a strong negative correlation ($R= - 0.788$) with serum folate, while its correlation with serum vitamin B12 is moderate negative (-0.499) and its correlation with serum IL-6 is weakly positive ($R=0.376$). The AUC value for homocysteine in predicting COVID-19 in the CDO group was 0.843, while 0.714 for the CD group, and 0.728 for the CO group. The serum homocysteine concentration test for all study groups was compared to the serum IL-6 test and the sensitivity was equal to 95% and its specificity was 67.5%. Serum homocysteine has potential predictive power in COVID-19 patients, and the severity of COVID-19 infection and the type of comorbidity is associated with higher sensitivity and specificity of homocysteine serological tests.

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Introduction

Homocysteine is an unnecessary sulfur-containing intermediate amino acid that is not present in the food; homocysteine is formed during the metabolism of methionine, homocysteine can be converted into cysteine or recycled into methionine with the help of B vitamins. The normal range of homocysteine is between 5-15 µmol/L, and the range is varied between males and females (1,2).

An increase in the level of homocysteine is called hyperhomocysteinemia which is said if the level of homocysteine is above 15 µmol/L. Hyperhomocysteinemia is an independent risk factor related to many diseases and has a synergistic effect on them like cardiovascular diseases, cerebrovascular diseases, and thromboembolic diseases; however, hyperhomocysteinemia can be asymptomatic (3,4).

The evaluation and management the hyperhomocysteinemia remain a matter of debate as results of previous studies revealed conflicting findings in their effects on lowering the risk of cardiovascular diseases and cerebrovascular diseases. In patients with homocystinuria, which is a rare autosomal recessive disease and the patient develops atherosclerotic diseases at a young age, there is clear evidence that lowering the homocysteine level will minimize the possibility of acquiring atherosclerotic diseases in young adults (5); also, it is found that lowering homocysteine level will slow down the brain atrophy (6). On the other hand, the use of homocysteine-lowering drugs did not prevent stroke (7) and did not reduce the probability of ischemic heart disease (8).

The level of Homocysteine is generally characterized into three major groups: moderate rise (16 to 30 µ/L), intermediate rise (31 to 100 µ/L), and severe rise (over 100 µ/L) (9).

The enzyme Methylene Tetrahydrofolate Reductase (MTHFR), a significant gene in the folate pathway, with the help of B vitamins (vitamin B6, vitamin B12, and folate) prepares a methyl group for the re-methylation of homocysteine back to methionine and cysteine (10,11). The conversion of homocysteine into methionine is catalyzed by a process called re-methylation, this conversion has happened with the help of vitamin B12. Then methionine may be broken to reform homocysteine. The conversion of homocysteine into cysteine is occurred by the enzyme cystathionine B-synthase. Disruption in the previous steps of homocysteine metabolism can lead to hyperhomocysteinemia. There are multiple causes of abnormalities in the metabolism of homocysteine metabolism, but the most common etiologies of increased homocysteine level

* Corresponding author. Email: haithim.shawket@yahoo.com

are insufficient MTHFR enzyme activity because of genetic defects (12). In addition, the homocysteine plasma concentrations increment can happen due to the genetic deficiencies in enzymes that existed in the homocysteine metabolic pathway, lack of vitamins like these enzymes cofactors, drug usage and some clinical situations (1).

Because B vitamins have a crucial role in homocysteine metabolism, deficiencies in B vitamins are associated with hyperhomocysteinemia, so conditions associated with vitamin B12, B6, or folate deficiency as in alcoholics, the use of proton pump inhibitors may theoretically predispose to hyperhomocysteinemia (13). Other conditions and diseases that are associated with hyperhomocysteinemia include hip fracture, hypothyroidism, Alzheimer's disease, schizophrenia, osteoporosis, and chronic kidney failure (6).

It is estimated that 5-7% of general populations have mild hyperhomocysteinemia and researchers proposed that hyperhomocysteinemia is related to an increased risk of thrombotic conditions, and lowering the homocysteine level will partially reduce the risk of stroke (2).

The elevated level of homocysteine is a risk factor for endothelial cell injury, which will promote vascular injury and induce an inflammatory response, which will cause thrombus formation (13). Since COVID-19 infection has a prothrombotic effect and because hyperhomocysteinemia has a similar consequence, it is important to understand the role of hyperhomocysteinemia in disease progression among COVID-19 cases (14).

Elevation in homocysteine level may be asymptomatic, or the patient is presented with the underlying cause of hyperhomocysteinemia such as vitamin B12 deficiency, vitamin B6 deficiency, or folate deficiency, or the presentation may be related to features of hyperhomocysteinemia complications like stroke, ischemic heart diseases, osteoporosis, or cognitive dysfunction (15).

In the evaluation of hyperhomocysteinemia, the patient is subjected to history taking, then a thorough physical examination with special attention to homocystinuria which is a rare dangerous disease (4). If homocystinuria is excluded, then extra investigations and management for hyperhomocysteinemia remain controversial. Some researchers and health institutes do not recommend any further management, while others advise the use of folic acid would reduce the disease progression in those with thrombotic complications such as carotid atherosclerosis; furthermore, patients with cognitive dysfunction will get benefit from B vitamin supplements in the management of cognitive impairment (5). Carotid atherosclerosis may cause carotid stenosis, a decrease in blood supply to the brain tissue and finally, severe stroke. Han et al proved the relation between serum homocysteine levels and carotid atherosclerosis in H-type hypertension patients (16).

One of the possible risk markers in hematological complications of COVID-19 is homocysteine, it is a non-proteinogenic amino acid resulting as an intermediate during methionine metabolism. Hyperhomocysteinemia, the increase in the level of homocysteine, is suggested to be a risk factor and/or marker of cardiovascular disease (17). The homocysteine is metabolized to methionine or cysteine with the help of B vitamins. Hyperhomocysteinemia may contribute to atherogenesis and increase the risk of atherosclerosis and coronary heart diseases (18). In other word, according to the studies, homocysteine is very related to coronary heart disease occurrence, hypertension and has a vital relationship with heart failure intensity (19). Moreover, abnormalities in homocysteine metabolism can cause an imbalance in the body oxidation-reduction reactions with an increase in oxidative stress which will result in increased oxidation of proteins, carbohydrates, and lipids (17). Many previous studies revealed that some viral infections are associated with an increase in homocysteine levels as HIV infection, viral hepatitis, and human papillomaviral infections (20).

This study aimed to know the significance of homocysteine as a biomarker for COVID-19, and the relation of homocysteine with COVID-19 severity in obese people and diabetic patients. This study addresses the levels of homocysteine amino acid in COVID-19 cases having DM and obesity simultaneously. The study tries to predict the main interactions between this biochemical marker and COVID-19 infections in DM with obese people. In addition to homocysteine, five other blood markers will be elucidated, these are IL -6, D-Dimer, vitamin B12, Folate, and blood sugar.

Materials and Methods

This case-control study was conducted at Shaheed Heman Hospital /Sulaimani City/Iraq. It enrolled 200 participants, the study group, COVID-19 patients with diabetes mellitus and obesity group (n=50) abbreviated as CDO, and three other groups: COVID-19 patients with diabetes mellitus group (CD group, n=50), the COVID-19 patients with obesity group (CO group, n=50), and the third group include healthy persons (H group, n=50). The study extended from 1 December 2022 till July 2022.

Approval was obtained from the ethical committee of the College of Medicine/Sulaimani University and informed consent was obtained from each participant before he/she was enrolled in the study.

Age and gender were recorded for each participant. Nearly 7 ml of blood was aspirated from each participant, then blood was centrifuged and serum separated then stored at – 20 ºC till performing the laboratory tests. Measurement of serum levels of total homocysteine, IL-6, D-dimer, vitamin B 12, and folate was performed by fully automated biochemistry device Cobas 6000 analyzer in the serum of participants using homocysteine Kit (HCYS Roche/Germany), Il-6 Kit (ELECSYS IL-6 Roche/Germany), D-Dimer Kit (D-DI2Roche/Germany), vitamin B12 Kit (ELECSYS VITAMIN B12 Roche/Germany), and Folate Kit (ELECSYS FOLATE III Roche/Germany) respectively.

Results

The current study included 200 participants with an overall mean ± Sd of ages 65.32 ± 8.33, with ages, range 41 – 84 years old. The overall gender distribution was 81(40.5%) male and 119 (59.5%) female. The participants had distributed into four groups, CDO group, CD group, CO group, and H group, their mean ± Sd of age was 66.66 ± 7.7, 65.98 ± 9.39, 63.70 ± 8.37, and 64.96 ± 7.68 respectively.

In this study, we measured the concentrations of homocysteine, IL-6, D-dimer, vitamin B 12, and folate in three COVID-19 groups and healthy control groups In Sulai-

mani City/Iraq.

The means serum concentration of homocysteine in the CDO group, CD group, CO group, and healthy group were 32.0114, 23.604, 19.4154, 9.3206 umol/l respectively, the highest homocysteine range was in the CD group (6.44-120 umol/l) while the lowest range was in H group (5.5-12.28 umol/l); the differences in means of homocysteine levels among study groups were statistically highly significant (p<0.001), Table 1).

The concentrations of IL-6 mean serum levels for the CDO group, CD group, CO group, and H group were 187.534, 33.6174, 33.4952, 4.0292 pg/mL respectively, with the widest range in the CDO group equal to 2.53-1918 pg/mL while least range in H group (2-6.5 pg/mL), the differences in the means among the four groups were statistically significant (p=0.0001), Table 1).

The mean D-dimer concentrations were 3744.12, 3676.06, 2308.1, 181.362 ng/mL for the CDO group, CD group, CO group, and H group respectively; the highest range in D-dimer was in the CDO group (110-13150 ng/mL) while its lowest range in H group (60-398 ng/mL) and the differences among these results were statistically highly significant (p<0.001), Table 1).

The means of vitamin B12 concentrations in sera of studied groups, CDO group, CD group, CO group, and H group, were 733.533, 716.339, 688.02, 345.378 pg/mL respectively, the range of vitamin B12 was highest in CD group (73.23-2970 pg/mL) and lowest in H group (114.9-701 pg/mL), the difference among these results were statistically significant with a p-value equal to 0.005. The folate level was measured for all participants and the means in level for the CDO group, CD group, CO group, and H group were 8.8012, 5.995, 5.0394, 10.063 ng/ml respectively; the range was highest in the CD group at 0.72-28 ng/ml and lowest in H group 7.4-15.3 ng/ml, the differences in results were statistically highly significant (p<0.001); all the above results are illustrated in the table (1).

The analysis of differences in the mean concentrations of homocysteine levels between every two groups of the following groups: CDO group, CD group, CO group, and H group, revealed the presence of statistically significant differences (p<0.05) except the differences between CD group and CO group was not statistically significant (p=0.957), as explained in Table 2.

The differences in the mean homocysteine levels in males and females were analyzed (Table 3). In the CDO group, the males have higher mean concentrations (34.4595 ±23.30167 umol/l) than females (30.511±19.32223 umol/l), (p<0.001). comparable results were found in the CD group with mean homocysteine concentration in males equal to 24.3465±22.52687 umol/l while for females equal to 22.9715±16.53789 umol/l, the p value<0.001. While the

Table1.Distribution of the serum parameters among the participant groups.

Parameters	Participant groups	N	Mean	Std. Deviation	Minimum	Maximum	p value
Homocysteine	Diabetes and Obese	50	32.0114	20.7795	8.9	80	<0.001
	Diabetes	50	23.604	19.3246	6.44	120	
	Obese	50	19.4154	8.42164	5.9	37.1	
	Healthy	50	9.3206	1.82283	5.5	12.28	
IL-6	Diabetes and Obese	50	187.534	501.303	2.53	1918	0.001
	Diabetes	50	33.6174	45.5511	2	200.9	
	Obese	50	33.4952	55.5184	2	287	
	Healthy	50	4.0292	1.04097	2	6.5	
D-dimer	Diabetes and Obese	50	3744.12	4368.88	110	13150	<0.001
	Diabetes	50	3676.06	4319.78	36.8	13000	
	Obese	50	2308.1	2772.46	189	13200	
	Healthy	50	181.362	95.7178	60	398	
Vitamin B12	Diabetes and Obese	50	733.533	679.854	49.7	2710	0.005
	Diabetes	50	716.339	703.46	73.23	2970	
	Obese	50	688.02	751.977	74.93	2900	
	Healthy	50	345.378	112.245	114.9	701	
Folate	Diabetes and Obese	50	8.8012	6.74073	2.1	28	<0.001
	Diabetes	50	5.995	5.92451	0.72	28	
	Obese	50	5.0394	2.63103	1.14	14.73	
	Healthy	50	10.063	1.81206	7.4	15.3	

Table 2. The significance of homocysteine means differences among the study groups.

Serum parameter	Group	Groups	Mean difference	P value
Homocysteine	Diabetic and obese	Diabetes	8.41	0.03
		Obese	12.60	<0.001
		Healthy	22.69	<0.001
	Diabetics	Obese	4.19	0.957
		Healthy	14.28	<0.001
	Obese	Healthy	10.09	0.005

means of concentration of homocysteine in the CO group and H group were higher in women than in men with mean concentrations in the CO group was 18.8395±8.49199 umol/l in males and 19.7684±8.49914 umol/l in females; whereas for H group they were 9.1475±1.72289 umol/l and 9.436±1.90657 umol/l for males and females respectively, and the differences in CO group and H group were statistically significant (p<0.001), as clarified in Table 3. the estimated marginal difference revealed that males have higher concentrations in CDO and CD groups than females but the inverse happens as concentrations decline in CO and H groups, Figure 1.

The participants in each of the four study groups were divided into three age groups, these are 40-55 years, 56-70years, and >70 years. The means of homocysteine concentrations in the CDO group among the three different age groups were different and these differences were statistically significant (p-value <0.001), similar results were observed in the other three groups, as clarified in Table 4.

The highest mean homocysteine concentrations were in the age group >70 years old in the CDO group, in the age group 55-70 years among the CD group, in the age group 40-55 years old for the CO group, and in the age group >70 years among healthy participants; these results are shown in Figure 2.

The serum homocysteine level in the CDO group has a strong positive correlation (r=0.748) with D-dimer and a strong negative correlation (r= - 0.788) with serum folate,

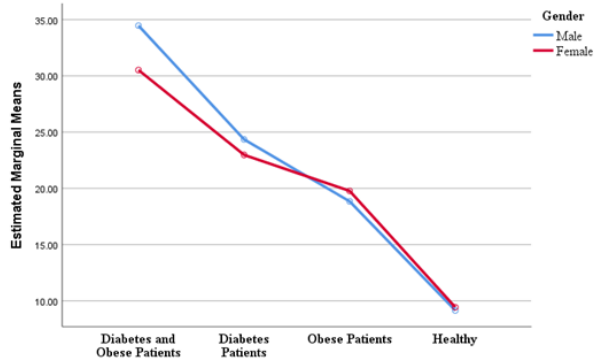

Figure 1. The estimated marginal means of homocysteine concentrations among males and females in different study groups.

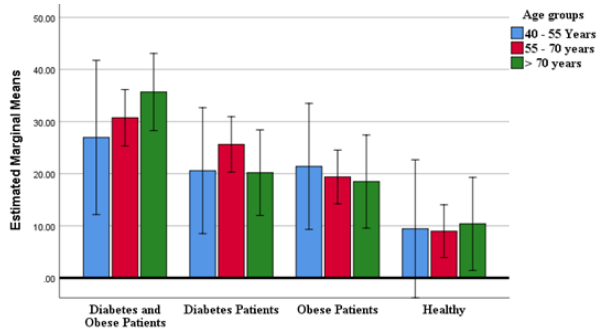

Figure 2. Means of homocysteine concentrations in different age groups of study populations.

Table 3. The gender differences in mean levels of homocysteine in different study groups.

Groups	Homocysteine µmol/l			N	p value
	Gender	Mean	Std. Deviation		
Diabetes and Obese	Male	34.4595	23.30167	19	<0.001
	Female	30.511	19.32223	31	
Diabetic	Male	24.3465	22.52687	23	<0.001
	Female	22.9715	16.53789	27	
Obese	Male	18.8395	8.49199	19	<0.001
	Female	19.7684	8.49914	31	
Healthy	Male	9.1475	1.72289	20	<0.001
	Female	9.436	1.90657	30	

Table 4. The mean homocysteine concentrations among different age groups of the study populations.

Groups	Age groups/years	Homocysteine µmol/l		N	p value
		Mean	Std. Deviation		
Diabetes and Obese	40 – 55	26.96	19.85	4	<0.001
	56 – 70	30.73	20.02	30	
	> 70	35.68	23.08	16	
	Total	32.01	20.78	50	
Diabetic	40 – 55	20.57	8.51	6	<0.001
	56 – 70	25.62	23.54	31	
	> 70	20.20	9.05	13	
	Total	23.60	19.32	50	
Obese	40 – 55	21.39	6.02	6	<0.001
	56 – 70	19.36	8.81	33	
	> 70	18.49	8.82	11	
	Total	19.42	8.42	50	
Healthy	40 – 55	9.42	1.88	5	<0.001
	56 – 70	8.97	1.85	34	
	> 70	10.37	1.39	11	
	Total	9.32	1.82	50	

while its correlation with serum vitamin B12 is moderate negative (-0.499); finally, its correlation with serum IL-6 is weakly positive (r=0.376). In the CD group, the serum homocysteine levels have a strong positive correlation (r=0.615) with serum IL-levels, whereas homocysteine has a weak positive correlation (r=0.298) with D-dimer, while weak negative relation (r=-0.224) with vitamin B12 and moderate negative relation (r=-0.412) with folate. The correlation among all other markers (serum IL-6, serum D-dimer, serum vitamin B12, and serum folate) was either weak positive or weak negative. The CO group revealed weak or very weak correlation either positive or negative between the concentrations for every two parameters including homocysteine, IL-6, D-dimer, vitamin B12, and folate. In the healthy group, only the correlation between D-dimer concentrations and IL-6 concentrations have a moderate positive correlation (r=0.397), all other correlations among the parameters were either very weak or weak, positive, or negative, as clarified in Table 5 and Figure 3.

A receiver operator characteristic (ROC) curve was performed to assess the potential predictive power of homocysteine for COVID-19 patients in all patients, (Figure 3). The area under the curve (AUC) value for homocysteine in predicting COVID-19 was 0.854. The results of the ROC curve analysis indicated that homocysteine had very good predictive efficiency for predicting COVID-19 in all patients.

The serum homocysteine concentration test for all study groups was compared to the serum IL-6 test and the results revealed that homocysteine sensitivity was equal to 95% and its specificity was 67.5%. In CDO group was having a sensitivity equal to 100% and a specificity of 80%, while in CD group, it has a sensitivity equal to 90% with a 62.5% specificity, in the CO group, had a sensitivity equal to 80% with a 55% specificity.

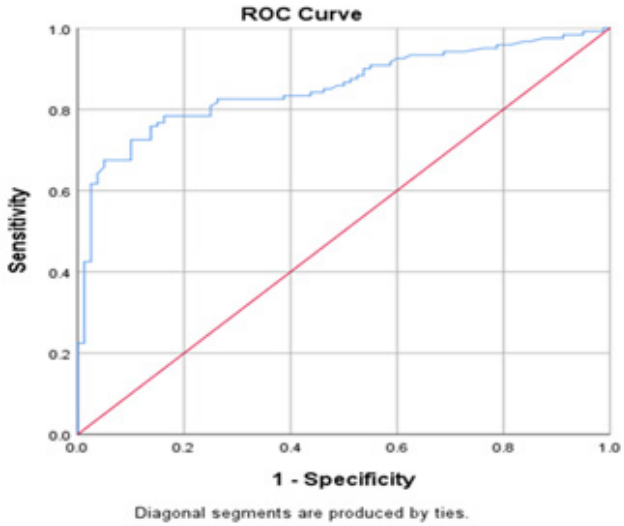

Figure 3. Receiver operating characteristic curve of homocysteine for predicting COVID-19 in all patients.

Discussion

The homocysteine levels in different COVID-19 study groups were elevated above the normal range which is equal to 5-15 µmol/L, and the elevation in the mean concentration of each COVID-19 group was progressive and starting from 19.4154 in obese COVID-19 patients, then 23.604 in diabetic COVID-19 group, the highest is 32.0114 in diabetic and obese COVID-19 group; these results clarify the association between the presence of comorbidity and higher homocysteine concentration, in addition, the presence of more than one comorbidity is related to more increment in homocysteine level than if the patient has only one comorbidity.

Wang, J, et al, (21) recorded the relationship between obesity and hyperhomocysteinemia (HHcy), which is comparable to the results of the current study; both obesity and HHcy are risk factors for cardiovascular diseases, and their presence in the elderly COVID-19 patient will increase the mortality rate from ischemic heart diseases. Previous studies reported that abnormal accumulation of fat in the abdomen is a risk factor for HHcy (22,23).

HHcy is mostly due to improper metabolism of homocysteine, this metabolism needs B vitamins, so any decrease in these vitamins will cause HHcy, it is a common

Table 5. The correlation coefficients among different parameters in different study groups.

Group	Parameter	Correlation coefficient	P value
CDO N=50	IL-6	0.165	0.25
	D-dimer	0.123	0.396
	Vitamin B12	– 0.254	0.075
	Folate	0.013	0.929
CD N=50	IL-6	0.6155	0.008
	D-dimer	0.298	0.035
	Vitamin B12	– 0.224	0.117
	Folate	– 0.412	0.003
CO N=50	IL-6	0.165	0.25
	D-dimer	0.123	0.396
	Vitamin B12	– 0.254	0.175
	Folate	0.013	0.929
H	IL-6	– 0.21	0.14
	D-dimer	– 0.096	0.51
	Vitamin B12	0.162	0.26
	Folate	– 0.065	0.64

finding in COVID-19 hospitalized patients to be associated with folate deficiency (24); the folate deficiency will cause HHcy; so, patients with COVID-19 are at risk of developing HHcy.

DM patients usually have a normal level of homocysteine, but high and low homocysteine levels are also recorded in diabetic patients (25); it is recognized previously that each of DM, HHcy, and COVID-19 is associated with vascular inflammation and injury, thus patient has all these three vascular risk factors will be at high risk of developing cardiovascular disease due to their synergistic effect (26).

The homocysteine level in COVID-19 patients groups revealed a relationship between the level of this amino acid and the type of morbidity; if the COVID-19 patients have DM with obesity their homocysteine was significantly different from the healthy group and this difference was decreased when homocysteine level of CDO group was compared to CO group and the least difference when it compared to CD group; this result reflects the effect of DM on an elevation of homocysteine level is higher than obesity or healthy people, and obesity has a more negative effect on homocysteine level than in healthy people. DM may affect the homocysteine level by inducing homocysteine biosynthesis enzyme/enzyme dysfunction, another possible cause is drugs that are used for the management of DM, and their side effect may cause hyperhomocysteinemia (27).

On the other hand, obesity alone may affect homocysteine levels as obese patients may have a reduced in methylenetetrahydrofolate reductase enzyme which in turn important to produce 5-methyltetrahydrofolate, the crucial vitamin in the conversion of homocysteine into methionine (28), thus hyperhomocysteinemia may be a feature in some obese patient even without COVID-19 due to effect of obesity on homocysteine metabolism (29). The present study showed that a COVID-19 patient with DM and obesity at the same time has more effect on homocysteine level than if one of them is present in the COVID-19 patient, thus multiple comorbidities in the same COVID-19 patient cause a significant elevation in homocysteine level. The findings of the current study revealed no significant differences in homocysteine levels in the COVID-19 DM group or COVID-19 obesity group, thus the effect of DM on homocysteine levels in COVID-19 patients is comparable to obesity.

In the current study, the mean homocysteine level in the healthy group was significantly higher in females than males which is against the results of Mayer O Jr, et al (30); actually, men need more folate than women to proceed with the folate concentration in RBCs due to higher body mass in men, so relatively men are more prone to develop folate deficiency than women, the folate deficiency will affect the homocysteine metabolism causing higher homocysteine concentration in men, Cohen E, et al, did a large cross-sectional study and found that plasma homocysteine level and its prevalence was higher in males than females among the population (31).

For the CDO group and CD group, the means homocysteine levels for males were significantly higher for males than females, this is the first study that compared gender differences in homocysteine levels in COVID-19 patients with DM and obesity; during searching in the literature, no similar study was found.

Kazuhiro Oishi, et al (32), found higher homocysteine levels in diabetic patients compared with non-diabetic patients but did not include gender and COVID-19 as risk factors for hyperhomocysteinemia, it seems that the level of serum homocysteine in the presence of multiple factors like gender, COVID-19, DM, vitamin B12 and folate concentrations, diabetic control, and different medications for DM and COVID-19 in single male or female make it difficult to appoint the exact risk of hyperhomocysteinemia.

The mean homocysteine levels remained within the normal level in all age groups in healthy adults with the highest mean value in age group >70 years (10.37±1.39µmol/l); Hsu-Ko Kuo, et al (33) did a systematic review on homocysteine and found an increase in homocysteine level with an increase in an age which is in accordance to findings in this study, while in COVID-19 patients, the current study revealed a highest mean level equal to 21.39±6.02µmol/l in the age group 40-55 years in CO group, in CD group the highest was in 56-70 years old (25.62±23.54), and in CDO group in age group >70 years old (35.68±23.08); these findings showed an increase in mean homocysteine level with an increase in age among COVID-19 patients with different comorbidities.

The results revealed a strong positive correlation between homocysteine and D-dimer this reflects the high thromboembolic situation in COVID-19 and may have bad complications in COVID-19 patients as both two markers in favor and reflect thrombus formation in the blood vessels. This concomitant elevation in homocysteine and D-dimer can be used to assess the patient's condition and the COVID-19 severity. The findings of Adem Keskin, et al, were like the results of the current study (20); while, Fridon Todua, et al, found a positive correlation between homocysteine and D-dimer in patients with pulmonary embolism (34).

The homocysteine level in the current study had a negative correlation with serum folate; the mechanism behind this result is related to the metabolic association between them, as folate is needed in homocysteine metabolism for the conversion of homocysteine to methionine, so if there is a decline in folate levels as described in some COVID-19 patients in the current study, there will be an accumulation of homocysteine (35).

The association between homocysteine level and vitamin B-12 is moderately negative. Vitamin B-12 is important in homocysteine metabolism so a decrease in vitamin B-12 is associated with an increase in homocysteine (36). Shakoor H, et al, (37) recorded results in line with the findings of this study.

The homocysteine had a positive weak correlation with IL-6, this positive association is expected as COVID-19 is associated with the inflammatory process which is manifested by elevated IL-6 and homocysteine; Mu, ZJ., et al, reported a comparable association between homocysteine and IL-6 among elderly people (38).

The ROC was utilized to clarify the importance of homocysteine as a serological diagnostic marker in COVID-19 infection; the potential predictive power was improved when all COVID-19 study groups were included, while when studied separately, the homocysteine was having the pest predictive value in the CDO study group, followed by CD, then CO groups. In line with current results, Ponti G, et al, concluded that homocysteine is an important

predictor of cardiovascular complications in COVID-19 (39). When the sensitivity and specificity of the homocysteine test in the COVID-19 study, groups were compared to each other, the best way in the CDO group, followed by the CD group, then the COa group, these findings indicate that the severity of a patient's condition and the severity of comorbidity is associated with homocysteine test sensitivity and specificity.

In COVID-19 patients, comorbidity like obesity or DM is associated with a high level of homocysteine and both DM and obesity have a strong relationship with hyperhomocysteinemia, and their presence in the same COVID-19 patient has a synergistic effect on the elevation of homocysteine levels. The high homocysteine level in COVID-19 tends to be associated with a high level of D-dimer, a high level of IL-6, and a low level of folate and vitamin B-12. Serum homocysteine has potential predictive power in COVID-19 patients, and the severity of COVID-19 infection and the type of comorbidity is associated with higher sensitivity and specificity of homocysteine serological tests.

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Interest conflict
The authors have no conflicts of interest to declare.

References

1. Ziaee A, Zakeri H, Johari Moghadam MM, Hejrati A, Eskandari D. Medical and Pharmacological Evaluation of Hyperlipidemia and Lipid Profile Status in Iranian Patients with Coronary Artery Disease. J Med Chem Sci 2022;5:413–21.
2. Veeranki S, Gandhapudi SK, Tyagi SC. Interactions of hyperhomocysteinemia and T cell immunity in causation of hypertension. Can J Physiol Pharmacol 2016;95:239–46.
3. Shen S, Mao Y, Shen H, Wang Q, Tian X. Correlation of serum homocysteine, cystatin c and uric acid and cerebral circulation dynamics in patients with h-type hypertension. Acta Medica Mediterr 2021;37:3051–5.
4. Malinow MR, Bostom AG, Krauss RM. Homocyst (e) ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation 1999;99:178–82.
5. Smith AD, Smith SM, De Jager CA, Whitbread P, Johnston C, Agacinski G, et al. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PLoS One 2010;5:e12244.
6. Lee M, Hong K-S, Chang S-C, Saver JL. Efficacy of homocysteine-lowering therapy with folic acid in stroke prevention: a meta-analysis. Stroke 2010;41:1205–12.
7. Li Y, Huang T, Zheng Y, Muka T, Troup J, Hu FB. Folic acid supplementation and the risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. J Am Heart Assoc 2016;5:e003768.
8. Morris AAM, Kožich V, Santra S, Andria G, Ben-Omran TIM, Chakrapani AB, et al. Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency. J Inherit Metab Dis 2017;40:49–74.
9. Park W-C, Chang J-H. Clinical implications of methylenetetrahydrofolate reductase mutations and plasma homocysteine levels in patients with thromboembolic occlusion. Vasc Spec Int 2014;30:113.
10. Ali Ramaji G, Nazemi A. Development of ARMS-PCR method to detect two mutations of MTHFR gene(C677T,A1298C)in suspected cases of thrombosis. Int J Adv Biol Biomed Res 2020;8:268–83.
11. Zhang H, Li W, Hu F, Hao D, Sun Q. Relationship between mthfr polymorphism, mitochondrial function, and susceptibility to acute lymphoblastic leukemia in children. Acta Medica Mediterr 2021;37:3227–33.
12. Hirschowitz BI, Worthington J, Mohnen J. Vitamin B12 deficiency in hypersecretors during long-term acid suppression with proton pump inhibitors. Aliment Pharmacol Ther 2008;27:1110–21.
13. Son P, Lewis L. Hyperhomocysteinemia., Treasure Island (FL): 2022.
14. Carpenè G, Negrini D, Henry BM, Montagnana M, Lippi G. Homocysteine in coronavirus disease (COVID-19): a systematic literature review. Diagnosis 2022.
15. Dietrich-Muszalska A, Malinowska J, Olas B, Głowacki R, Bald E, Wachowicz B, et al. The oxidative stress may be induced by the elevated homocysteine in schizophrenic patients. Neurochem Res 2012;37:1057–62.
16. Han S, Kong F, Han B, Wang Z, Chen Q, Zhao Z, et al. The relationship between serum homocysteine levels, inflammatory response and plaque stability in patients with h-type hypertension and carotid atherosclerosis. Acta Medica Mediterr 2022;38:3065–70.
17. Škovierová H, Vidomanová E, Mahmood S, Sopková J, Drgová A, Červeňová T, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci 2016;17:1733.
18. Kim J, Kim H, Roh H, Kwon Y. Causes of hyperhomocysteinemia and its pathological significance. Arch Pharm Res 2018;41:372–83.
19. Hu Z, Ye J, Wu W, Yi Y, Chen L, Li R, et al. Mir-21/pten and mir-21/pdcd4 signaling pathways mediate homocysteine induced heart failure. Acta Medica Mediterr 2022;38:2263.
20. Keskin A, U Ustun G, Aci R, Duran U. Homocysteine as a marker for predicting disease severity in patients with COVID-19. Biomark Med 2022;16:559–68.
21. Wang J, You D, Wang H, Yang Y, Zhang D, Lv J, et al. Association between homocysteine and obesity: a meta-analysis. J Evidence-Based Med 2021;14:208–17.
22. Liu C, Liu L, Wang Y, Chen X, Liu J, Peng S, et al. Hyperhomocysteinamia Increases Risk of Metabolic Syndrome and Cardiovascular Death in an Elderly Chinese Community Population of a 7-year Follow-up Study Short Title: HHcy/HWC and MetS/CVD Death in Elderly Chinese. Front Cardiovasc Med 2021:2193.
23. Fu S, Yao Y, Zhao Y, Luan F. Relationships of hyperhomocysteinemia and hyperuricemia with metabolic syndrome and renal function in chinese centenarians. Front Endocrinol (Lausanne) 2018;9:502.
24. Meisel E, Efros O, Bleier J, Beit Halevi T, Segal G, Rahav G, et al. Folate levels in patients hospitalized with coronavirus disease 2019. Nutrients 2021;13:812.
25. van Guldener C, Stehouwer CDA. Diabetes mellitus and hyperhomocysteinemia. Semin. Vasc. Med., vol. 2, Copyright© 2002 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New …; 2002, p. 87–96.
26. Wee AKH. COVID-19's toll on the elderly and those with diabetes mellitus–is vitamin B12 deficiency an accomplice? Med Hypotheses 2021;146:110374.
27. Afriyie-Gyawu E, Ifebi E, Ampofo-Yeboah A, Kyte B, Shrestha S, Zhang J. Serum folate levels and fatality among diabetic

adults: A 15-y follow-up study of a national cohort. Nutrition 2016;32:468–73.
28. Becker A, Smulders YM, Van Guldener C, Stehouwer CDA. Epidemiology of homocysteine as a risk factor in diabetes. Metab Syndr Relat Disord 2003;1:105–20.
29. Fu L, Li Y, Luo D, Deng S, Hu Y-Q. Plausible relationship between homocysteine and obesity risk via MTHFR gene: a meta-analysis of 38,317 individuals implementing Mendelian randomization. Diabetes, Metab Syndr Obes Targets Ther 2019;12:1201.
30. Mayer O J, Simon J, Rosolová H. Pohlavní rozdíly v sérových hladinách homocysteinu a asociovaných faktorech [Gender differences in serum homocysteine levels and associated factors]. Cas Lek Ces 1999;138:525–7.
31. Cohen E, Margalit I, Shochat T, Goldberg E, Krause I. Gender differences in homocysteine concentrations, a population-based cross-sectional study. Nutr Metab Cardiovasc Dis 2019;29:9–14.
32. Oishi K, Nagake Y, Yamasaki H, Fukuda S, Ichikawa H, Ota K, et al. The significance of serum homocysteine levels in diabetic patients on haemodialysis. Nephrol Dial Transplant 2000;15:851–5.
33. Kuo H-K, Sorond FA, Chen J-H, Hashmi A, Milberg WP, Lipsitz LA. The role of homocysteine in multisystem age-related problems: a systematic review. Journals Gerontol Ser A Biol Sci Med Sci 2005;60:1190–201.
34. Todua F, Akhvlediani M, Vorobiova E, Tsivtsivadze G, Baramidze A. Homocysteine and D-dimer levels and multilayer computed tomography for diagnosing pulmonary artery thromboembolism. Vessel Plus 2017;1:38–42.
35. Malahayati I, Serudji J, Sulastri D. Correlation between Folic Acid and Homocysteine Plasma in Severe Pre-Eclampsia and Normal Pregnancy. Makara J Heal Res 2018:74–9.
36. Mikkelsen K, Stojanovska L, Prakash M, Apostolopoulos V. The effects of vitamin B on the immune/cytokine network and their involvement in depression. Maturitas 2017;96:58–71.
37. Shakoor H, Feehan J, Mikkelsen K, Al Dhaheri AS, Ali HI, Platat C, et al. Be well: A potential role for vitamin B in COVID-19. Maturitas 2021;144:108–11.
38. Mu Z-J, Fu J-L, Sun L-N, Chan P, Xiu S-L. Associations between homocysteine, inflammatory cytokines and sarcopenia in Chinese older adults with type 2 diabetes. BMC Geriatr 2021;21:1–9.
39. Ponti G, Ruini C, Tomasi A. Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19. Med Hypotheses 2020;143:109859.