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Serum SCUBE-1 levels can predict endothelial dysfunction in healthy young adults

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ABSTRACT

Objectives: This study aims to investigate the predictive role of the signal peptide-CUB-EGF domain-containing protein-1 (SCUBE-1) in the presence of endothelial dysfunction.

Patients and methods: Between December 2014 and August 2015, 120 healthy young adults (68 males, 52 females; mean age 29.7±4.1 years; range, 20 to 35 years) without a previous history of cardiovascular disease were included. The participants were divided into two groups according to normal (n=73) and abnormal flow-mediated dilatation (FMD) response (n=47). Simultaneously, the standard biochemical markers, blood urea-nitrogen, creatinine, liver enzymes, glucose, lipids and SCUBE-1 levels were determined from the blood samples.

Results: Multivariable logistic regression revealed a strong association between SCUBE-1 and FMD ($p<0.001$). We found that for a cut-off point of >136.37 ng/mL, SCUBE-1 had 85.0% sensitivity and 81.2% specificity to identify abnormal FMD.

Conclusion: Our study findings indicate that there is a favorable inverse correlation between the SCUBE-1 and FMD.

Keywords: Atherosclerosis progression; endothelial dysfunction; flow-mediated vasodilatation; SCUBE-1.

Atherosclerosis is a systemic disorder which leads to important clinical outcomes with impaired blood supply to the vital tissues.[1,2] The first visible formation of atherosclerosis is fatty deposit in the tunica intima of arteries which develops in the early ages, even before birth.[1,2] Progression of fatty streaks results in endothelial dysfunction (ED) which is an early predictor of atherosclerosis and has a pivotal role in the atherogenesis process. In addition, ED is a preventable and reversible pathology, and timely diagnosis and management is critical to avoid irreversible arterial occlusions.[1-3]

Acetylcholine can be used for the assessment for ED invasively, which provokes endothelium-dependent dilation and smooth muscle-mediated constriction. Vessel diameters are angiographically compared before and after the infusion of acetylcholine.[3] Furthermore, non-invasive methods can be used for ED examination. Flow-mediated dilatation (FMD), which depends on vasodilatory response of an artery to the increase in the blood flow-associated shear stress, can be used to assess ED via the brachial artery.[4,5] It is a qualified, less subjective, and cost-effective method for the evaluation of atherosclerosis and associated complications.[5,6]

The signal peptide-CUB-EGF domain-containing protein-1 (SCUBE-1) is a recently available biomarker which is expressed by activated and adhered platelets and identified in human vascular endothelial cells.[7] Increased SCUBE-1 has been shown to be associated with acute ischemia in response to endothelial injury.[8] Although the underlying mechanism is still unclear,

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immunohistochemically SCUBE-1 deposits can be identified in the sub-endothelial matrix of the atherosclerotic plaques.[8,9] Similarly, nitric oxide (NO) is an endothelial-released ischemic mediator which is responsible for the endothelium-dependent relaxation. Also, NO regulates the arterial wall homeostasis and, in the absence of this regulatory mechanism, endothelium becomes dysfunctional.[6] In addition, FMD is used in the evaluation of this impaired homeostasis.[4-6] Based on these findings, it can be suggested that SCUBE-1 and FMD may be closely associated and SCUBE-1 can be used for the evaluation of ED as FMD.

In the present study, we aimed to investigate the predictive role of SCUBE-1 and FMD for the assessment of ED in healthy young adults.

PATIENTS AND METHODS

Between December 2014 and August 2015, 120 healthy young adults (68 males, 52 females; mean age 29.7±4.1 years; range, 20 to 35 years) without a previous history of cardiovascular disease were included in this prospective study. Individuals having ED-related risk factors including smoking more than five years, familial hyperlipidemia, hypertension, diabetes, and obesity were excluded. The participants were divided into two groups according to normal (n=73) and abnormal FMD response (n=43). A written informed consent was obtained from each participant. The study protocol was approved by the Medical School of Dicle University Ethics Committee (No. 2014/337). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Blood samples were withdrawn from the antecubital vein following an overnight fasting. The samples were obtained by centrifugation at 3,000 rpm for 10 min before analysis. Thereafter, the standard biochemical markers, blood urea-nitrogen, creatinine, liver enzymes, glucose, lipids, and SCUBE-1 levels were measured.

Measurement of SCUBE-1

The measurement of SCUBE-1 levels was performed according to the method described by Turkmen et al.[10] Commercially available enzyme-linked immunosorbent assay kit (Catalog No. CSBE15005 h, Cusabio Biotech Co., Wuhan, Hubei, P.R. China) was used for the detection of the SCUBE-1 levels. The results were defined as a 0.16 ng/mL minimally detectable dose and were expressed in ng/mL.

Evaluation of FMD

Flow-mediated dilatation (FMD) was evaluated in all participants before blood sampling with an overnight fasting (water was permitted) in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C by a single sonographer according to the guidelines previously reported by Corretti et al.[11] Before the evaluation, all individuals were permitted to have 10-min rest. A linear array transducer with

Table 1. Comparison of main characteristic between groups

	Normally FMD ≥5% (n=73)			Abnormally FMD <5% (n=47)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			30.4±3.5			29.4±2.6	0.096
Smoking (less than 5 years)	12	16		13	27		0.063
Body Mass Index (kg/m²)			27.7±4.1			28.4±5.2	0.106
Systolic blood pressure (mmHg)			109.8±9.5			123.7±10.7	<0.001
Diastolic blood pressure (mmHg)			71.9±7.7			83.0±10.4	<0.001
Glucose (mg/dL)			92.4±24.0			105.2±11.3	0.096
Total cholesterol (mg/dL)			162.2±42.1			160.4±33.3	0.544
Triglyceride (mg/dL)			144.0±62.3			161.1±83.6	0.080
Low-density lipoprotein cholesterol (mg/dL)			96.7±31.5			110.4±15.9	0.148
High-density lipoprotein cholesterol (mg/dL)			32.4±6.6			30.2±5.2	0.248
Creatinine (mg/dL)			0.76±0.27			0.83±0.30	0.210
Hemoglobin (gr/dL)			14.9±2.1			14.6±3.7	0.936
SCUBE-1 (ng/mL)			81.7±18.16			198.1±31.42	<0.001

FMD: Flow-mediated dilatation; SD: Standard deviation; SCUBE1: Signal peptide-CUB-EGF domain-containing protein 1.

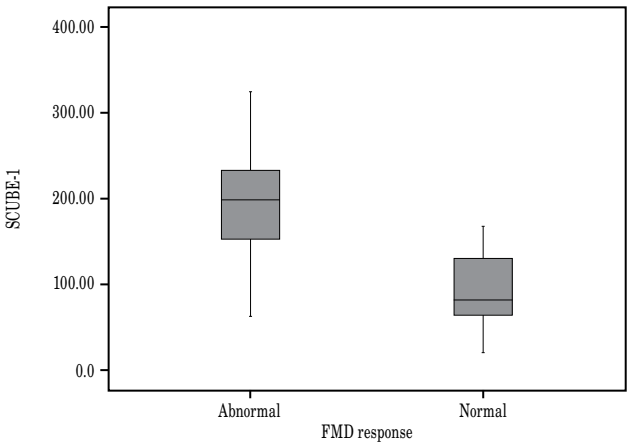


Figure 1. Comparison of SCUBE-1 levels and FMD response with box-plot graphic between two groups. FMD: Flow-mediated dilatation; SCUBE-1: Signal peptide-CUB-EGF domain-containing protein 1.

a frequency of 10 MHz (Vivid 4-Cardiovascular Ultrasound System, 10L transducer) was used to acquire the images. The baseline diameter of the brachial artery was measured ~5-cm above the antecubital fossa. Then, the sphygmomanometer cuff was placed above the antecubital fossa and the cuff was inflated to suprasystolic pressure (50 mmHg above systolic pressure) for 5 min to induce ischemia at the brachial artery. The brachial artery was evaluated on a longitudinal image continuously from 30 sec before to 2 min after cuff deflation and the percentage change was observed in the vessel diameter during reactive hyperemia. A FMD value of <5% was defined as an abnormal FMD response and ≥5% as a normal FMD response.[4,11]

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed in mean ± standard deviation (SD). Significant differences were analyzed using the Mann-Whitney U test. Multiple logistic regression and univariate analysis were performed to analyze abnormal FMD responses in dependent variables (p<0.20). The optimal cut-off point of SCUBE-1 (at which sensitivity and specificity would be maximal) for the prediction of abnormal FMD response was determined through the receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was calculated to identify the accuracy of the test. A p value of <0.05 was considered statistically significant.

RESULTS

The age distribution and Body Mass Index (BMI) values were similar between the groups. In addition both groups were similar in terms of smoking history. However, systolic and diastolic blood pressures were slightly higher in the abnormal FMD group (p<0.05). There were no statistically significant differences between two groups according to the standard blood markers (fasting glucose, total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, creatinine, and hemoglobin) (p>0.05). Demographic and clinical characteristics of both groups are shown in Table 1.

Higher SCUBE-1 levels were found in the abnormal FMD group (198.1±31.42 ng/mL), compared to the normal FMD group (81.7±18.16 ng/mL) (p<0.001). The SCUBE-1 remained the strongest independent predictor of abnormal FMD response (Figure 1), followed by systolic and diastolic pressures according to multiple logistic regression analysis (Table 2).

The optimal cut-off level of SCUBE-1 in detecting abnormal FMD and in predicting ED response was found to be >136.37 ng/mL with 85.0% sensitivity and 81.2% specificity (AUC 0.774, 95% CI: 0.711-0.854) (Figure 2).

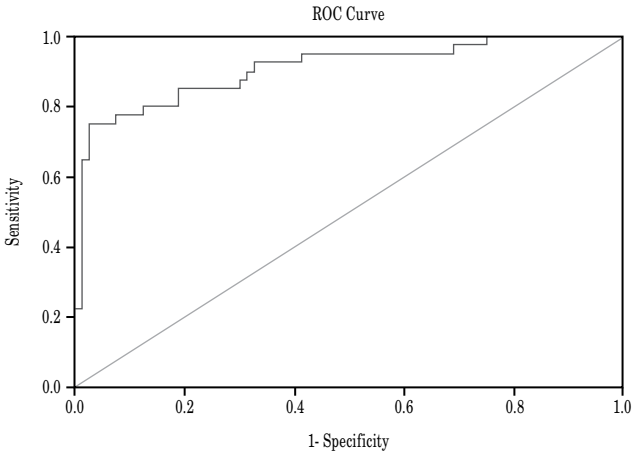
DISCUSSION

To the best of our knowledge, there is no study yet which defines what is the main role of SCUBE-1 in human atherosclerosis. The present study attempted to bridge a different horizon for this gap in the literature. The SCUBE-1 levels were found to be significantly

Table 2. Multiple logistic regression analysis for participants with abnormal flow-mediated dilatation

	p	OR	95% CI	Cut-off value
Systolic blood pressure (mmHg)	0.001	1.128	0.101-0.269	117.5
Diastolic blood pressure (mmHg)	0.001	1.048	0.125-0.313	77.5
SCUBE-1	<0.001	0.150	0.847-0.969	136.37

SCUBE-1: Signal peptide-CUB-EGF domain-containing protein 1.



Area under the curve				
Test result variable(s): SCUBE-1				
Area	Standard error*	Asymptotic significance†	Asymptotic 95% CI	
			Lower bound	Upper bound
0.908	0.031	0.000	0.847	0.969

SCUBE-1: Signal peptide-CUB-EGF domain-containing protein 1; * Under the non-parametric assumption; † Null hypothesis: true area= 0.5; CI: Confidence interval.

Figure 2. ROC curve analysis of SCUBE-1 values for predicting abnormal FMD responses. ROC: Receiver Operating Characteristic; SCUBE-1: Signal peptide-CUB-EGF domain-containing protein 1; FMD: Flow-mediated dilatation.

higher in the participants with ED. Moreover, the SCUBE-1 was found as an independent indicator of ED with high specificity and sensitivity. These results can be interpreted as a starting point for further comprehensive studies on atherosclerosis and ischemia with SCUBE-1.

In clinical practice, ED can be evaluated with different methods. However, some of them are invasive and difficult to access in all medical centers. Evaluation of endothelium-dependent vasodilator responses to specific agonists such as acetylcholine or shear stress which normally provoke vasodilation is one example for these methods. It can be infused directly to the coronary artery and response can be observed. However, it is an invasive and not easily repeatable method. Another method which is relatively fast and easy-to-use is the fingertip infrared light transmission photoplethysmography. Data from photoplethysmography can partially indicate cardiac risk factors; however, this method has unintended features such as low reproducibility and its ability to detect changes with intervention is unknown. Therefore, easily available method FMD is more appropriate technique to evaluate endothelial functions and atherosclerosis, in a non-invasive fashion.[6,12] However, in the literature, there is no definitive biomarker to monitor endothelial functions. Different endothelium-dependent markers have been investigated in the literature such as inflammatory markers (serum reactive protein), fibrin degradation products, adhesion molecules (E- and P-selectin), endocan, endothelial cadherin, selectin, growth factors such as angiopoietin-1 and -2 and vascular endothelial growth factor, as well as von Willebrand factor antigen.[13,14] According to controversial results, some of these markers were found with higher plasma levels in ED, but the unique specificity or isolated sensitivity was not determined for these markers.

After SCUBE-1 has been suggested as a new scope in vascular biology, it has been increasingly studied for different types of vascular disorders.[7] Initially experimental adhesion and aggregation studies revealed that SCUBE enhanced platelet adhesion and aggregation.[7] Although SCUBE deposits were determined in the atherosclerotic plaques, the role of SCUBE-1 in the atherosclerotic plaques and thrombosis still remains unclear.[7,15] Previous studies reported that SCUBE-1 levels could increase due to endothelial injury or endothelial response to ischemia. Even, it was claimed that SCUBE-1 levels simultaneously increased with NO in endothelial injury in a correlated manner.[8] It is also well-known that NO is an essential mediator in the vessel wall and it also acts an active role in endothelial functions.[16]

The SCUBE-1 has been suggested as a promising biomarker in different ischemic disorder such as myocardial, cerebral, and mesenteric ischemia. Furthermore, monitoring of SCUBE-1 levels has been proposed as a solution for insufficiency of the ischemic marker in recent cardiology studies.[8,10,17] Higher SCUBE-1 levels were reported in hypertensive patients, compared to healthy individuals in a study conducted by Ozkan et al.[18,19] They claimed that it was related with rising in the platelet activation of blood pressure and its association with ED in hypertension.[18] The most striking study on the relationship between atherosclerosis progression and SCUBE family has been recently reported by Ali et al.[15] The authors gave a high-fat diet to the mice to induce an atherosclerotic human model and they designed an experimental intimal thickening model with carotid artery ligation in rats for mimicking diffuse intimal thickening in human. Finally, they found the presence of secreted and membrane-settled SCUBE-2 in diffuse intima thickening and advanced lesions of atherosclerotic plaques. Different from the aforementioned study, we examined the SCUBE-1 levels in a human model and, to the best of our knowledge, this is the unique study

to investigate the SCUBE-1 levels together with FMD in the literature. Additionally, our results support the thesis of relationship existence between the SCUBE-1 and endothelium.

One of the major limitations of the study is its reflection on a healthy young population. Therefore, these result should be confirmed with comparison studies with atherosclerotic patients. Another limitation of this study is that it is non-ivasive and lackage of tissue analysis and therefore it is impossible to determine the mechanism action between development of atherosclerosis and SCUBE-1.

In conclusion, our study results suggest that SCUBE-1 is closely related with endothelial functions. Therefore, this marker can be an alternative for biomarker gap in both ischemic and atherosclerotic events. Even, it can be beneficial in the early detection of plaque progress of atherosclerosis. However, these findings should be supported with further large-scale clinical studies also including patients with systemic atherosclerosis.

Declaration of conflicting interests

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