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COVID-19 Hypercoagulability Association with Serum Levels of Homocysteine and SCUBE-1

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ABSTRACT

Background: Hypercoagulable state is a major complication of the coronavirus disease-2019 (COVID-19), with a possible result of debility and/or mortality. Homocysteine and SCUBE-1 are plasma biomarkers; their abnormal levels are relatable to coagulation as a cause or an effect.
Objectives: To investigate the association of homocysteine and SCUBE-1 with COVID–19–associated hypercoagulability.
Materials and methods: This is a cross-sectional study with ninety adult COVID-19 patients with variable severity. Patients were classified according to D-dimer level at the time of hospital admission into two groups: with and without hypercoagulability. Serum was extracted from centrifuged blood (collected in gel tubes) and stored at -20 C. Serum levels of homocysteine and SCUBE-1 were measured utilizing Chemiluminescense Immunoassay and Enzyme-Linked Immunosorbent Assay, respectively, using commercially available kits.
Results: Thirty-eight patients (42.22%) out of the ninety had a hypercoagulable state, and the vast majority of patients with hypercoagulability (89.47%) had severe disease. The median (IQR) levels of homocysteine and SCUBE-1 in patients with hypercoagulability were 9.56 (8.75) mol/L and 0.19 (0.11) ng/ml, respectively, which were higher than that of normal coagulable patients (8.15 [5.85] mol/L and 0.16 [0.06] ng/ml, respectively) with highly significant differences (P-value = 0.044 and 0.01, respectively).
Conclusion: Homocysteine and SCUBE-1 serum levels are significantly associated with COVID-19 hypercoagulability and disease severity, and may be utilized as adjunct biomarkers for prediction/diagnosis of hypercoagulability in COVID-19 patients.
Keywords: COVID-19; Hypercoagulability; Homocysteine; SCUBE-1.

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INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) is a highly contagious illness caused by a new strain of coronavirus called Severe Acute Respiratory Syndrome-Coronavirus Disease 2 (SARS-CoV-2) of the betacoronavirus genus [1]. It was first reported as an outbreak of pneumonia in Wuhan, China in late December 2019, and rapidly spread across the world to be declared as a global pandemic by World Health Organization (WHO) on March 11, 2020. Since then, COVID-19 has wreaked havoc on different nations, strained numerous healthcare systems, and caused livelihood losses on account of protracted closures, which have had a ripple impact on the world economy [2].

Symptoms of SARS-CoV-2 may appear 2-14 days after exposure to the virus [3]. The most common symptoms are fever, cough, dyspnoea, nausea, vomiting, abdominal pain, diarrhea, and anorexia [4].

Many pulmonary and extra-pulmonary complications have been reported in the severe-critical spectrum [5], and hypercoagulability is one of the morbid complications. It may manifest as pulmonary embolism (PE), deep venous thrombosis (DVT), myocardial infarction (MI), ischemic strokes, or arterial thrombosis [6].

Homocysteine is a non-essential amino acid, not supplied by diet. It is a metabolite of the essential amino acid methio-

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nine and exists at a critical biochemical intersection in the methionine cycle - between S-adenosylmethionine, the indispensable ubiquitous methyl donor, and vitamins B12 and folic acid. Hyperhomocysteinemia is associated with an increased risk of thrombosis [7].

SCUBE-1 is a member of the secreted and membrane-anchored SCUBE protein family. It is highly expressed in human platelets; in addition, it plays an important role in inflammation and thrombosis [8].

Prediction and diagnosis of COVID-19 hypercoagulability can be challenging [9]. In addition, the association of high serum levels of homocysteine and SCUBE-1 with arterial/venous thrombosis remains controversial [10, 11].

Hence, we conducted the current study to evaluate the association of homocysteine and SCUBE-1 with COVID-19 hypercoagulability as possible predictive criteria for unfavorable outcomes.

MATERIALS AND METHODS

Study Design and Population

This is a cross-sectional study, which was conducted at Al-Nahrain University/ College of Medicine/ Research Medical Unit and Medical City/ National Centre of Teaching Laboratories, Baghdad, Iraq. The current study covered the period from the 1st of March to the 2nd of October 2022. The study was approved by the Institute Review Board (the local Ethical Committee of the College of Medicine, Al-Nahrain University, No. 99/M.M.M, Date 31/1/2022). All participants were informed about the study and their consent was taken before any sample or data collection had taken place.

All adult patients who were confirmed with SARS-CoV-2 infection were included in the study. Patients younger than 18 years old, who decline to participate, or those on anticoagulant treatment started within 48 hours before sampling were excluded. Demographic and clinical characteristics of the patients including age, gender, body mass index (BMI), and comorbidities were collected in preformed questionnaires. Patients disease severity status was defined according to World Health Organization as the following:

- Mild disease: symptomatic patients without evidence of viral pneumonia or hypoxia.
- Moderated disease: patients with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia, including SpO2 $\geq$ 90% on room air.
- Severe disease: patients with clinical signs of pneumonia plus one of the following: respiratory rate $\geq$ 30 breaths/min; severe respiratory distress; or SpO2 $<$ 90% on room air.
- Critical disease: patients with pneumonia plus the new onset of acute respiratory distress syndrome (ARDS) within 1 week, sepsis, septic shock, or acute thrombosis [12].

Furthermore, the serum D-dimer values at the time of admission were obtained from patient's records, and according to these values, patients were classified into two groups, with and without hypercoagulability. Hypercoagulable status was defined as $>$ 1.5 times increase in D-dimer serum level beyond the upper normal limit [220500 ng/mL] based on a previous study [13].

Methods

The study used samples of 1 milliliter of a preserved serum at -20°C from a previous study by authors [14]. Ready commercial kits (Human Homocysteine Kits/Shijiazhuang Hipro Biotechnology/ China; Human Signal peptide, CUB domain, and EGF-like domain-containing protein 1 (SCUBE-1) ELISA Kit/ YL Biont/ China) were used for measuring serum levels of homocysteine and SCUBE-1, respectively, according to manufacturers instructions.

Statistical Analysis

Statistical analyses were carried out utilizing Statistical Package for the Social Sciences (SPSS) software version 25 (SPSS, Chicago, IL, USA). The normality test was applied to continuous data (Shapiro-Wilk test). Data were reported as numbers and percentages, means and standard deviations, and/or medians and interquartile ranges (IQR) depending on their distribution (normal or non-normal). Student t-test and Mann Whitney U test were used to evaluate data with a normal distribution and a non-normal distribution, respectively. The receiver operating characteristic curve (ROC) was used to evaluate homocysteine and SCUBE-1 in the context of the detection of hypercoagulability. Spearman's correlation test was used to explore the possible correlation of homocysteine and SCUBE-1 with other variables. A statistically significant difference was determined to exist when the P-value was less than 0.05.

RESULTS

Demographic and Clinical Characteristics of the Patients

The mean ± SD of the age of the patients was 48.81 ± 17.67 years (median [IQR] = 45.0 [33.0] years). The majority of patients (71.1%) were males. Patients mean ± SD BMI was 25.17 ± 4.18 kg/m^2 (median [IQR] = 25.0 [6.0] kg/m^2). The minority of patients had type-2 diabetes mellitus (T2DM) and hypertension (25.6% and 18.9%, respectively) as indicated in Table 1.

D-dimer, Homocysteine, and SCUBE-1

It was observed that the parameters values were not regularly distributed. The median (IQR) levels of D dimer, homocysteine, and SCUBE-1 were 631.5 (3184.25) ng/mL, 8.9 (7.27) mol/L, and 0.17 (0.07) ng/mL, respectively (Table 2).

Table 1. Demographic and clinical data of the study population (N=90) *.

Variable	Value
Age, years	
Mean ± SD	48.81 ± 17.67
Median (IQR)	45.0 (33.0)
Gender	
Male	64 (71.1%)
Female	26 (28.9%)
BMI, kg/m^2	
Mean ± SD	25.17 ± 4.18
Median (IQR)	25.0 (6.0)
Comorbidities	
Type 2 diabetes mellitus	23 (25.6%)
Hypertension	17 (18.9%)

* SD = Standard Deviation; IQR = Interquartile Range; BMI = Body Mass Index.

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Table 2. Inflammatory and coagulation biomarkers of the study population (N = 90) *.

Variable	Mean ± SD	Median	IQR
D-dimer, ng/mL	2041.2 ± 2575.5	631.5	3184.25
Homocysteine,μmol/L	10.1 ± 5.39	8.9	7.27
SCUBE-1, ng/mL	0.27 ± 0.32	0.17	0.07

* SD = Standard Deviation; IQR = Interquartile Range .

Hypercoagulability State

Out of the 90 patients with COVID-19 infection, 38 patients (42.22%) had a hypercoagulable state, while the other 52 patients (57.78%) had a normal coagulable state.

Association of Demographic and Clinical Characteristics with Hypercoagulability

No significant differences were found between the hypercoagulability state and each of age, gender, BMI, and T2DM (P-values: 0.361, 0.155, 0.837, and 0.263, respectively). However, the vast majority of patients with hypercoagulability (89.47%) had a severe disease as compared with only 11.54% of patients with normal coagulability, with a significant difference (P-value = 0.001). Furthermore, hypertension was significantly (P-value = 0.002) more common among patients with hypercoagulability (34.21%) than those with normal coagulability (7.69%) as shown in Table 3.

Association of Homocysteine and SCUBE-1 with Hypercoagulability

The median (IQR) level of homocysteine in patients with hypercoagulability was 9.56 (8.75) μmol/L which was higher than that of patients with normal coagulability (8.15 [5.85] mol/L) with a significant difference (P-value = 0.044). Similarly, the median (IQR) level of SCUBE-1 in hypercoagulable patients was 0.19 (0.11) ng/mL which was higher than that of patients with normal coagulable state (0.16 [0.06] ng/mL) with a significant difference (P-value = 0.01).

Table 3. Association of demographic and clinical characteristics with hypercoagulability *.

Variable	Hypercoagulability		P-value
	No(n=52)	Yes(n=38)	
Age, years			0.361
Mean ± SD	44.71±16.99	54.42±17.23	
Median (IQR)	43.0 (25.0)	57.0 (31.0)	
Gender			0.155
Male	40 (76.2%)	24 (63.16%)	
Female	12 (23.08%)	14 (36.84%)	
BMI, kg/m^2			0.837
Mean ± SD	25.53±4.2	24.68±4.17	
Median (IQR)	25.0 (6.0)	23.0 (5.5)	
Disease severity			0.001
Severe	6(11.54%)	34 (89.47%)	
Mild-moderate	46 (88.46%)	4 (10.53%)	
Comorbidities			
Type 2 diabetes mellitus	11 (21.15%)	12 (31.58%)	0.263
Hypertension	4 (7.69%)	13 (34.21%)	0.002

* SD = Standard deviation; IQR = Interquartile range; BMI = Body mass index.

Predictive Value of Homocysteine and SCUBE-1 for Hypercoagulability in Patients with COVID-19

The receiver operating characteristic curve (ROC) was used to evaluate the value of homocysteine and SCUBE-1 in predicting hypercoagulability in patients with COVID-19.

For homocysteine, the area under the curve (AUC) was 0.625 (95% CI = 0.504-0.746, P-value = 0.044). The sensitivity and specificity of the test at a cut-off value of homocysteine = 8.9 mol/L were 58% and 56%, respectively.

For SCUBE-1, the AUC was 0.66 (95% CI = 0.546-0.722, P-value = 0.010). The sensitivity and specificity of the test at a cut-off value of SCUBE-1 = 0.17 ng/mL were 66% and 60%, respectively (Figure 1).

Correlation of Homocysteine and SCUBE-1 with Other Variables

Both homocysteine and SCUBE-1 demonstrated a positive significant correlation with D–dimer (P-value = 0.300, P-value= 0.004; P-value = 0.217, P-value = 0.040, respectively) as shown in Table 4.

Association of Demographic and Clinical Data with Homocysteine and SCUBE-1 LEVEL

Gender, T2DM, and hypertension had no significant association with homocysteine (P-values: 0.786, 0.097, and 0.335, respectively) or SCUBE-1 levels (P-values: 0.807, 0.904, and 0.951, respectively). However, the median (IQR) levels of homocysteine and SCUBE-1 in patients with severe disease were 10.35 (10.28) μmol/L and 0.19 (0.12) ng/mL, respectively, which were higher than that of patients with mild/moderate disease (8.15 [5.6] μmol/L and 0.16 [0.06] ng/mL, respectively) with significant differences (P-values: 0.0017 and 0.003, respectively) as shown in Table 5.

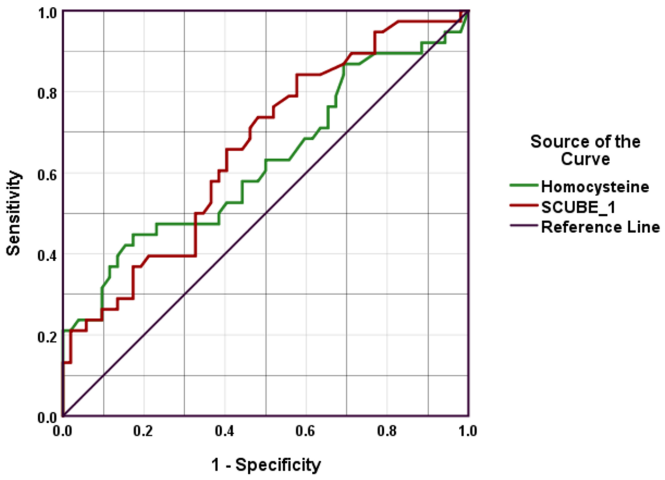

Figure 1. Receiver operating characteristic curve for homocysteine and SCUBE-1 in predicting hypercoagulability in patients with COVID-19.

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Table 4. Pearson's and Spearman's correlation of demographic and clinical data, and inflammatory and coagulation biomarkers with homocysteine and SCUBE-1 levels in patients with COVID-19 *.

Variable		Homocysteine level, μmol/L	SCUBE-1, ng/mL
Age, years	r	0.127	0.103
	p	0.233	0.334
BMI, kg/m^2	r	-0.143	0.106
	p	0.178	0.332
Dimer, ng/mL	r_s	0.300	0.217
	p	0.004	0.040
SCUBE-1, ng/mL	r_s	0.192	
	p	0.067	

* r = Pearsons Coefficient; ρ = Spearmans Coefficient; BMI = Body Mass Index.

Table 5. Association of demographic and clinical data with homocysteine and SCUBE-1 levels in patients with COVID-19 *.

Variable	Homocysteine level, μmol/L	SCUBE-1, ng/mL
Gender		
Male	8.5 (8.0)	0.17 (0.08)
Female	9.4 (5.95)	0.17 (0.04)
P-value	0.786	0.807
Type 2 diabetes mellitus		
Yes	10.1 (9.6)	0.17 (0.1)
No	8.3 (6.7)	0.17 (0.07)
P-value	0.097	0.904
Hypertension		
Yes	9.2 (9.5)	0.18 (0.08)
No	8.8 (7.1)	0.17 (0.07)
P-value	0.335	0.951
Disease Severity		
Mild/moderate	8.15 (5.6)	0.16 (0.06)
Severe	10.35 (10.28)	0.19 (0.12)
P-value	0.0017	0.003

* All data are given as median (IQR).

DISCUSSION

COVID-19 predisposes patients to thrombotic/ thromboembolic events with a relatively high prevalence and can be fatal [15]. The prediction and diagnosis of these events (e.g. PE, DVT) can be challenging because symptoms of PE overlap with that of COVID-19, and imaging studies may not be feasible in all instances and cases. Moreover, some laboratory parameters are not specific to venous thromboembolisms (VTEs), like D-dimer, which can be elevated by inflammation itself. The present study revealed an association of each of homocysteine and SCUBE-1 with COVID-19 hypercoagulability, which can be utilized as adjunct markers for predicting/diagnosing hypercoagulability in COVID-19 patients [9].

To the best of our knowledge, this is the first study to investigate the role of SCUBE-1 in COVID-19 patients in Iraq, and the second on a worldwide level after the Toprak et al. study [16]. According to the result of this study, hypercoagulability in COVID-19 patients is significantly associated with elevated serum levels of homocysteine and SCUBE-1.

For homocysteine, the above-mentioned result harmonizes with the work of Vuckovic et al. on 65 patients with either arterial or venous thrombosis and 65 controls, where they found that cases had significantly higher mean ± SD homocysteine levels than controls (12.3 ± 4.94 μmol/L vs 9.2 ± 3.68 mol/L; P-value < 0.001) [17]. Additionally, Aday and colleagues conducted a study on two prospective cohorts of women from two previously completed randomized trials (WHS and WAFACS). From the WHS study, 27,555 women ≥ 45 years old and free of cardiovascular disease and VTE were assessed for the association of homocysteine and other thrombotic biomarkers with future VTE (n = 743), PE (n = 363), and DVT (n = 545). From the WAFACS study, 2,672 women (of whom 102 had VTE events) were assessed. They reported that high homocysteine levels were associated with unprovoked VTE in both cohorts [18], which is accordant with this study's result.

Regarding SCUBE-1, Toprak and colleagues conducted a study on 553 COVID-19 patients and 553 healthy controls and observed that patients with COVID-19 had significantly greater SCUBE-1 levels in the group with thrombotic outcomes compared to the group with no thrombotic outcomes (P-value < 0.001) [16], which is in agreement with our study's result.

The current study revealed a significant predictive value of each of homocysteine and SCUBE-1 for hypercoagulability in patients with COVID-19; in addition, sensitivity and specificity for both were reported. In the study of Chen et al. on 26 patients with DVT after operation for lower limb fracture and 49 controls in China, the AUC value was 0.7804 for homocysteine, with sensitivity and specificity of 76.92% and 71.44%, respectively, at a cut-off value of 9.54 μmol/L. They concluded that homocysteine could predict DVT in patients who underwent surgery for lower limb fracture [19].

In another cross-sectional study by Yao and co-authors, homocysteine was evaluated in 888 Chinese patients with non-valvular atrial fibrillation and CHA2DS2-VASc score of 0 and 1 of whom 32 patients had left atrial/left atrial appendage thrombus. Homocysteine showed a significant predictive value with an AUC of 0.722 (P-value < 0.009), a sensitivity of 67%, and a specificity of 65% for an optimal cut-off value of 13.5 μmol/L. They concluded that homocysteine should be taken into account in the prediction of thromboembolism [20].

In Toprak et al. study, 30 out of 553 COVID-19 developed thrombotic complications. The optimal cut-off value for SCUBE-1 was calculated to be 2.30 ng/mL, with an AUC of 0.967 (P-value < 0.001) according to thrombotic complications. The predictive values of SCUBE-1, D-dimer, and fibrinogen for thrombotic complications were compared pairwise. While there was no statistically significant difference between SCUBE-1 and D-dimer (P-value = 0.3601), there was a significant difference between SCUBE-1 and fibrinogen (P-value = 0.0044); sensitivity and specificity were not reported in their study [16]. Additionally, Xiao and colleagues conducted a study on 177 patients who underwent computerized tomography pulmonary angiography (CTPA), of whom 143 were confirmed with PE diagnosis (PE group), 34 with negative CTPA test (PE suspicious), and an additional 89 healthy controls. ROC analysis showed that at the cut-off of 7.789 ng/mL, SCUBE-1 has significant diagnostic value in differentiating PE patients from healthy control (AUC=0.919,

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sensitivity=81.25%, specificity=92.13%) but not the PE patients from suspicious PE group [21].

In comparison to the aforementioned studies, the current study shows relatively lower values for homocysteine and SCUBE-1, which may be attributed to the effect of the lower sample size and the definition of a hypercoagulable" patient that was used in our study.

Moreover, the present study reports a significant correlation of D-dimer level with each of homocysteine and SCUBE-1, which harmonizes with the results of numerous worldwide studies. Todua et al. conducted a case-control study on levels of homocysteine, D-dimer, and multilayer computerized tomography (CT) for diagnosing pulmonary artery thromboembolism, 53 patients with 27 healthy controls were included. They reported a significant positive correlation between homocysteine and D-dimer levels (r = 0.557) [22].

Homocysteine is associated with vascular inflammation, and this association with large vessels has been confirmed in cardiovascular disease, stroke/cardiovascular accident, T2DM, hypertension, and metabolic syndrome [23]. SARS-CoV-2 infection can lead to elevated levels of homocysteine. Although there is limited information explaining this rise, it possibly can be attributed to the reshaping of cellular metabolism as the virus hijacks it, and to the redirection of cellular resources to fulfill the viral RNA synthesis, which requires de novo purine biosynthesis involving folate and one-carbon metabolism. Many aspects of host sulfur amino acid metabolism, especially glutathione metabolism underlying antioxidant defenses, are also taken over by the SARS-CoV-2 virus. One of the consequences is depleted glutathione (the most important antioxidant in human physiology) and increased SAM and homocysteine levels [24]. Elevated homocysteine level causes injury to endothelium and, consequently, activates the coagulation cascade through different pathways [25]. This may be a possible explanation for the increased levels of homocysteine in COVID-19 patients with hypercoagulable states.

Regarding SCUBE-1, a retrospective study by Mentee and colleagues on 205 Turkish patients with Crimean-Congo hemorrhagic fever displayed a positive correlation between SCUBE-1 and each of the D-dimer and LDH values [26]. Toprak et al. study reported a similar correlation [16] SCUBE-1 is highly expressed in human platelets playing an important role in inflammation and thrombosis. SCUBE-1 molecules are stored in alpha granules in inactivated platelets. After platelet activation by thrombin, these molecules are translocated to the platelet surface and released in the form of small, soluble particles to be incorporated into the thrombus [18, 27]. In addition, EGF-like domain repetitions of SCUBE-1 are capable of stimulating platelet-platelet interaction or supporting platelet-matrix adhesion in vitro, which is similar to the known roles of the EGF-like domains in facilitating homophilic protein-protein interactions and cell adhesion [28, 29]. This may explain the elevated serum levels of SCUBE-1 in COVID-19 patients with D-dimer levels and hypercoagulable states.

The current study reported a significant association between homocysteine and SCUBE-1 with severe disease status. Many studies' results are in agreement with this result. A systematic review was conducted by Carpen and colleagues on three studies totaling 694 hospitalized COVID-19 patients. Despite the limited evidence, the researchers concluded that elevated homocysteine levels seem to be a potentially useful biomarker for the prediction of adverse progression in COVID-19 patients because the difference between the mean homocysteine values in severe *vs.* non-severe COVID-19 patients was always significant in each one of the three studies [30]. Toprak and colleagues reported in their study that higher SCUBE-1 levels were associated with disease severity [16].

There are many limitations to the current study: First, the relatively small sample size, which can limit the generalizability of the results. Second, limited data access (e.g. insufficient information about the patient's daily lifestyle, eating habits and intake of vitamin B complex, other chronic diseases, and the chronic use of some medications, which can affect the coagulation activity, homocysteine metabolism, and serum levels). Third, the horizontal study design (cross-sectional) limits the ability to follow up with patients and construct temporal and cause-effect relationships between the variables (which is beyond the scope of this study). Finally, we could not control for every possible lifestyle factor, and the observational nature of this design leaves the possibility of residual confounding factors.

CONCLUSION

Hypercoagulability and D-dimer levels are significantly associated with higher serum levels of homocysteine and SCUBE-1 in patients with COVID-19. As of this moment, there is still no well-demarcated strategy to predict COVID-19 clinical progress accurately. The results of this study can be of supplementary value in practice, possibly by providing additional criteria that help in better classification and prediction of COVID-19 patients' clinical course and the customization of the best treatment regimen, which can lead eventually to more successful results. We recommend studies that are more extensive with longitudinal design ensuring follow-up of changes in inflammatory parameters and disease progression, larger sample size, and more detailed data about daily lifestyles and vitamin oral intake which affect homocysteine metabolism.

ETHICAL DECLARATIONS

Acknoweldgements

None.

Ethics Approval and Consent to Participate

Written approval had been gained from the Ethics Committee of the College of Medicine, Al-Nahrain University, Baghdad, Iraq. Study data/information was used for the research purpose only. Informed consent from every participant was taken.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

All the authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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