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Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1) level in hemodialysis patients and parameters affecting that level

Sukru Ulusoy [a,*], Gulsum Ozkan [a], Ahmet Menteşe [b], Adnan Yavuz [c], S. Caner Karahan [b], Ayşegül Uzun Sümer [b]

[a] Department of Nephrology, Karadeniz Technical University, School of Medicine, Turkey
[b] Department of Medical Biochemistry, Karadeniz Technical University, School of Medicine, Turkey
[c] Department of Hemodialysis, Trabzon RTS Hemodialysis Center, Turkey

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ABSTRACT

Background: Signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor)-domain-containing protein 1 (SCUBE1) is a cell surface protein belonging to the SCUBE gene family. SCUBE1 has been shown to rise in parallel with platelet activation in acute ischemic events. However, there are no studies showing levels in the hemodialysis patient group, in which there is known to be an increase in platelet function impairment and activation. The purpose of this study was to investigate SCUBE1 levels in a hemodialysis patient group and the factors affecting those levels.

Materials and methods: One hundred three hemodialysis patients and 21 age-matched healthy controls were included. SCUBE1 and sCD40L levels were investigated from blood specimens collected on pre- and post-hemodialysis sessions. We investigated the correlation between SCUBE1 levels and sCD40L, patients' demographic data, parameters with hemodialysis treatment and routine biochemical tests.

Result: SCUBE1 levels were significantly higher in the hemodialysis patient group compared with the controls ($p = 0.000$). There was a significant rise in SCUBE1 levels in the post-hemodialysis session ($p = 0.000$). We determined a positive correlation between SCUBE1 and sCD40L ($p = 0.016$, $r = 0.215$). Gender, blood pressure, BUN, creatinine, hematocrit and high-sensitivity C-reactive protein (hsCRP) levels, hemodialysis membrane surface area, amount of ultrafiltration, blood flow rate, dialysis flow rate and carnitine use significantly affected SCUBE1 levels.

Conclusion: We have shown, for the first time in the literature, that SCUBE1 level, a potential acute ischemia marker, is elevated in hemodialysis patients with no clinical ischemic event, and that various factors affect this elevation.

Introduction

SCUBE1 (signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor)-domain-containing protein 1) is a newly described cell surface protein belonging to the SCUBE gene family [1]. The SCUBE gene family contains three different isoforms, including SCUBE1 (SCUBE1–3). During mouse embryogenesis, the SCUBE gene family has been shown to be expressed particularly in developing tissues such as the gonads, central nervous system, dermomyotome and limb buds [1–3]. In addition to expression during the embryological development stage, SCUBE1 has been shown to be secreted in the endothelium and platelets [1,4,5]. Tu et al. showed that SCUBE1 is stored in thrombocytes' α granules and that it moves to the cell surface with thrombocyte stimulation and activation [4]. Dai et al. showed that SCUBE1 levels rise with platelet activation in acute coronary syndrome (ACS) and acute ischemic stroke (AIS), but that there is no rise in chronic ischemic events [6]. Contemporary studies regarding SCUBE1 function and level in various patient groups are limited, and new research is needed.

Numerous studies have shown that patients receiving hemodialysis treatment are prone to thrombotic complications such as myocardial infarction (MI) and vascular access thrombosis [7,8]. Some studies have shown that there is an increase in platelet activation and coagulation cascade in blood specimens collected at the end of hemodialysis despite the administration of heparin, and that the hemodialysis process gives rise to a prothrombotic process [9,10]. At the same time, platelet aggregation and functions are impaired in uremic patients [11]. However, there have been no studies determining SCUBE1 levels in hemodialysis patients, known to have impairment of both endothelial and platelet functions, and showing the effect of hemodialysis treatment on SCUBE1.

* Corresponding author at: Karadeniz Technical University, School of Medicine, Department of Nephrology, 61080 Trabzon, Turkey. Fax: +90 462 325 22 70.
E-mail address: sulusoy2002@yahoo.com (S. Ulusoy).

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The primary purpose of this study was to determine levels of SCUBE1, which have only recently been described and with no knowledge about its levels in hemodialysis patients, and to show whether there is a difference in these levels pre- and post-hemodialysis. The secondary aim was to determine the parameters affecting SCUBE1 levels. For that purpose, we used patients' demographic data, parameters related to hemodialysis treatment and routine biochemical tests and levels of sCD40L, a thrombocyte activation marker.

Materials and methods

Approval for the study was obtained from the Karadeniz Technical University Medical Faculty Ethical Committee. Informed consent forms were obtained from patients. One hundred three patients with chronic renal failure (CRF) on the 3 hemodialysis weekly program, who agreed to take part and who used a synthetic membrane during hemodialysis treatment, with no use of oral anticoagulants, antiaggregants and antihypertensive drugs or cigarettes and with no malignity or thyroid function impairment were enrolled, together with 21 healthy controls. Peripheral blood samples were collected for SCUBE1 and sCD40L investigation before and after dialysis sessions. Plasma was obtained by centrifugation at 1600 *g* for 10 min within 15 min of collection. Plasma was frozen and stored at −70 °C for subsequent analysis after a single thaw. Patients' demographic data (age, gender, duration of hemodialysis, duration of arteriovenous fistula (AVF), vascular access pathway and vascular access problems), blood pressure, drugs used (erythropoietin (EPO), phosphorus binders, carnitine, etc.), hemodialysis session data (blood flow rate, membrane surface area, dialysate flow rate, amount of ultrafiltration (UF)), and the biochemical parameters blood urea nitrogen (BUN), creatinine, sodium (Na), potassium (K), calcium (Ca), phosphorus (P), high-sensitivity C-reactive protein (hsCRP), intact-parathormone (iPTH) and Kt/V values were recorded. Biochemical parameters were investigated using their own solutions on Roche modular autoanalyzers. Kt/V values were calculated using the formula

$$Kt/V = -\log(Upost/Upre - 0.008t) + (4 - 3.5\,Upost/Upre) \times (Wpre - Wpost)/Wpost$$

Upost	post-hemodialysis urea
Upre	pre-hemodialysis urea
Wpost	post-hemodialysis weight
Wpre	pre-hemodialysis weight
t	hemodialysis duration [12].

Measurement of sCD40L level

Levels of human serum sCD40L were determined using an enzyme-linked immunosorbent assay kit (eBioscience, BMS239CE, Vienna, Austria), according to the manufacturer's protocol. Absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (designed by Molecular Devices in California, USA). The results were expressed as ng/mL.

Measurement of human signal peptide, CUB and EGF-like domain-containing protein 1 (SCUBE1) levels

Levels of human signal peptide, CUB and EGF-like domain-containing protein 1 (SCUBE1) were determined using an enzyme-linked immunosorbent assay kit (Cusabio Biotech Co., Catalog no. CSB-E15005h, P.R. China), according to the manufacturer's protocols. Absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (designed by Molecular Devices in California, USA). The results were expressed as ng/mL. The minimum detectable dose of human SCUBE1 is typically less than 0.16 ng/mL.

Statistical analysis

Normal distribution of data was analyzed using the Kolmogorov–Smirnov test. Comparisons in groups with parametric conditions were performed using Student's *t* test and in groups without parametric conditions using the Mann Whitney *U* test. Comparisons between values in the patient groups were performed using the paired *t* test in groups with parametric conditions and the Wilcoxon test in groups without parametric conditions. The chi square test was used to analyze demographic data. Pearson correlation analysis was used for correlation analyses. Data were given as mean ± standard deviation (SD). A $p<0.05$ was regarded as statistically significant.

Results

Demographic data and biochemical parameters

One hundred three hemodialysis patients (mean age 61.0 ± 14.7) and 21 healthy controls (mean age 60.8 ± 13.6) were enrolled. Eleven (52.4%) members of the control group were female and 10 (47.6%) were male, while 36 (35%) members of the hemodialysis patient group were female and 67 (65%) were male. Patients' demographic data and biochemical parameters are given in Table 1. Blood pressure, BUN, creatinine, P and hsCRP values in the hemodialysis patient group were significantly higher than those in the control group, while Htc and Ca were significantly lower. Primary disease, comorbid diseases, drugs used and hemodialysis-associated parameters in the hemodialysis patient group are given in Table 2. The most common primary kidney disease was hypertension (HT) at 38.8%, and the most comorbid disease was diabetes mellitus (DM) at 31.1%. AVF was used as vascular access pathway by 96.1% of patients, while 16.5% had AVF problems (a history of thrombectomy or failure to establish sufficient blood flow rate for hemodialysis). Mean AVF operation in patients was 1.0 ± 0.2, mean duration of AVF was 41.3 ± 33.5 months and mean duration of hemodialysis was 46.3 ± 35.7 months. Amount of UF was 2.6 ± 0.8 L, blood flow rate was 355.2 ± 58.4 mL/min, hemodialysis membrane surface area was 1.5 ± 0.2 m^2 and dialysate flow rate was 523.3 ± 42.4 mL/min.

Table 1
Comparison of the laboratory and demographic parameters of the control and hemodialysis groups.

	Control group (n = 21)	Hemodialysis patients (n = 103)	p values
Age (year)	60.8 ± 13.6	61.0 ± 14.7	NS
Gender (F/M)	11/10	36/67	NS
BMI	26.0 ± 4.3	26.3 ± 4.3	NS
SBP (mm Hg)	117.8 ± 5.8	132.1 ± 20.5	0.000
DBP (mm Hg)	62.8 ± 6.0	76.4 ± 12.1	0.000
BUN (mg/dL)	13.4 ± 2.8	70.0 ± 16.5	0.000
Creatinine (mg/dL)	0.7 ± 0.1	8.5 ± 2.5	0.000
Na (mmol/L)	138.9 ± 2.1	138.3 ± 2.8	NS
K (mmol/L)	5.2 ± 0.8	5.2 ± 0.7	NS
Ca (mg/dL)	9.4 ± 0.2	8.9 ± 0.6	0.000
P (mg/dL)	4.4 ± 0.8	5.3 ± 1.2	0.000
Albumin (g/dL)	3.9 ± 0.2	4.0 ± 0.4	NS
Htc (%)	40.6 ± 3.1	33.7 ± 3.0	0.000
Plt (U/L)	235.2 ± 50.1	219.5 ± 72.4	NS
WBC (1/nL)	6242.8 ± 2439.3	6819.6 ± 2817.5	NS
CRP (mg/dL)	0.3 ± 0.1	0.9 ± 1.4	0.000
iPTH (pg/mL)		295.0 ± 216.2	
Kt/V		1.4 ± 0.1	

Abbreviations: BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, BUN; blood urea nitrogen, Na; sodium, K; potassium, Ca; calcium, P; phosphorus, Htc; hematocrit, Plt; platelet, WBC; white blood cell, hsCRP; high-sensitivity C-reactive protein, iPTH; intact-parathormone.
Data are presented mean ± SD.
A $p<0.05$ was regarded as statistically significant.

Table 2
The demographic characteristics of hemodialysis patients.

Hemodialysis patients		n (%)
Primary renal disease	Chronic glomerulonephritis	5 (4.9)
	Urological causes	9 (8.7)
	DM	28 (27.2)
	HT	40 (38.8)
	PKD	21 (21.4)
Comorbid disease	DM	32 (31.1)
	CAD	16 (15.5)
	CHF	7 (6.8)
	ACS and CVE history in 6 months	9 (8.7)
Medications	EPO	89 (86.4)
	Phosphorus binder	101 (98.1)
	Carnitine	74 (71.8)
Vascular access type	AVF	99 (96.1)
	Catheter	4 (3.9)
AVF problems		17 (16.5)

Abbreviations: DM; diabetes mellitus, HT; hypertension, PKD; polycystic kidney disease, CVE; cerebrovascular event, ACS; acute coronary syndrome, CAD; coronary artery disease, CHF; congestive heart failure, EPO; erythropoietin, AVF; arteriovenous fistula.
Data are presented mean ± SD and %.
A $p<0.05$ was regarded as statistically significant.

SCUBE1 and sCD40L levels

SCUBE1 level was 99.9 ± 41.9 ng/mL in the control group compared to 310.9 ± 41.9 ng/mL in the hemodialysis group, the difference being statistically significant ($p=0.000$). Control group sCD40L level was 1.7 ± 0.8 ng/mL, compared to 3.5 ± 1.8 ng/mL in the hemodialysis group. The difference was again significant ($p=0.000$). Post-hemodialysis SCUBE1 level was 535.6 ± 145.2 ng/mL and sCD40L level was 3.7 ± 1.8 ng/mL. The post-hemodialysis SCUBE1 level was significantly elevated ($p=0.000$), and although there was a rise in sCD40L levels, this was not statistically significant. There was a positive correlation between pre-hemodialysis SCUBE1 and sCD40L levels ($p=0.016$, $r=0.215$). There was also a negative correlation between pre- and post-hemodialysis SCUBE1 levels ($p=0.000$, $r=-0.502$).

Correlation between SCUBE1 and sCD40L levels and demographic data

Pre-hemodialysis SCUBE1 level in female patients was 210.4 ± 115.4 ng/mL and 314.7 ± 157.1 ng/mL in male patients. Male patients' pre-hemodialysis SCUBE1 level was significantly higher than that of females ($p=0.000$). There was no difference between the sexes' post-hemodialysis SCUBE1 and sCD40L and pre-hemodialysis sCD40L values. Primary disease did not affect hemodialysis patients' SCUBE1 and sCD40L levels (Table 3). There was no correlation between pre- and post-hemodialysis SCUBE1/sCD40L values and age and body mass index. There was a positive correlation between pre-hemodialysis SCUBE1 and systolic blood pressure (SBP) and diastolic blood pressure (DBP) ($p=0.019$, $r=0.21$ and $p=0.001$, $r=0.285$, respectively). There was also a positive correlation between pre-hemodialysis sCD40L and DBP ($p=0.003$, $r=0.268$). There was no positive correlation between duration of HD and pre- and post-hemodialysis SCUBE1, though there was a negative correlation with pre-hemodialysis sCD40L ($p=0.034$, $r=-0.209$) and a positive correlation with post-hemodialysis sCD40L ($p=0.028$, $r=0.216$). There was a positive correlation between duration of AVF and pre-HD sCD40L ($p=0.013$, $r=0.244$). There was a negative correlation between amount of UF and pre-hemodialysis SCUBE1 ($p=0.031$, $r=-0.213$). We also determined a positive correlation between surface area of the hemodialysis membrane used and pre-hemodialysis SCUBE1 ($p=0.000$, $r=0.368$) and a negative correlation with post-hemodialysis SCUBE1 ($p=0.000$, $r=-0.361$). There was a positive correlation between hemodialysis membrane surface area and pre- and post-hemodialysis sCD40L ($p=0.026$, $r=0.219$; $p=0.046$, $r=0.215$, respectively). There was also a positive correlation between blood flow rate and pre-hemodialysis SCUBE1 ($p=0.037$, $r=0.206$). There was a positive correlation between dialysate flow rate and pre-hemodialysis SCUBE1 ($p=0.000$, $r=0.418$), a negative correlation with post-hemodialysis SCUBE1 ($p=0.000$, $r=-0.371$) and a positive correlation with post-hemodialysis sCD40L ($p=0.001$, $r=0.323$) (Table 3).

Table 3
Correlation of SCUBE1 and sCD40L levels with demographic and biochemical data.

	Pre-HD	Post-HD	Pre-HD	Post-HD
	SCUBE1	SCUBE1	sCD40L	sCD40L
Age	None	None	None	None
BMI	None	None	None	None
SBP (mm Hg)	$p=0.019$, $r=0.210$	None	None	None
DBP (mm Hg)	$p=0.001$, $r=0.285$	None	$p=0.003$, $r=0.268$	None
BUN	$p=0.000$, $r=0.387$	None	$p=0.004$, $r=0.256$	None
Creatinine	$p=0.000$, $r=0.614$	None	$p=0.037$, $r=0.187$	None
Ca	None	None	None	None
P	None	None	None	$p=0.001$, $r=-356$
Htc	$p=0.000$, $r=-0.362$	None	$p=0.034$, $r=-191$	None
Plt	None	None	None	None
WBC	None	None	None	None
hsCRP	$p=0.010$, $r=0.230$	None	None	None
iPTH	None	None	None	None
Kt/V	None	None	None	None
AVF duration	None	None	$p=0.013$, $r=0.244$	None
HD duration	None	None	$p=0.034$, $r=-0.209$	$p=0.028$, $r=0.216$
HD msa	$p=0.000$, $r=0.368$	$p=0.000$, $r=-0.361$	$p=0.026$, $r=0.219$	$p=0.046$, $r=0.215$
UF amount	$p=0.031$, $r=-0.213$	None	None	None
Blood flow rate	None	$p=0.037$, $r=0.206$	None	None
Dialysate flow rate	$p=0.000$, $r=0.418$	$p=0.000$, $r=-0.371$	None	$p=0.001$, $r=0.323$
Primary disease	None	None	None	None

Abbreviations: SBP; systolic blood pressure, DBP; diastolic blood pressure, BUN; blood urea nitrogen, Ca; calcium, P; phosphorus, Htc; hematocrit, Plt; platelet, WBC; white blood cell, hsCRP; high sensitive C-reactive protein, iPTH; intact-parathormone, HD msa; hemodialysis membrane surface area.
A $p<0.05$ was regarded as statistically significant.

Diabetic patients' pre-hemodialysis SCUBE1 level was 265.9 ± 125.4 ng/mL, compared with 331.2 ± 142.0 ng/mL in the non-diabetics. There was no significant difference between the groups. Post-hemodialysis SCUBE1 values of patients with coronary artery disease (CAD) were significantly higher compared to those of patients without CAD ($p=0.026$). There was no significant difference in terms of pre-hemodialysis SCUBE1 and pre- and post-hemodialysis sCD40L values. There was no significant difference between subjects with or without congestive heart failure (CHF) in terms of pre- and post-hemodialysis SCUBE1 and sCD40L values. There was also no significant difference between the pre- and post-hemodialysis SCUBE1 and sCD40L values of patients who had undergone cerebrovascular event (CVE) or CAD in the previous 6 months. There was also no difference between pre- and post-hemodialysis SCUBE1 and sCD40L values of patients with or without AVF problems (Table 4).

Correlation between SCUBE1 and sCD40L levels and biochemical parameters

There was a positive correlation between BUN and pre-hemodialysis SCUBE1 ($p=0.000$, $r=0.387$) and sCD40L ($p=0.004$, $r=0.256$). There was also a positive correlation between creatinine level and pre-

Table 4
Correlation of SCUBE1 and sCD40L levels with comorbidity and drugs used.

	Pre-HD	Post-HD	Pre-HD	Post-HD
	SCUBE1 (ng/mL)	SCUBE1 (ng/mL)	sCD40L (ng/mL)	sCD40L (ng/mL)
Comorbid disease				
DM present	265.9 ± 125.4	512.2 ± 166.3	3.5 ± 1.7	4.0 ± 2.2
DM not present	331.2 ± 142.0	546.0 ± 134.7	3.5 ± 1.8	3.6 ± 1.7
CAD present	242.8 ± 64.0	592.0 ± 96.4	3.2 ± 2.0	3.3 ± 1.4
CAD not present	323.5 ± 146.4	525.2 ± 150.7[a]	3.5 ± 1.8	3.8 ± 2.0
CHF present	289.9 ± 80.2	617.4 ± 10.5	2.6 ± 1.1	2.5 ± 1.0
CHF not present	312.5 ± 143.8	529.6 ± 148.7	3.5 ± 1.8	3.8 ± 1.9
CVE/ACS present	264.8 ± 59.7	587.8 ± 10.1	2.4 ± 0.7	3.8 ± 2.0
CVE/ACS not present	315.4 ± 144.6	530.6 ± 148.2	3.6 ± 1.8	2.7 ± 0.9
Medications				
EPO present	318.0 ± 141.7	533.5 ± 144.1	3.3 ± 1.6	3.6 ± 1.9
EPO not present	265.8 ± 121.8	549.1 ± 157.1	4.3 ± 2.5	4.5 ± 1.7
PB present	312.3 ± 140.3	534.0 ± 146.2	3.5 ± 1.8	3.7 ± 1.9
PB not present	243.6 ± 128.8	617.2 ± 28.7	1.3 ± 0.1	2.9 ± 1.3
Carnitine present	263.6 ± 64.8	449.5 ± 158.9	3.3 ± 1.6	3.3 ± 1.4
Carnitine not present	431.6 ± 198.2[b]	569.3 ± 125.3[c]	3.7 ± 2.2	4.9 ± 2.5[d]
Vascular access type				
AVF	317.6 ± 138.5	534.7 ± 145.4	3.5 ± 1.8	3.7 ± 1.9
Catheter	246.7 ± 140.7	556.6 ± 162.7	3.1 ± 2.2	4.2 ± 3.6
AVF problems				
Present	319.7 ± 179.6	534.4 ± 134.8	3.1 ± 2.0	4.2 ± 2.6
Not present	309.2 ± 131.7	535.8 ± 148.0	3.5 ± 1.7	3.6 ± 1.7

Abbreviations: DM; diabetes mellitus, CAD; coronary artery disease, CHF; congestive heart failure, CVE; cerebrovascular event, ACS; acute coronary syndrome, EPO; erythropoietin, PB; phosphorus binder, AVF; arteriovenous fistula.
A $p<0.05$ was regarded as statistically significant.
[a] $p=0.026$.
[b] $p=0.002$.
[c] $p=0.002$.
[d] $p=0.005$.

hemodialysis SCUBE1 ($p=0.000$, $r=0.614$) and sCD40L ($p=0.037$, $r=0.187$). There was no correlation between pre- and post-hemodialysis SCUBE1 and post-hemodialysis sCD40L values and Ca and P values, though there was a negative correlation between post-hemodialysis sCD40L and P. There was a positive correlation between hsCRP level and pre-hemodialysis SCUBE1 ($p=0.010$, $r=0.230$). There was no correlation between pre- and post-hemodialysis SCUBE1 and sCD40L values and platelet count, Kt/V or iPTH levels. There was a negative correlation between pre-hemodialysis SCUBE1 and sCD40L and Htc level ($p=0.000$, $r=-0.362$, $p=0.034$, $r=-191$, respectively) (Table 3).

Correlation between SCUBE1 and sCD40L levels and EPO, phosphorus binder and carnitine use

There was no correlation between pre- and post-hemodialysis SCUBE1 and sCD40L values and EPO and phosphorus binder use. Pre-hemodialysis SCUBE1 level of patients using carnitine was 263.6 ± 64.8 ng/mL, compared to 431.6 ± 198.2 ng/mL for patients not using carnitine, the difference between the groups being significant ($p=0.002$). Carnitine using patients' post-hemodialysis SCUBE1 value was 449.5 ± 158.9 ng/mL, compared to 569.3 ± 125.3 ng/mL for patients not using carnitine, and the difference was again significant ($p=0.002$). There was no significant difference in carnitine using patients' pre-hemodialysis sCD40L levels. Post-hemodialysis sCD40L level was 3.3 ± 1.4 ng/mL among carnitine users and 4.9 ± 2.5 ng/mL among non-users, and the difference was significant ($p=0.005$) (Table 4).

Discussion

The main findings of this analysis of SCUBE1 levels in hemodialysis patients and the factors affecting those levels were: I) SCUBE1 levels in the hemodialysis patient group were higher compared to those in healthy individuals; II) SCUBE1 levels were significantly higher at the end of the hemodialysis sessions compared to the beginning; III) sCD40L levels were also higher in the hemodialysis patient group, and there was also a rise at the end of the hemodialysis session, though this was not statistically significant; IV) SCUBE1 level was positively correlated with sCD40L level; and V) parameters affecting SCUBE1 level are gender, SBP, DBP, BUN, creatinine, Htc, blood flow rate, hemodialysis membrane surface area, amount of UF, dialysate flow rate, carnitine use and CAD.

Platelet functions in uremic patients have been known to be impaired for many years. Such impairments particularly take the form of defective thrombocyte adhesion and aggregation and a tendency to hemorrhage [13]. Although the cause of thrombocyte function impairment in uremic patients is not yet fully understood, guanidosuccinic acid, the effect of urea and middle molecule uremic toxins, intrinsic platelet defects, anemia and vessel abnormality are all factors involved in this impairment [14,15]. Since hemodialysis and peritoneal dialysis eliminate some uremic toxins they establish partial improvement in platelet functions. However, there are studies showing that contact with a foreign surface (the hemodialysis membrane) during hemodialysis therapy causes a rise in platelet activation [14]. Platelet function impairment and increased activation in the hemodialysis patient group are factors that give rise to cardiovascular complications that lead to mortality and morbidity in this patient group [7,8,16].

sCD40L is a 50 kD transmembrane protein and member of the tumor necrosis factor superfamily. sCD40L was originally identified on the CD4 T cell, although it has also been located on activated platelets in recent years [17,18]. Various studies have shown that sCD40L levels are high in the hemodialysis patient group and that the level is correlated with cardiovascular mortality and morbidity [16,19]. We also determined a higher sCD40L level in the hemodialysis patient group compared to the control group. A post-hemodialysis rise confirms that hemodialysis therapy leads to platelet activation.

SCUBE1 is a cell surface protein and member of the SCUBE gene family. SCUBE1 has been shown to be stored in rapidly proliferated tissues during embryogenesis, in the endothelium and finally in thrombocyte alpha granules [1–5]. SCUBE1 protein is expressed by predominant thrombocytes and can be proteolytically released by turning into smaller, active fragments. These active fragments are associated with thrombus formation and localized in the subendothelial matrix of the atherosclerotic plaque. Molecular and biochemical studies have also shown that SCUBE1 is a novel adhesive molecule and plays a role in platelet–matrix interaction and ristocetin-induced platelet agglutination [4]. The only clinical study performed revealed high levels in situations of atherosclerotic plaque rupture and thrombocyte activation, such as ACS and acute ischemic stroke, and these can be used as a marker in ischemic events [6]. Numerous studies have evaluated the sensitivity and specificity of the cardiac biomarkers Troponin T, Troponin I and CK-MB levels in determining ACS in the hemodialysis patient group. These studies showed that these markers can be elevated in hemodialysis patients without ACS, and that elevated Troponin T in particular is closely correlated with mortality in hemodialysis patients without ACS [20]. Korkmaz et al. determined low CK-MB positivity in a hemodialysis patient group without ACS and suggested that Troponin I and CK-MB can be used together for the diagnosis of ACS [21]. In our study, we show for the first time that SCUBE1 level is high at pre- and post-hemodialysis in the hemodialysis patient group, known to be predisposed to thrombotic complications, even in the absence of an ischemic event. There may be several reasons why SCUBE1 levels are high in this patient group with no acute ischemic event. 1) A positive correlation between sCD40L level and SCUBE1 suggests that there may have been a thrombocyte activation-associated increase in SCUBE1 level in this patient group. 2) It is known that chronic inflammation is present in the

hemodialysis patient group. This inflammation may have caused a rise in SCUBE1 level. Our finding that supported this hypothesis was the positive correlation between hsCRP level and SCUBE1. 3) Uremic toxins may have led to a rise in SCUBE1 level. A positive correlation between BUN and creatinine level and SCUBE1 supported this idea. Moreover, SCUBE1 elimination is through the renal path and may be high since there is no excretion with urine in this patient group.

Male gender is an immutable risk factor for cardiovascular disease [22]. SCUBE1 level was higher in the male gender in our study. We therefore thought that SCUBE1 might be one of the contributory factors to the predisposition to cardiovascular events in males.

Hypertensive patients' predisposition to thrombosis is today a known fact. Factors causing this predisposition to thrombosis include endothelial damage, impairment of the equilibrium between coagulation and fibrinolysis, and impaired thrombocyte morphology and functions [23]. SCUBE1 and sCD40L levels were elevated in patients with high SBP and DBP in this study. SCUBE1 may contribute to the thrombotic process alongside thrombocyte activation in the hypertensive patient group, which is predisposed to thrombosis.

Dai et al. showed that SCUBE1 levels rise together with platelet activation in acute thrombotic events (ACS, AIS) [6]. In our patient group, neither a history of CAD nor having undergone ischemic stroke or ACS in the previous 6 months affected SCUBE1 levels. However, SCUBE1 level at the end of hemodialysis was higher in patients with a history of ACS. This finding may be a reflection of hemodynamic instability arising during hemodialysis treatment. In addition, the positive correlation between hemodialysis blood flow rate and post-hemodialysis SCUBE1 may be a result of hemodynamic instability developing as blood flow rate rises.

Since the foreign body surface area the platelets encounter increases as the hemodialysis membrane surface area rises, this leads to a rise in pre- and post-hemodialysis sCD40L levels and pre-hemodialysis SCUBE1 levels. SCUBE1 level decreased at post-hemodialysis, probably since elimination by diffusion increases as membrane surface area rises. Another indicator that SCUBE1 is eliminated through diffusion during hemodialysis treatment is the decrease in SCUBE1 levels at post-hemodialysis. Pre-hemodialysis SCUBE1 level was lower in patients with a larger amount of UF. This finding suggests that SCUBE1 can be eliminated by ultrafiltration in addition to diffusion.

Another parameter affecting SCUBE1 levels in our study was Htc level. Erythrocytes occupy an important place in hemostasis. Erythrocytes can lead to the formation of thrombin mediated by procoagulant phospholipids on the outer membrane surface in the absence of platelets [24]. Various studies have shown that acute or chronic falls in Htc levels can lead to a hypercoagulable condition [25,26]. The fact that SCUBE1 levels, previously shown to be correlated with thrombotic events, were higher in subjects with low Htc levels in our study, which in other words indicate a negative correlation between SCUBE1 and Htc levels, supports these studies.

In terms of the effect on SCUBE1 levels of the drugs routinely used in our patient group, we observed that with the exception of L-carnitine, the medications used had no impact on SCUBE1 levels. Pre- and post-hemodialysis SCUBE1 and sCD40L levels of the patients using L-carnitine were significantly lower compared to the levels of the patients not using it. Carnitine is a quaternary ammonium compound biosynthesized by the amino acids lysine and methionine. It is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids (fats) for the generation of metabolic energy in organisms. Several studies have shown that L-carnitine has coagulation cascade and platelet activation-reducing effects [27,28]. Our study supported the idea that L-carnitine reduces platelet activation and the rise in SCUBE1.

In conclusion, we have determined for the first time in the literature that SCUBE1 level, shown to be a novel thrombocyte activation marker and to rise in acute ischemic events, is elevated in the hemodialysis patient group with no ischemic event. The correlation with sCD40L corroborates a platelet activation-associated rise. Our hemodialysis patient group's demographic characteristics and several parameters related to hemodialysis treatment itself affected the SCUBE1 level. As a result of this study we think that this marker, which may perhaps also be a marker of acute ischemia in future studies performed with different patient groups, is elevated in the hemodialysis patient group with no acute ischemic event. As with other cardiac markers, it should not be forgotten that measuring it before or after hemodialysis treatment will affect this marker's level. Further clinical studies are needed to evaluate this novel marker's sensitivity in determining ACS in the hemodialysis patient group and its correlation with long-term mortality and morbidity.

Conflict of interest

We report no conflict of interest.

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