Research Article

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Can SCUBE1 be used to predict the early diagnosis, lesion volume and prognosis of acute ischemic stroke?

SCUBE1 Akut İskemik İnmenin Erken Tanısını, Lezyon Volümünü ve Prognozunu Tahmin Etmede Kullanılabilir mi?

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Abstract

Objective: SCUBE1 [signal peptide-CUB (complement C1r/C1s)-EGF (epidermal growth factor)-like domain-containing protein 1] is a novel biochemical marker. SCUBE1 is thought to play roles both in platelet activation and inflammation, which are important stages for the development of acute ischemic stroke (AIS). The purpose of our study was to determine the diagnostic and prognostic values and temporal change of plasma SCUBE1 levels in AIS patients.

Materials and methods: Thirty-five patients diagnosed with AIS at the Cumhuriyet University Faculty of Medicine Neurology Department, between June and December 2017, and a control group of 35 healthy volunteers were included.

Results: Median first day SCUBE1 value in the patient group was 97.51 ng/mL, and the median 7th day SCUBE1 value was 32.72 ng/mL. Median control group SCUBE1 value was 27.51 ng/mL. The first day SCUBE1 levels were significantly higher than the 7th day and the control group SCUBE1 levels ($p=0.001$, $p<0.001$, respectively). The ROC analysis showed that SCUBE1 levels above 68.8 ng/mL can be used as an indicator with high sensitivity and specificity for AIS diagnosis. Multivariate analysis revealed that the first day SCUBE1 had significant independent effects on development of AIS. In correlation analysis, plasma SCUBE1 levels showed a significantly positive correlation with lesion volume, NIHSS and MRS values ($p<0.01$).

Conclusion: First day plasma SCUBE1 values in AIS patients rised at significant levels compared to the control group. SCUBE1 could use both in the early diagnosis and prediction of prognosis and lesion volume of AIS patients.

Keywords: SCUBE1; acute ischemic stroke; platelet activation; inflammation.

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Öz

Amaç: SCUBE1[sinyal peptid-CUB(kompleman C1r/C1s)-EGF(epidermal büyüme faktörü)-benzeri zincir içeren protein) yeni bir biyokimyasal göstergedir. SCUBE1'in akut iskemik inme(Aİİ) gelişimi için önemli basamaklar olan platelet aktivasyonu ve inflamasyonda rol oynadığı düşünülmektedir. Bizim çalışmamızdaki amacımız Aİİ hastalarında SCUBE1 seviyesinin tanıs ve prognostik değerini ve zamana bağlı değişimini belirlemektir.

Gereç ve yöntem: Haziran ve Aralık 2017 tarihleri arasında Cumhuriyet Üniversitesi Tıp Fakültesi Nöroloji

Bölümü'nde Aİİ tanısı almış 35 hasta ile 35 gönüllüden oluşan bir hasta grubu çalışmaya dahil edildi.

Tartışma: Hasta gruptaki birinci gün ortanca SCUBE1 değeri 97.51 ng/ml, ve yedinci gün ortanca SCUBE1 değeri 32.72 ng/ml' ydi. Ortalama kontrol grup SCUBE1 değeri ise 27.51 ng/ml'ydi. Birinci gün SCUBE1 seviyesi yedinci günkü ve kontrol grup SCUBE1 seviyelerinden anlamlı şekilde yüksekti. ($p = 0.001$, $p < 0.001$, sırasıyla). ROC analizi 68.8'in üzerindeki SCUBE1 düzeylerinin Aİİ tanısında yüksek sensitivite ve spesifiteye sahip bir gösterge olarak kullanılabileceğini gösterdi. Çok değişkenli analiz ise birinci gün SCUBE1 seviyesinin Aİİ gelişiminde anlamlı bağımsız etkisi olduğunu ortaya koydu. Korelasyon analizlerinde ise birinci gün plazma SCUBE1 seviyeleri lezyon volümü, NIHSS ve MRS değerleri ile anlamlı pozitif korelasyon gösterdi ($p < 0.01$).

Sonuçlar: Aİİ'li hastaların birinci gün plazma SCUBE1 seviyeleri kontrol gruba göre yüksektir ve SCUBE1 seviyeleri Aİİ'li hastalarda hem erken tanı hem de prognoz ve lezyon volümünün tahmini için kullanılabilir.

Anahtar kelimeler: SCUBE1; akut iskemik inme; platelet aktivasyonu; inflamasyon.

Introduction

Stroke is a clinical syndrome that is characterized by quickly progressing signs of the focal loss of cerebral function, without evident reason except for the mentioned one of vascular origin, and the stroke symptoms must last for a period longer than 24 h or cause death [1]. Stroke takes the third place among the most significant reasons for death following coronary artery disease and cancer, and the most significant reason for disability, particularly in the elderly population [2]. Stroke can be divided into two subtypes as ischemic and hemorrhagic. Moreover, acute ischemic strokes (AIS) make up 85% of all strokes [3].

The three most common etiologic factors of AIS are large vessel atherosclerosis, cardioembolic stroke and lacunar infarcts [1]. The inflammatory process represents a significant stage for developing atherosclerosis in the AIS pathogenesis [2]. It is known that platelet activation has a key role in the development of both cardioembolic stroke and large-vessel atherothrombotic stroke (LAT) [4, 5]. In studies carried out on experimental cerebral ischemia, an increase has been determined in the generation of a number of molecules, such as vascular cell adhesion molecules and various inflammatory markers [2, 3].

SCUBE1 [signal peptide-CUB (complement C1r/ C1s, U egf, and Bmp1)-EGF (epidermal growth factor)-like

domain-containing protein 1] represents a cell surface molecule that has been described recently and is secreted and expressed during early embryogenesis [6]. An N-terminal signal peptide, nine consecutive EGF-like repeats, a spacer region, cysteine-rich repeat motifs, and a CUB domain at the C terminal constitute the protein in question [7]. The storage of the mentioned molecules occurs in the alpha-granules in inactive platelets [8]. The migration of the molecules in question to the platelet surface occurs after activation by thrombin. At the current stage, small and soluble particles of SCUBE1 take significant place as a result of the activation of platelets in cardiovascular biology [9]. The immunohistochemical determination of SCUBE1 deposition has been performed in the subendothelial matrix in advanced atherosclerotic lesions in humans. EGF-like repeats are accountable for adhesive interactions which are among the most important steps in inflammatory processes [6, 10]. The SCUBE1 level has also been shown by a recent study to be increased by oxidative stress [11], and it has been accepted as an inflammatory marker [12].

This study was planned for the purpose of determining the diagnostic and prognostic value of SCUBE1, which represents a new biochemical marker that can be used for this purpose in AIS patients.

Materials and methods

Study group

The current research represents a single-center prospective study involving 35 patients referred to our clinic during the first 24 h of symptoms and diagnosed with AIS between June 2017 and December 2017 and 35 age- and sex-matched controls. In this period, 115 AIS patients were hospitalized in our clinic. Forty six patients of this AIS group did not agree to participate in the study, while seven patients were discharged earlier than the seventh day. Furthermore, 27 patients could not meet the exclusion criteria of our study.

The patients' medical history, computed tomography (CT) and cranial magnetic resonance (MR) imaging results demonstrated the ischemic lesion that occurred recently, and the patients' neurological examination results were utilized for the purpose of making the definitive diagnosis of AIS. Patients who volunteered to participate in the study and signed the consent form were included in the study. All participants' rights were protected and written informed consent signatures were obtained before the procedures according to the Declaration of Helsinki. The

study has no restrictions in terms of the patients' gender and age. Patients aged under 18, patients diagnosed with systematic acute/chronic inflammatory/autoimmune or infectious diseases, idiopathic cardiomyopathies, significant valvular heart disease, chronic connective tissue diseases, cancer, hematological disorders, severe kidney, liver or heart failure, prior acute myocardial infarction, acute coronary syndrome during the last three months, having a history of major surgery or trauma, and hemorrhagic stroke, cardiopulmonary arrest, acute pulmonary edema, acute mesenteric ischemia or pulmonary thromboembolism were excluded from the study.

The clinical study was conducted in compliance with all the related national regulations, institutional policies, and the principles of the Declaration of Helsinki, and approval was received from the institutional review board or corresponding committee of the authors. The first-degree relatives of the patients completed the consent form instead of the patients who could not give the approval by themselves.

The demographic characteristics of the patients, their symptoms, the history of previous diseases, detailed neurological examination results, Glasgow Coma Scores (GCS), the National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scores (MRS) were recorded on a form.

CT was performed in the Emergency Department by means of a Siemens Sensation 16 Slice device. A Siemens Symphony Magnetom 1.5 tesla device was used to perform cranial MR imaging for each patient. The volume of the ischemic lesion in the AIS patients was computed from MR imaging by the ABC/2 formula (ellipsoid). Acute lacunar infarction was defined as a small infarction ($<2 \text{ mm}^3$) in the regions supply to which is provided by the penetrating arterioles [13–15].

CT angiography of the neck and brain was performed for the patients who required it to determine the etiologic causes. Two patients underwent digital subtraction angiography (DSA). Classification of ischemic stroke subtypes was performed in accordance with the Trial of 10,172 in Acute Stroke Treatment criteria [16]. LAT was described as acute cerebral infarction that includes the trunk or branched vessels of the major cerebral arteries. Electrocardiography (ECG) with 12 derivations and echocardiography were carried out for every patient involved in the study. The patients who required monitorization were monitored by 24-h rhythm Holter tests. The diagnosis of cardioembolic stroke was based on these results.

Our control group comprised 35 individuals matched with the patient group with regard to age and gender, not having an uncontrolled systemic disease, such as

cardiovascular disease, hypertension, diabetes, who arrived at our clinic due to reasons except for AIS (headache, dizziness or peripheral vertigo) between June and December 2017, and who provided blood samples.

Evaluation of biochemical and hematological parameters

Complete blood count, routine biochemistry, high sensitive C-reactive protein (hsCRP) were examined from the blood samples taken from the patient and control groups. A BC-6800 analyzer (Mindray, Toshiba, Tokyo, Japan) with a Diagon kit (Sysmex, Kobe, Japan) was used to perform the complete blood count. The routine biochemistry analyses were evaluated using a BC-2000 analyzer (Mindray, Toshiba, Tokyo, Japan) with a Diagon kit via photometric methods. HsCRP levels were measured by means of an Immage 800 analyzer (Beckman Coulter, Yokohama, Japan) by utilizing the manufacturer's kit and employing a completely automated nephelometric method.

Additionally, 5-mL blood samples were taken once from the control group and twice (first within the 24 h after onset and on the seventh day) from the patient group in yellow top tubes with gel separator without anticoagulant for examination. Centrifugation of the samples was performed for the period of 15 min using a 4000-cycle centrifuge device at the temperature of (+4) degrees. The obtained serums were put in Eppendorf tubes and kept at the temperature of -80°C to be studied for the relevant parameters. Twenty-four hours prior to the SCUBE1 examination, the removal of the Eppendorf tubes was performed, and they were put in a medium with the temperature of +4 degrees. Sera thawed in a gradual way during the period of 24 h, and after raising them to room temperature, SCUBE1 levels were measured by using an ELISA kit according to the manufacturer's protocol (catalogue number: SL2410Hu, SunLong, Biotech, China). Serum SCUBE1 levels were given as ng/mL (CV values for SCUBE1: intra- and inter-assay precision $<10\%$).

Statistical analysis

The Statistical Package for the Social Sciences for Windows 21.0 (SPSS Inc., Chicago, IL, USA) was utilized for statistical analyses. The Kolmogorov-Smirnov test was conducted for the purpose of determining whether the continuous variables exhibited a normal distribution. The continuous variables that exhibited a normal distribution were presented as mean $\pm$ standard deviation, whereas the variables that did not exhibit a normal

distribution were presented as median (25th–75th IQR) values. For quantitative data, the independent-sample t-test was conducted for comparing the two groups of parameters indicating a normal distribution. Additionally, the Mann-Whitney U-test was utilized for comparing the two independent groups, and the Wilcoxon signed-rank test was conducted for the purpose of comparing the matched two groups exhibiting a nonnormal distribution. Besides, the Kruskal-Wallis test was utilized in the evaluation of three independent groups. The comparison of the categorical variables was performed by a χ^2 -test, and they are presented as a percentage and absolute values. Data analysis was carried out for the purpose of determining whether SCUBE1 was independently related to the AIS risk by employing univariate logistic and multivariate logistic regression models. The variables, such as age, gender, LV, smoking, body mass index, platelet count, hsCRP, fasting blood glucose and creatinine, were assessed by univariate analyses. Covariates with the value of $p < 0.01$ from univariate logistic regression were included for performing a multivariate analysis. A receiver operating characteristic (ROC) analysis was conducted for the purpose of

identifying the most sensitive SCUBE1 cut-off level in order to determine AIS patients. For the purpose of determining the cutoff level of SCUBE1, the highest Youden Index was utilized. A value of $p < 0.05$ was considered as statistically significant. A post-hoc power analysis was conducted on the basis of the SCUBE1 results, with the effect size of 0.60 ($\alpha: 0.05$), which showed the study power as 86% with the assumption of the parent distribution as a Laplace distribution.

Results

Table 1 contains information on the baseline features and laboratory parameters of the patient and control groups. No significant differences in terms of age, gender, the presence of diabetes mellitus, body mass index, smoking, and platelet count were determined. The patient group had considerably higher levels of low-density lipoprotein cholesterol and higher ratios of hypertension presence than the control subjects. The first-day plasma

Table 1: Baseline characteristics and laboratory parameters of the study population.

	Control group (n = 35)	AIS group (n = 35)	χ^2	p-Value
Age (years)	75 ± 6.4	79.4 ± 7.2		0.13
Men, n (%)	15 (42)	16 (46)	1.21	0.31
Hypertension, n (%)	18 (51)	27 (77)	4.22	0.01
Diabetes mellitus, n (%)	14 (40)	17 (48)	0.54	0.09
BMI (kg/m ²)	22.5 (19.5–25.7)	20.8 (19.4–25.5)		0.11
Smoking, n (%)	8 (22.9)	4 (11.4)	0.46	0.31
Total cholesterol (mg/dL)	184.9 ± 39.4	192.3 ± 39.4		0.47
Low density lipoprotein cholesterol (mg/dL)	130.2 ± 39.4	172.8 ± 35.7		0.02
High density lipoprotein cholesterol (mg/dL)	46 (32–74)	44 (31–86)		0.86
Triglycerides (mg/dL)	108 (49–284)	129 (59–296)		0.06
Creatinine (mg/dL)	0.82 (0.61–1.33)	0.74 (0.52–1.01)		0.42
Fasting glucose (mg/dL)	92 (79–114)	85 (53–109)		0.18
Hemoglobin (g/dL)	14.2 ± 1.5	14.2 ± 1.7		0.14
WBC (10 ³ /mm ³)	8.2 (4.9–11.2)	8.1 (5.2–14.1)		0.83
Platelet Count (10 ⁶ /mL)	220.34 ± 63.4	238.02 ± 72.2		0.76
Lesion volume (mm ³)		52.35 ± 12.34		
GCS		13.6 ± 3.02		
NIHSS		7.47 ± 4.12		
MRS		2.69 ± 1.51		
Etiology, n (%)				
LAT		11 (31.4)		
CE		9 (25.7)		
LI		15 (42.9)		
Others		2 (0.6)		

Bold values are used to demonstrate the statistical significance. All values are presented as mean value and SD, median value (25th–75th IQR) or n (%). AIS, Acute ischemic stroke; BMI, body mass index; CE, cardioembolic stroke; GCS, Glasgow Coma Score; HsCRP, high-sensitivity C-reactive protein; LAT, large-vessel atherothrombotic stroke; LI, lacunar infarct; MRS, modified Rankin Score; NIHSS, National Institutes of Health Stroke Scale; WBC, white blood cell.

hsCRP concentrations in the AIS group were found to be significantly higher than both the 7th day in the same group (Wilcoxon signed-rank test) and the control group (Mann-Whitney U-test) ($p=0.023$, $p=0.021$, respectively) (Table 2) (Figure 1). When the SCUBE1 levels were compared (Table 2), the first-day SCUBE1 levels were found to be considerably higher compared to the 7th day (Wilcoxon signed-rank test) and the control group SCUBE1 levels (Mann-Whitney U-test) ($p=0.001$, $p<0.001$, respectively). No difference was determined between the 7th-day SCUBE1 levels and the SCUBE1 levels of the control group (Mann-Whitney U-test) ($p>0.05$) (Figure 1).

As a result of the univariate logistic regression analyses, it was found out that the parameters presented below

had a considerable impact on the development of AIS: platelet count, body mass index (BMI), first-day hsCRP and SCUBE1 ($p<0.05$, Table 3). As a result, the mentioned variables were included in the multivariate reduced-rank model, demonstrating that the first-day hsCRP and SCUBE1 had considerable independent impacts on the development of AIS ($p=0.044$, $p=0.021$, respectively, Table 3).

Then, the patient group was separated into non-lacunar and lacunar stroke subgroups according to the lesion volume. Lesion volumes less than 2 mm^3 were accepted as lacunar stroke [13–15]. Then, the first-day SCUBE1 levels in the patients with non-lacunar and lacunar stroke were compared to those in the control group. Significantly

Table 2: AIS(1st day/7th day) and control group mean HsCRP and SCUBE1 levels.

	AIS group (n=35)		Control group (n=35)	p^1	p^2
	1st day	7th day			
HsCRP (mg/L)	4.29 (2.71–6.82)	3.17 (1.31–5.97)	3.03 (1.23–4.72)	0.023^a	0.021^b
SCUBE1 (ng/mL)	97.51 (27.34–105.71)	32.72 (9.87–42.25)	27.51 (8.21–41.97)	0.001^a	<0.001^b

Bold values are used to demonstrate the statistical significance.

p^1 , the p-values of the comparison of 1st and 7th days.

p^2 , the p-values of the comparison of 1st day and control group.

^aWilcoxon signed rank test, ^bMann-Whitney U-test. All values are presented median value (25th–75th IQR). AIS, Acute ischemic stroke; HsCRP, high-sensitivity C-reactive protein.

Figure 1: Comparison of hsCRP and SCUBE1 levels between AIS group (1st and 7th days) and control group and comparison of SCUBE1 levels between AIS group (non lacunar and lacunar stroke) and control group.

AIS, Acute ischemic stroke; hsCRP, high sensitive C-reactive protein; NS, non-significant.

Table 3: Univariate and multivariate logistic regression analysis representing the independent predictors of AIS.

Variables	Univariate regression analysis		Multivariate regression analysis	
	Exp(β) (95% CI)	p-Value	Exp(β) (95% CI)	p-Value
Age	1.034 (0.975–1.097)	0.260	–	–
Female sex	0.561 (0.217–1.449)	0.233	–	–
Smoking	1.000 (0.229–4.361)	1.000	–	–
Platelet count	0.736 (0.522–1.038)	0.081	0.777 (0.532–1.137)	0.194
Fasting glucose	0.975 (0.940–1.011)	0.177	–	–
Creatinine	0.209 (0.016–2.718)	0.231	–	–
WBC	1.015 (0.991–1.040)	0.225	–	–
BMI	0.787 (0.608–1.018)	0.068	0.789 (0.589–1.056)	0.111
HsCRP	1.221 (1.045–1.426)	0.012	1.201 (1.005–1.435)	0.044
SCUBE1 (1st day)	1.008 (1.003–1.014)	0.004	1.007 (1.001–1.013)	0.021

AIS, Acute ischemic stroke; BMI, body mass index; CI, confidence interval; HsCRP, high-sensitivity C-reactive protein; WBC, white blood cell.

higher plasma SCUBE1 levels were determined in the patients with non-lacunar stroke in comparison to those in the lacunar stroke patients and the control group (Kruskal-Wallis test) ($p < 0.01$). Likewise, the patients with lacunar infarction and the patients in the control group did not differ significantly in terms of their plasma SCUBE1 levels (Mann-Whitney U-test) ($p > 0.05$) (Figure 1).

When the Pearson analysis was performed, it was revealed that there was a positive linear association between the lesion volume and first-day plasma SCUBE1 levels in the AIS group ($p < 0.001$, $r = 0.832$) (Figure 2). The comparison of the relationship of the NIHSS and MRS values with the first-day SCUBE1 levels in the patient group was performed for the purpose of investigating the effect of SCUBE1 levels on the prognosis in patients with AIS. In the correlation analysis, plasma SCUBE1 levels showed a significantly positive correlation with NIHSS ($p < 0.01$, $r = 0.824$) and MRS values ($p < 0.01$, $r = 0.845$).

Figure 2: Positive correlation between lesion volume and plasma SCUBE1 levels.

Additionally, for the purpose of testing the diagnostic value of the first-day plasma SCUBE1 levels in order to predict AIS, ROC curves were produced. As can be seen from Figure 3, the first-day plasma SCUBE1 levels were comparable to the first-day hsCRP levels to distinguish patients with AIS from the controls (SCUBE1: Area under the curve (AUC) = 0.858, 95% CI: 0.788–0.929, $p < 0.001$;

Figure 3: ROC curve analyses of the predictive power of plasma SCUBE1 level.

AUC, Area under the curve; hsCRP, high sensitive C-reactive protein; CI, confidence interval; ROC, receiver operating characteristic.

hsCRP: AUC = 0.665, 95% CI: 0.557–0.773, $p = 0.006$). The plasma SCUBE1 concentration had a particular value in the prediction of AIS with a sensitivity of 78.4% and a specificity of 87.8%. 68.8 ng/mL was the best cut-off value computed by means of the Youden index.

Discussion

This study is the first study in the literature which shows that there may be a positive linear correlation between the lesion volume and acute period SCUBE1 levels in human AIS patients. Furthermore, it is revealed in our study that elevated plasma SCUBE1 levels could also be associated with high NIHSS and MRS values in the acute phase of AIS. On the other hand, SCUBE1 levels were determined to be a statistically significant independent predictor of the AIS development in our study's population. Additionally, we determined the probable dynamic levels of SCUBE1 to be varying based on time by comparing the 1st-day, 7th-day and control group SCUBE1 levels. The inflammatory process is a well-known stage of atherosclerosis development in the pathogenesis of AIS [2, 3]. It is known that the plasma levels of various molecules, including vascular cell adhesion molecules and inflammatory markers, increase in the acute phase of cerebral ischemia [2, 3].

C-reactive protein (CRP) represents a non-specific acute phase protein, which increases in inflammatory, infectious and neoplastic condition and in tissue damage [17]. HsCRP has been recently obtained by the use of high sensitivity assays for CRP. Although CRP and hsCRP define the same biochemical molecule, hsCRP is commonly used for stroke risk assessment and prognosis after stroke [18, 19]. There is a large number of studies showing the elevation of hsCRP levels in the acute period of stroke [18–22]. In addition, the dynamic course of hsCRP levels after acute cerebral ischemia was also revealed in the study of Yoldas et al. [19]. Likewise, in our study, the hsCRP levels were found to be higher on the first day than on the seventh day, and there was no difference between the values of the 7th day and the control group.

Besides, platelet activation is more pronounced in the acute period of AIS [4, 5]. Dai et al. found out that the elevation of plasma SCUBE1 level could be detected following 6 h at earliest, and it was maintained until the 84th h in AIS patients [9]. In the experimental study by Turkmen et al. the increase in SCUBE1 levels could be detected after 1 h at earliest [10]. In agreement with these studies, in the present study, the first-day SCUBE1 levels of the AIS patients were higher than those of the control

group, whereas the 7th-day SCUBE1 levels were the same as those among the controls (Table 2) (Figure 1). This result supports the view that SCUBE1 levels may be used as an indicator in human AIS patients in the acute phase. This is important because studies carried out recently have determined that the early diagnosis and treatment of AIS patients may decrease the impact of the disease on mortality and disability [23]. Novel reliable biochemical markers that indicate brain damage are needed for the purpose of excluding conditions that imitate stroke immediately to use novel therapeutic methods, including thrombolytics, thrombus aspiration and thrombectomy [24]. Based on these results, it may be claimed that SCUBE1 levels could be one of these biomarkers. Besides, the study by Dai et al. showed that plasma SCUBE1 had an independent association in AIS patients with plasma sCD40L, which represents a marker of platelet activation, in addition to inflammation [9]. In our study, the first-day SCUBE1 levels were compared to the first-day hsCRP level, which is a well-known inflammatory marker, and we found a linear correlation between the first-day SCUBE1 and hsCRP levels (Figure 3). The AUC values of SCUBE1 were greater than the AUC values of hsCRP in the ROC analysis (Figure 3). Based on these results, it may be stated that the first-day SCUBE1 levels can be used as a more valuable indicator than hsCRP for the early diagnosis of AIS patients. Additionally, we determined with the ROC analysis that SCUBE1 levels above 68.8 ng/mL can be used as an indicator with high sensitivity and specificity for the AIS diagnosis (Figure 3). Moreover, in our study, the multivariate analysis revealed that SCUBE1 level could be a statistically significant independent predictor of the AIS development (Table 3).

Then, the patient group was separated into two sub-groups in accordance with the lesion volume; the first-day SCUBE1 levels were found to be higher in the non-lacunar stroke group compared to both the lacunar stroke ($<2 \text{ mm}^3$) and the control groups (Figure 1). No significant difference was found between the lacunar stroke and control groups. LAT and cardioembolic stroke included acute platelet activation and inflammation, whereas lacunar stroke included the occlusion of the small penetrating artery because of lipohyalinosis from sustained hypertension, with fewer pieces of evidence of platelet activation and inflammation [15]. Therefore, our results also supported this finding.

Another result that we showed in this study was the positive linear correlation between the acute phase SCUBE1 levels and lesion volumes (Figure 2). Turkmen et al. revealed a strong association between atrophic neuron percentages and SCUBE1 levels in their histopathological

study of the rat brain in ischemia of thrombotic origin. In agreement with our study, it could be suggested that the above-mentioned association indicates an increase in SCUBE1 levels with the severity of brain damage. It may be predicted that AIS patients with higher SCUBE1 levels may have higher lesion volumes by measuring the SCUBE1 levels in the acute phase. When the relationship between the lesion volume and prognosis is considered, it may be stated that AIS patients with higher SCUBE1 levels may have a poor prognosis. Besides, according to our analysis, the elevation in the SCUBE1 levels was also associated with higher NIHSS and MRS scores. Baseline NIHSS and MRS are an evaluation of stroke severity with high reliability, and these have good correlation with stroke prognosis [25, 26].

In summary, the findings obtained from the present study revealed that SCUBE1 levels arising by the early period of ischemic stroke could be utilized as a biomarker in the early AIS diagnosis. Additionally, it may be claimed that higher SCUBE1 levels in patients having high lesion volumes show a strong association between SCUBE1 levels and neuronal injury in our study. Furthermore, plasma SCUBE1 concentration may predict the AIS severity in an independent way as evaluated by NIHSS and MRS. Additional studies including wider samples of cases, in addition to clinical studies with a validation group, are needed to validate these findings.

Our study has several limitations. It has a comparatively small sample size, which has limited statistical power for determining small alterations in subgroup analyses. Besides, only the plasma level of hsCRP was assessed as an inflammatory marker, other platelet activation markers such as sCD40L and inflammatory markers were not detected in this study. Additionally, the prognosis of the patients was evaluated only with NIHSS and MRS, and these patients were not followed up in the long-term.

Conflict of interests statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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