

# New Marker of Platelet Activation, SCUBE1, Is Elevated in Hypertensive Patients

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## BACKGROUND

Hypertension is associated with an increase in platelet activation and endothelial dysfunction and leads to a tendency to cardiovascular events (CVEs). Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1) is a novel platelet activation marker. There are currently no studies showing the level of SCUBE1 in hypertensive patients. The purpose of this study was to determine the level of SCUBE1 in this patient group and to investigate the parameters affecting that level.

## METHODS

Forty-five newly diagnosed, untreated, stage 1 hypertensive patients and 21 healthy individuals were included. Blood specimens were collected to determine SCUBE1, soluble CD40 ligand, prothrombin time, partial thromboplastin time, fibrinogen, D dimer, hemogram, lipid parameters, blood urea nitrogen, creatinine, and uric acid levels. The relation between SCUBE1 level and demographic data and biochemical parameters was then investigated.

## RESULTS

SCUBE1 and sCD40L levels obtained from plasma specimens from the hypertensive group were significantly higher than those of the control group ( $P < 0.001$ ;  $P < 0.05$ , respectively). Hypertensive group blood pressure (BP) values and uric acid, low-density lipoprotein, total cholesterol, and triglyceride levels were also statistically higher than those of the control group. Parameters affecting SCUBE1 levels were systolic and diastolic BP, sCD40L, lipid parameters, and uric acid levels.

## CONCLUSIONS

We show elevated levels of SCUBE1, a novel platelet activation marker, in primary hypertensive patients. We think that, when supported by further clinical studies, this newly described marker may be useful in the monitoring of CVEs in this patient group, in which platelet activation is known to be associated with such events.

**Keywords:** blood pressure; hypertension; platelet activation; sCD40L; signal peptide-CUB-EGF domain-containing protein 1; SCUBE1.

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Hypertension (HT) is a disease thought to affect approximately one-quarter of the global adult population in 2000 and is predicted to affect approximately one-third of the population by 2025.<sup>1</sup> HT is a risk factor for atherothrombotic complications, whose etiology involves endothelial function impairment and impaired platelet morphology and functions.<sup>2,3</sup> Many studies have shown that hypertensive patients exhibit endothelial vascular injury and endothelial dysfunction, which trigger platelet activation and adhesion.<sup>4</sup> Platelets respond to HT with changes in calcium metabolism, increased reactive oxygen species production, modification in platelet membrane proteins, increased  $\beta$ -thromboglobulin and P-selectin expression, impaired nitric oxide (NO) bioavailability, and an increase in release of some vasoactive agents and growth factors (platelet-derived growth factor, vascular endothelial growth factor).<sup>4-7</sup>

Platelet activation is evaluated by measuring morphology, function, and various plasma marker levels.<sup>7</sup> One of these activation markers is soluble CD40 ligand (sCD40L). CD40L is a transmembrane protein structurally related to the tumor necrosis factor  $\alpha$  family and is expressed on the cell surface

of active platelets.<sup>8</sup> sCD40L is a form of CD40L released into plasma from the active thrombocyte surface. More than 95% of plasma sCD40L is known to originate from thrombocytes.<sup>9</sup> sCD40L is regarded as one of the predictor markers of cardiovascular events (CVEs) in healthy individuals.<sup>10,11</sup>

The signal peptide-CUB (signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor) domain-containing protein (SCUBE) gene family contains 3 isoforms (SCUBE1-3).<sup>12</sup> SCUBE1 is expressed in fast-growing tissues during embryological development and has, in recent years, been shown to be secreted in the endothelium and platelets.<sup>13,14</sup> SCUBE1 has been shown to be stored in thrombocyte  $\alpha$  granules and to translocate to the cell surface in the event of thrombocyte activation.<sup>14</sup> One clinical study showed that SCUBE1 levels rise in acute ischemic stroke (AIS) and acute coronary syndrome (ACS) with platelet activation.<sup>15</sup> However, there are only limited studies showing the parameters affecting functions and levels of SCUBE1, a novel platelet activation marker. No clinical studies to date have investigated the levels of SCUBE 1 in a hypertensive population.

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The purpose of this study was to determine the level of SCUBE1 in a recently diagnosed, primary hypertensive patient group with no history of antihypertensive treatment and to evaluate the correlation with sCD40L levels to establish whether these levels are correlated with thrombocyte activation.

## METHODS

Forty-five patients diagnosed as stage 1 hypertensive on the basis of the Joint National Committee 7 (JNC) guideline<sup>16</sup> with no history of antihypertensive treatment, aged >18 years, with no exclusion criteria, and applying to the Karadeniz Technical University Medical Faculty Hospital Adult Nephrology and Hypertension Department, Turkey, were enrolled, together with 21 healthy control subjects. Patients with secondary HT, kidney function impairment, nephrotic syndrome, diabetes mellitus, collagen tissue disease, atrial fibrillation, thyroid function impairment, coronary artery disease, kidney and liver function impairment, or a history of lipid-reducing drugs or any drugs known to affect coagulation, and patients using alcohol or cigarettes were excluded. Patients were selected once the Karadeniz Technical University had confirmed that the study protocol conformed to the Second Helsinki Declaration. Patients were given a detailed physical examination. Blood pressure (BP) was measured in conformity with the JNC guideline.<sup>16</sup> Patients with BP of  $\geq 140/90$  mm Hg at 2 measurements were enrolled. Patients' age, sex, and body mass index were recorded. Abdominal and Doppler ultrasound were used to eliminate causes of secondary HT. Ten-milliliter blood specimens were collected from each patient enrolled from a large vein in the antecubital region without use of tourniquet between 8:00 AM and 10:00 AM after 12 hours of fasting at time of first diagnosis. Hemoglobin, platelet number, prothrombin time, activated partial thromboplastin time, fibrinogen, D dimer, glucose, potassium, blood urea nitrogen, creatinine, uric acid, total cholesterol, triglyceride, low-density lipoprotein (LDL), high density lipoprotein, and high-sensitivity C-reactive protein measurements were performed on the same day. Blood specimens placed into citrate-containing tubes for SCUBE1 and sCD40L analysis were centrifuged immediately for 20 minutes at 3,500 cycles. Plasmas were separated out and kept at  $-80^{\circ}\text{C}$  until assay.

### Measurement of sCD40L level

Levels of human serum sCD40L were determined using an enzyme-linked immunosorbent assay kit (BMS239CE; eBioscience, Vienna, Austria), according to the manufacturer's protocols. The absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (Molecular Devices, Sunnyvale, CA). The results were expressed as nanograms per milliliter.

### Measurement of SCUBE1 levels

Levels of SCUBE1 were determined using an enzyme-linked immunosorbent assay kit (Cusabio Biotech, Wuhan,

China), according to the manufacturer's protocols. The absorbance of samples was measured at 450 nm using VERSA max tunable microplate reader (Molecular Devices). The results were expressed as nanograms per milliliter. The minimum detectable dose of human SCUBE1 is typically  $< 0.16$  ng/ml.

### Statistical analysis

Compatibility of data with normal distribution was investigated using the Kolmogorov–Smirnov test. The *t* test was used in the comparison of normally distributed data, and the Mann–Whitney *U* test was used for nonnormally distributed data. The  $\chi^2$  test was used to analyze demographic data, and the Pearson correlation analysis was used for correlation analysis. Multiple linear regression analysis was performed for parameters affecting SCUBE levels (systolic BP (SBP), diastolic BP (DBP), sCD40L, total cholesterol, triglyceride, and uric acid).  $P < 0.05$  was considered statistically significant.

## RESULTS

### Demographic data and biochemical parameters

No statistically significant difference was determined between the hypertensive and control patient groups in terms of age, sex, or body mass index in this study involving 45 newly diagnosed, primary hypertensive patients (mean age =  $42.29 \pm 12.45$ ; 22 women, 23 men) and 21 healthy controls (mean age =  $40.81 \pm 8.48$ ; 11 women, 10 men). Mean SBP and DBP in the hypertensive group were  $149.36 \pm 5.26$  and  $91.33 \pm 4.18$  mm Hg, respectively, and  $103.80 \pm 5.89$  and  $63.80 \pm 4.97$  mm Hg, respectively, in the control group. There was a significant difference in SBPs and DBPs between the hypertensive and control groups ( $P < 0.001$  and  $P < 0.001$ , respectively). There was also no difference between the hypertensive and control groups in glucose, potassium, blood urea nitrogen, creatinine, and high-sensitivity C-reactive protein levels. No difference was again determined between the groups' prothrombin time, partial thromboplastin time, fibrinogen levels, D dimer levels, hemoglobin levels, and platelet numbers. Mean hypertensive group uric acid level was  $4.72 \pm 1.01$  mg/dl, which was significantly higher than the  $4.01 \pm 1.10$  mg/dl measured in the control group ( $P < 0.05$ ). Hypertensive group total cholesterol, LDL, and triglyceride levels were significantly higher than those of the control group. *P* values at comparison of the control and hypertensive groups' total cholesterol, LDL, and triglyceride levels were  $P < 0.05$ ,  $P < 0.05$ , and  $P < 0.05$ , respectively (Table 1).

### Hypertensive and control groups' SCUBE1 and sCD40L levels

The control group's mean plasma SCUBE1 level was  $29.13 \pm 8.41$  ng/ml, and that of the hypertensive group was  $73.44 \pm 32.86$  ng/ml. Plasma SCUBE1 level was significantly elevated in the hypertensive patient group ( $P < 0.001$ ). The control group's mean plasma sCD40L was  $1.04 \pm 1.18$  ng/ml, compared with  $1.84 \pm 1.24$  ng/ml in the hypertensive group.

**Table 1.** A comparison of the demographic and biochemical parameters of the hypertensive and control groups

Parameters	Hypertensive group (n = 45), mean ± SD or median (min–max)	Control group (n = 21), mean ± SD or median (min–max)	P value
Age	42.2 ± 12.4	40.8 ± 8.4	NS
Sex, female/male	22/23	11/10	NS
BMI	28.0 ± 4.2	26.6 ± 3.9	NS
SBP, mm Hg	149.3 ± 5.2	103.8 ± 5.8	<0.001
DBP, mm Hg	91.3 ± 4.1	63.8 ± 4.9	<0.001
Glucose, mg/dl	88.5 ± 5.4	90.3 ± 6.5	NS
Potassium, mmol/L	4.5 ± 0.4	4.5 ± 0.4	NS
BUN, mg/dl	12.0 (9–19)	12.0 (6–26)	NS
Creatinine, mg/dl	0.7 (0.5–1.1)	0.7 (0.5–1.2)	NS
Uric acid, mg/dl	4.7 ± 1.0	4.0 ± 1.1	<0.05
Total cholesterol, mg/dl	102.0 (41–281)	121.0 (41–410)	<0.05
Triglyceride, mg/dl	163.7 ± 100.2	111.4 ± 60.3	<0.05
HDL, mg/dl	45.3 ± 11.1	46.5 ± 11.6	NS
LDL, mg/dl	131.2 ± 43.5	103.4 ± 27.3	<0.05
HsCRP, mg/dl	0.3 (0–0.8)	0.3 (0–2.0)	NS
Hemoglobin, g/dl	13.4 ± 1.0	13.1 ± 1.0	NS
Platelet, ×10 <sup>9</sup> /μL	247.4 ± 54.0	253.9 ± 52.5	NS
PT, sec	13.8 ± 0.9	13.6 ± 0.7	NS
aPTT, sec	29.8 ± 2.6	29.9 ± 2.7	NS
Fibrinogen, mg/dl	339.9 ± 73.6	326.0 ± 66.4	NS
D dimer, ng/ml	0.7 ± 0.2	0.6 ± 0.3	NS

Statistical significance was set at  $P < 0.05$ .

Abbreviations: aPTT, activated partial thromboplastin time; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; NS, not significant; PT, prothrombin time; SBP, systolic blood pressure.

The hypertensive group's plasma sCD40L was also significantly elevated ( $P < 0.05$ ) (Figure 1).

#### Parameters affecting SCUBE1 and sCD40L levels

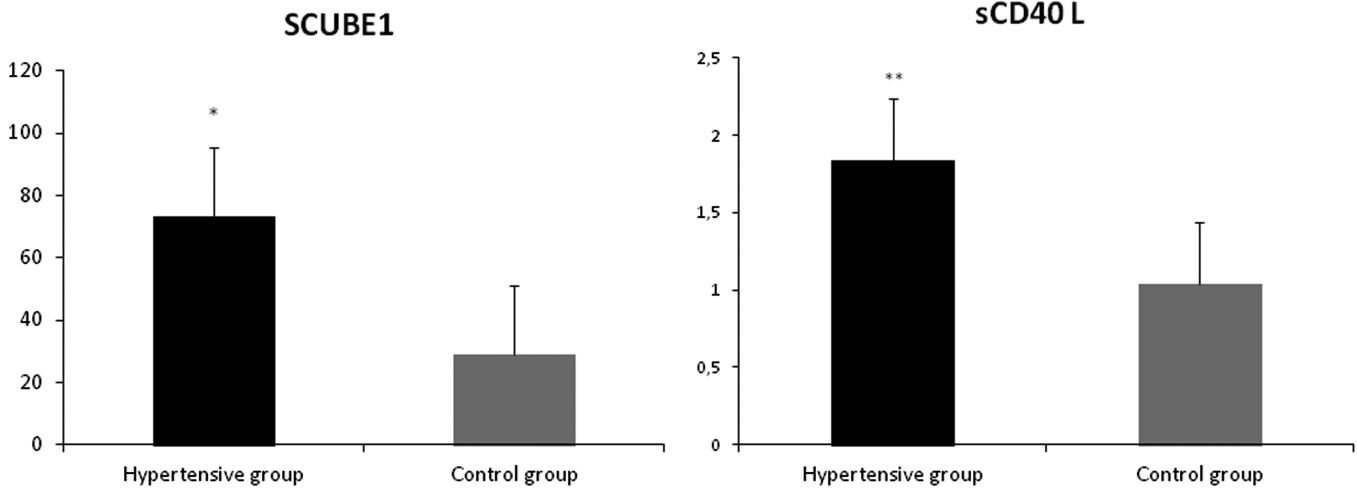
There was a positive correlation between SCUBE1 and sCD40L ( $P < 0.05$ ;  $r = 0.411$ ). Age, sex, and body mass index had no effect on SCUBE1 or sCD40L levels. Systolic and diastolic BP were significantly positively correlated with SCUBE1 ( $P < 0.001$ ,  $r = 0.67$ ; and  $P < 0.001$ ,  $r = 0.68$ , respectively). Systolic and diastolic BP were also significantly positively correlated with sCD40L ( $P < 0.05$ ,  $r = 0.38$ ; and  $P < 0.05$ ,  $r = 0.39$ , respectively). The biochemical and hematological parameters blood urea nitrogen, creatinine, glucose, potassium, high-density lipoprotein, high-sensitivity C-reactive protein, hemoglobin, platelet count, prothrombin time, partial thromboplastin time, fibrinogen, and D-dimer levels were not correlated with SCUBE1 or sCD40L. There was a positive correlation between uric acid level and both SCUBE1 and sCD40L ( $P < 0.05$ ,  $r = 0.26$ ; and  $P < 0.05$ ,  $r = 0.34$ , respectively). SCUBE 1 level was positively correlated with LDL ( $P < 0.05$ ;  $r = 0.35$ ), total cholesterol ( $P < 0.05$ ;  $r = 0.27$ ) and triglyceride ( $P < 0.05$ ;  $r = 0.24$ ). At multiple

linear regression analysis, SBP ( $P < 0.05$ ,  $\beta = 0.60$ ,  $R^2 = 0.38$ ), sCD40L ( $P < 0.05$ ,  $\beta = 0.27$ ,  $R^2 = 0.10$ ), and LDL ( $P < 0.05$ ,  $\beta = 0.25$ ,  $R^2 = 0.08$ ) were observed to affect SCUBE levels (Figure 2 and 3), whereas the effects of uric acid, DBP, total cholesterol, and triglyceride disappeared at linear regression analysis.

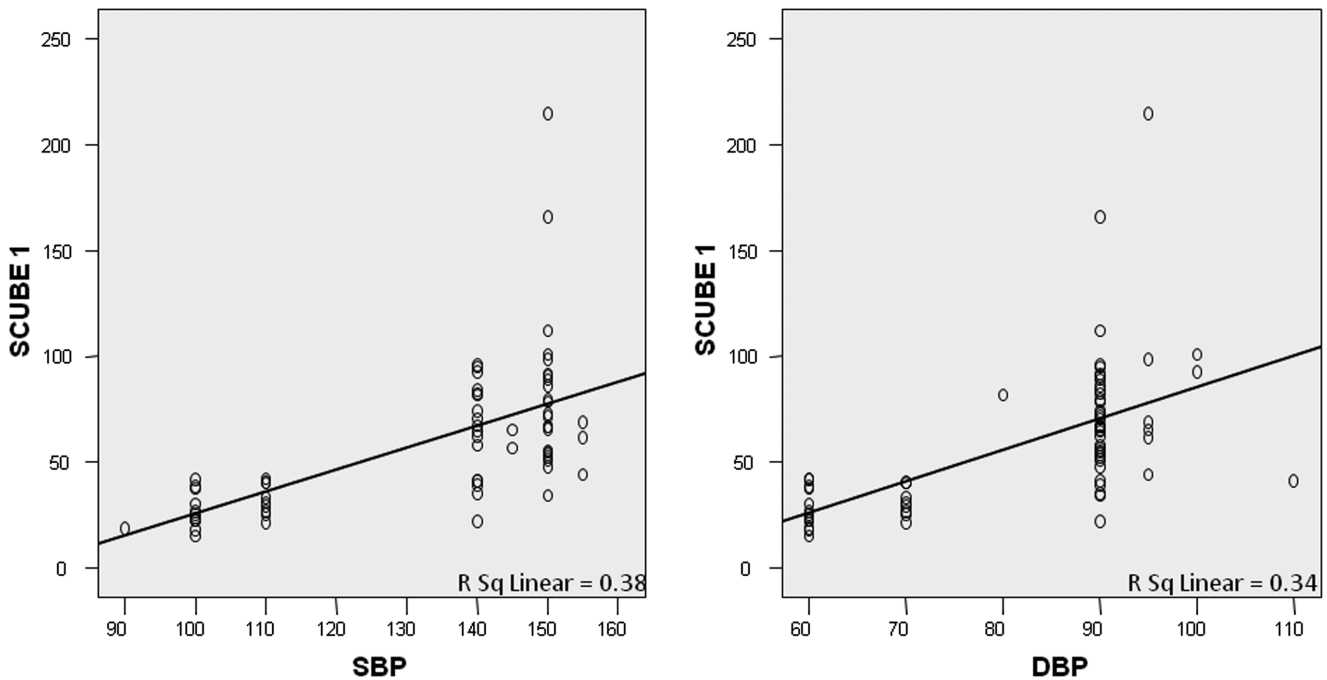
#### DISCUSSION

HT is associated with increases in platelet activation and endothelial dysfunction markers leading to activation in the coagulation system.<sup>5</sup> In this study, we demonstrated that the level of the novel platelet activation marker SCUBE1 is elevated in newly diagnosed primary hypertensive patients. sCD40L level was also significantly elevated in the hypertensive group, and there was a positive correlation between sCD40L and SCUBE1 levels. Parameters affecting SCUBE1 levels were SBP, DBP, uric acid, total cholesterol, triglycerides, and LDL.

SCUBE1 is a member of the SCUBE gene family. According to recent discoveries in mammals, it has 3 isoforms, SCUBE1–3.<sup>12</sup> SCUBE is expressed in fast-growing tissues during mouse embryogenesis. But there are no studies



**Figure 1.** Comparison of the signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1) and soluble CD40 ligand (sCD40L) levels (both ng/ml) of the hypertensive patients and control subjects. SCUBE1 and sCD40L levels in the hypertensive group, measured from plasma were significantly higher than those of the control group. \*SCUBE1 level of control subjects vs. hypertensive patients:  $P < 0.001$ . \*\*sCD40L level of control subjects vs. hypertensive patients:  $P < 0.05$ .

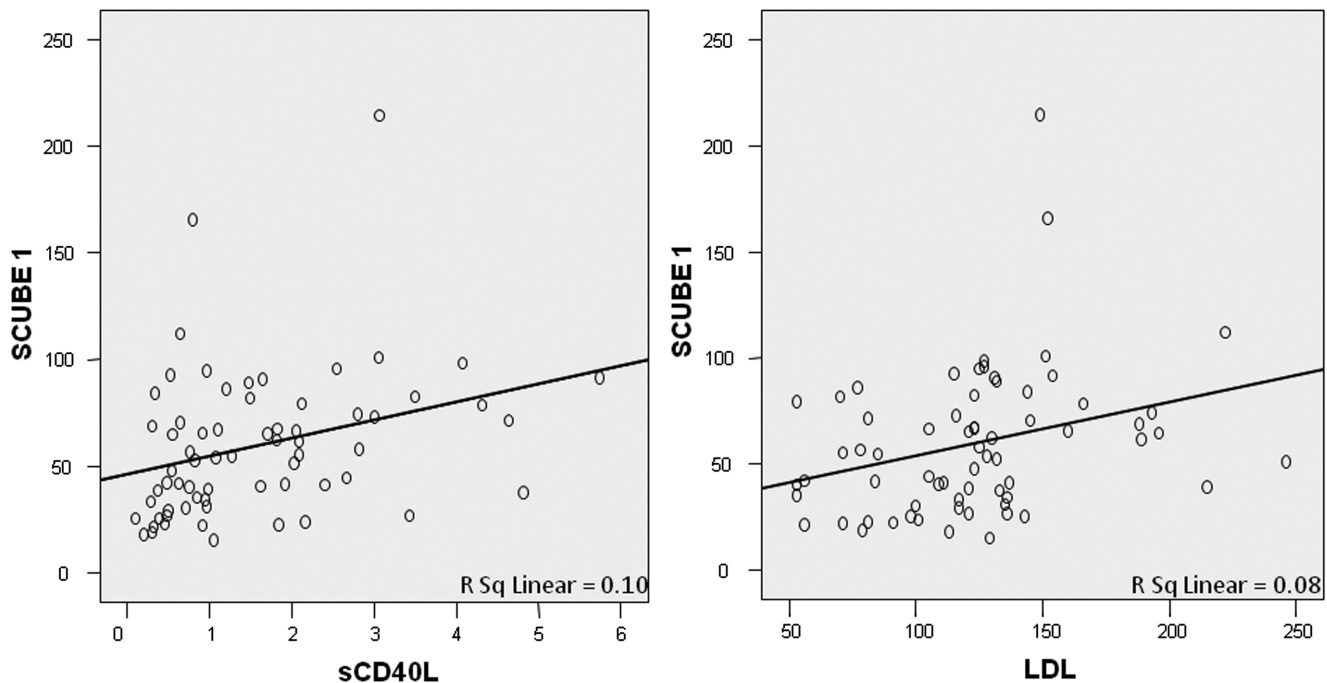


**Figure 2.** Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1), systolic blood pressure (SBP), and diastolic blood pressure (DBP) linear regression analysis. There was a significant correlation between SBP and SCUBE1 at linear regression analysis ( $P < 0.05$ ,  $\beta = 0.604$ ,  $R^2 = 0.38$ ), whereas that correlation between DBP and SCUBE1 had disappeared ( $P > 0.05$ ,  $\beta = 0.017$ ,  $R^2 = 0.34$ ).

showing the function of this expression in fast-growing tissues.<sup>13,14</sup> In addition to embryological development, SCUBE1 is also expressed from endothelial cells and platelets.<sup>14</sup> One clinical study showed an increase in sCD40L, a platelet activation marker, together with SCUBE 1 in ACS and AIS. That study suggested that SCUBE 1 levels do not rise apart from in acute ischemic events and that they may be a marker of such events.<sup>15</sup>

HT is a widely seen, chronic, treatable disease. It leads to a number of complications involving the vascular system,

particularly heart attack and stroke.<sup>16</sup> Many studies have shown that HT leads to endothelial dysfunction and impaired platelet morphology and functions.<sup>4,5</sup> Our study showed that levels of SCUBE1, known to be expressed from endothelial cells and activated platelets, but whose levels in hypertensive individuals were unknown until now, are elevated in untreated primary hypertensive patients. SCUBE1 level was positively correlated with both SBP and DBP. The fact that the hypertensive patients had a higher SCUBE1 level than the control group patients and that it was positively correlated



**Figure 3.** Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1), soluble CD40 ligand (sCD40L), and low-density lipoprotein (LDL) linear regression analysis. At linear regression analysis, sCD40L ( $P < 0.05$ ,  $\beta = 0.27$ ,  $R^2 = 0.10$ ) and LDL ( $P < 0.05$ ,  $\beta = 0.258$ ,  $R^2 = 0.08$ ) affected SCUBE1 levels.

with BP suggested that HT leads to a rise in SCUBE1 levels. In a study by Dai *et al.*,<sup>15</sup> there was a history of HT in 62% of coronary artery disease (CAD) patients, 28% of ACS patients, and 32% of AIS patients. Mean SCUBE1 level was 50 ng/ml in the CAD patients and 205 and 95.1 ng/ml, respectively, in the ACS and AIS patients. Control group SCUBE1 levels were generally below the detection limit of 50 ng/ml. There was no difference in that study between CAD patients and control group SCUBE1 levels, whereas the ACS and AIS patients' SCUBE1 levels were significantly elevated. In that study, despite there being a history of HT in 62% of the CAD cases, no difference was observed between them and the control group. However, because the aim of the study was to evaluate the effect of acute ischemic events on SCUBE1, patients' BP levels and antihypertensives used were not specified, with only HT recorded as medical history. In contrast with our study, although 62% of patients with CAD had a history of HT, SCUBE1 levels were not high. The absence of a correlation between SCUBE1 and a history of HT may be ascribed to patients' BP being under control or use of antihypertensive drugs affecting SCUBE1 levels by impacting on endothelial and platelet functions. No information was provided on this subject. Our hypertensive patients' mean SCUBE1 level was  $73.44 \pm 32.86$  ng/ml, close to that of Dai *et al.*'s asymptomatic CAD group. Our control group's mean SCUBE1 level was  $29.13 \pm 8.41$  ng/ml, below the values in that study. In conclusion, we show that BP is one of the parameters affecting SCUBE1 levels, which is shown to rise in ischemic events and may be elevated in hypertensive patients who have not received treatment.

sCD40L, a member of the tumor necrosis factor  $\alpha$  family that is released into plasma from activated platelets, has

been shown to be elevated in hypertensive patients. It has been suggested that this elevation may be correlated with atherosclerotic vascular complications.<sup>8-11,17</sup> We therefore used sCD40L as a platelet activation maker and determined an elevated level in our hypertensive patient group, in agreement with previous studies. Because of the positive correlation between sCD40L and SCUBE1, we thought that elevated SCUBE1 in hypertensive individuals was correlated with platelet activation. We think that when SCUBE1 is supported with clinical studies in addition to sCD40L, which has been shown to be correlated with development of CVEs in hypertensive patients, it can be used as a marker in monitoring development of CVEs.

One of the parameters affecting SCUBE1 levels in hypertensive patients was hyperlipidemia. This has been shown in both *in vitro* and clinical studies to lead to an increase in platelet activation and to a tendency to atherothrombotic complications.<sup>18,19</sup> The positive correlation between LDL and SCUBE1 and the association between SCUBE1 and LDL at linear regression analysis supports a correlation between elevated SCUBE1 and hyperlipidemia.

In conclusion, HT is a widely seen disease associated with platelet activation. Understanding the mechanism of platelet activation and identifying markers of that activation will help with the early identification of HT-related thrombotic complications and the development of new antiplatelet agents of use in treatment and prevention. We have demonstrated that the level of SCUBE1, a novel platelet activation marker, is elevated in hypertensive patients. We think that the determination of a correlation between SCUBE1 level and CVEs and mortality in this patient group through further studies can represent a significant parameter in clinical practice.

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**DISCLOSURE**

The authors declared no conflict of interest.

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