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The diagnostic value of SCUBE1 levels in acute ischemic stroke

[Akut iskemik inmede SCUBE1 düzeylerinin tanısal değeri]

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ABSTRACT

Aim: Stroke is the third most important cause of death after coronary artery disease and cancer, and the most important among those diseases leading to disability. Recent studies have shown that early diagnosis and treatment of patients presenting to the emergency department with stroke can reduce the effect of the disease on mortality and morbidity. The purpose of this study was to determine the diagnostic value of plasma SCUBE1, a novel biochemical marker thought to be capable of use in ischemic conditions, values in the diagnosis of acute ischemic stroke in the emergency department.

Materials and Methods: Thirty patients diagnosed with acute ischemic stroke at the Karadeniz Technical University Faculty of Medicine Emergency Department, Turkey, between May and October, 2011, and a control group of 30 healthy volunteers were included. An enzyme-linked immunosorbent assay kit was used to determine SCUBE-1 levels. Patient and control group plasma SCUBE1 values were compared.

Results: Mean age in the patient group was 74.50 ± 10.50, and 59.93 ± 12.63 in the control group. Mean 6th hour SCUBE1 value in the patient group was 25.104 ± 15.837 ng/ml, and the mean 12th hour SCUBE1 value was 27.395 ± 14.146 ng/ml. Mean control group SCUBE1 value was 35.019 ± 22.310 ng/ml. Control group SCUBE1 values were higher than those of the patient group. Sixth hour SCUBE value was statistically significant when the patient and control groups were compared with age-adjusted values ($p = 0.626$). No statistically significant difference was determined between 6th and 12th hour SCUBE1 values ($p = 0.334$).

Conclusion Plasma SCUBE1 values in acute ischemic stroke patients did not rise at significant levels compared to the control group, and are therefore not useful in the early diagnosis of acute ischemic stroke.

Key Words Emergency Department, acute ischemic stroke, SCUBE1

Conflicts of Interest The authors had not personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process and last twenty four months.

ÖZET

Amaç: İnme dünyada koroner arter hastalığı ve kanserden sonra üçüncü ana ölüm sebebi olup, sakatlığa yol açan hastalıklar arasında ise birinci sıradadır. Yapılan araştırmalar acil servise inme ilegelen hastaların erken teşhis ve tedavisi ile bu hastalığın mortalite ve morbidite üzerine etkilerini azaltabileceğini göstermiştir. Bu çalışmada acil servisde akut iskemik inme tanısında, iskemik durumlarda kullanılabileceği düşünülen yeni bir biyokimyasal belirteç olan plazma SCUBE1 düzeyinin tanısal değerini belirlemek amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya Karadeniz Teknik Üniversitesi Tıp Fakültesi Acil Servisinde Mayıs 2011-Ekim 2011 tarihleri arasında Akut İskemik İnme tanısı alan 30 hasta ve sağlıklı 30 gönüllüden oluşan kontrol grubu alınmıştır. SCUBE1 seviyelerini belirlemek için enzim bağlı immunosorbent deney kiti kullanılmıştır. Çalışmamızda hasta ve kontrol grubunun plazma SCUBE1 değerleri karşılaştırılmıştır.

Bulgular: Hasta grubunun yaş ortalaması 74.50 ± 10.50, kontrol grubunun yaş ortalaması 59.93 ± 12.63 idi. Hasta grubunun 6. saat SCUBE1 değeri ortalaması 25.104 ± 15.837 ng/ml, 12. saat SCUBE1 değeri ortalaması 27.395 ± 14.146 ng/ml, kontrol grubunun SCUBE1 değeri ortalaması 35.019 ± 22.310 ng/ml saptanmıştır. Kontrol grubu SCUBE1 değerleri hasta grubundan yüksek bulunmuştur. Yaşa göre düzeltilmiş değerlerle hasta ve kontrol grubu karşılaştırıldığında 6. saat SCUBE1 değeri istatistiksel olarak anlamlı bulunmamıştır ($p = 0.626$). 6. saat SCUBE1 ve 12. saat SCUBE1 değerleri karşılaştırılmış ve istatistiksel olarak anlamlı bulunmamıştır ($p = 0.334$).

Sonuç: Çalışmamızda akut iskemik inme hastalarında plazma SCUBE1 değerinin kontrol grubuna göre istatistiksel olarak anlamlı seviyelerde artmadığı, bu nedenle iskemik inme hastalarının erken tanısı için yararı olmayacağı sonucuna varılmıştır.

Anahtar Kelimeler: Acil Servis, Akut İskemik İnme, SCUBE1

Çıkar Çatışması: Yoktur.

Introduction

The World Health Organization (WHO) defines stroke as a clinical syndrome characterized by rapidly developing signs of focal loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin [1].

Stroke is the third most important cause of death after coronary artery disease and cancer, and the most important among those diseases leading to disability [2]. In the USA, approximately 795,000 people suffer strokes every year, of which 77% are first stroke and 23% repeat [3]. Ischemic strokes represent 85% of all stokes, the remaining 15% being hemorrhagic [4].

The inflammatory process is an important step for the development of atherosclerosis in the pathogenesis of cerebrovascular disease. Production of several molecules, such as vascular cell adhesion molecules, has been observed to increase in studies of experimental cerebral ischemia. Measurement of levels of adhesion molecules in plasma is thought to provide significant information regarding atherogenesis developing as a result of inflammation or endothelial dysfunction [5]. This study was planned in order to determine the diagnostic value of SCUBE1 [signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor)-like domain-containing protein 1], a novel biochemical marker thought to be capable of use for that purpose, in acute ischemic stroke patients.

SCUBE1 is a newly described cell surface molecule secreted and expressed throughout early embryogenesis. This protein consists of an N terminal signal peptide, 9 consecutive EGF-like repeats, a spacer region, cysteine-rich repeat motifs and a CUB domain at the C-terminal. SCUBE genes have been shown to be expressed in a number of developing tissues, such as the gonads, central nervous system, dermomyotome, the digital mesenchyme and limb buds during mouse embryogenesis. In addition to embryonic expression, SCUBE1 is also expressed in the endothelium and platelets [6].

These molecules are stored in alpha granules in inactive platelets, are translocated to the platelet surface following activation by thrombin, are released in the form of small, soluble particles and are incorporated into thrombus. SCUBE1 deposition has been determined immunohistochemically in the subendothelial matrix in advanced atherosclerotic lesions in humans [7]

This study was intended to determine the diagnostic value of plasma SCUBE1 values in the diagnosis of acute ischemic stroke in the emergency department.

Materials and Methods

Patients applying to the Karadeniz Technical University Faculty of Medicine Emergency Department, aged over 18, with a pre-diagnosis of ischemic stroke and who agreed to participate by completing the consent form were enrolled. Patients applying to the Karadeniz Technical University Faculty of Medicine Emergency Department aged under 18, patients diagnosed at the department with acute coronary syndrome, acute kidney failure, chronic kidney failure, hemorrhagic stroke, acute peripheral artery blockage, liver failure, acute pulmonary edema, cardiopulmonary arrest, sepsis, acute mesenteric ischemia or pulmonary thromboembolism, multitrauma patients, and patients applying to the emergency system more than 12 h after onset of symptoms were excluded. Ten patients attending the emergency department stroke clinic and included in the study were subsequently excluded for lack of data.

Karadeniz Technical University Faculty of Medicine Local Ethical Committee approval was granted for the study. Following granting of approval, patients applying to the Karadeniz Technical University Faculty of Medicine Emergency Department were consecutively enrolled over 5 months.

Once patients applying to the emergency department with suspected acute ischemic stroke had been evaluated at triage and their vital findings investigated, the hospital file and study form were given to the emergency department physician. Patients were examined by the emergency department physician and those meeting the inclusion criteria were enrolled.

Patients' demographic data, symptoms, previous diseases and physical examination findings were recorded on the study form. Detailed neurological examination findings and Glasgow Coma Score (GCS) were also recorded on the form. The National Institutes of Health Stroke Scale (NIHSS) was applied by the examining emergency department physician and scores were again recorded on the form. Electrocardiography (ECG) with 12 derivations was performed on all patients included in the study.

Patients' brain tomographies were evaluated together with a radiologist. All cases other than ischemic CVE were excluded.

Brain tomography was performed using a Siemens Sensation 16 Slice device. When required, cranial MRI was performed with a Siemens Symphony Magnetom 1.5 tesla device.

Full blood count, routine biochemistry, hemorrhage parameters and cardiac enzymes were investigated from blood specimens collected from the patient and control groups. In addition, 2 cc blood was collected in citrate tubes from both groups for investigation. Specimens were centrifuged for 15 min in a 4000-cycle centrifuge device at (+4) degrees. One cubic centiliter of serum was placed in Eppendorf tubes, and these were kept at -80 degrees until assay. Twenty-four hours before SCUBE1 investigation, the Eppendorf tubes were removed and placed in a +4 degree environment. Sera thawed gradually over 24 h and SCUBE1 levels were measured by raising them to room temperature.

An enzyme-linked immunosorbent assay kit (Catalog No. CSBE15005 h, Cusabio Biotech Co., Wuhan, Hubei, P.R. China) was used to determine SCUBE-1 levels, following the manufacturer's instructions. Specimen absorbances were determined

on a VERSA max tunable microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 450 nm. Results were expressed as ng/ml.

SPSS (Statistical Package for Social Sciences for Windows v.17.0) was used for statistical analysis. Data were evaluated using descriptive techniques (mean, standard deviation). In addition, for quantitative data the Independent Samples T Test (Independent Sampling t test) was used in the comparison among groups of parameters exhibiting normal distribution and the paired T test for determining the significance of differences between the two matched groups. Categoric data were expressed as number and percentage. Pearson's chi-square test was used in the analysis of categoric data. Pearson Correlation Analysis was used to determine how one variable was affected as the value of another variable changed. Analysis of Covariance was used to measure the common effect of more than one independent variable on a specific independent variable. Results were evaluated at a 95% confidence interval with significance set at $p<0.05$.

Results

Thirty patients diagnosed with acute ischemic stroke and 30 healthy volunteers to constitute a control group were included. Thirteen (43.4%) of the patient group were male and 17 (56.6%) female. Compared to 16 (53.4%) males and 14 (46.6%) females in the control group. Mean age ± standard deviation (SD) in the patient group was 74.50 ± 10.50, and 59.93 ± 12.63 in the control group. The difference between the patient and control groups in terms of age was significant (independent samples t test, p=<0.001). Patients' symptoms at time of presentation were sudden loss of consciousness in 12 (40%), impaired speech in 8 (26.6%), loss of strength on the left side in 7 (23.4%), loss of strength of the right side in 2 (6.6%) and facial numbness in 1 (3.4%).

Five (16.7%) members of the patient group had a history of coronary artery disease (CAD). HT was present in 20 (66.7%) patients, hyperlipidemia (HL) in 2 (6.7%), diabetes mellitus (DM) in 6 (20%), transient ischemic attack (TIA) 1 (3.3%), CVE in 7 (23.3%) and a history of cigarette use in 7 (23.3%), while no diseases such as malignity or alcohol use were present. Control group subjects had no history of diseases such as CAD, HT, HL, DM or malignity, or use of cigarettes or alcohol. The presence of HT was statistically significant when the patient and control group histories were examined (Pearson chi-square, $p<0.001$). No significant difference was determined between the groups in terms of CAD, HL, DM, TIA, CVE or cigarette use ($p > 0.005$).

Normal sinus rhythm was determined in 13 (43.4%) of the patient group, atrial fibrillation in 9 (30%), atrial flutter in 1 (3.3%), ST0T alteration in 3 (10%), ventricular extrasystole in 3 (10%) and left branch block in 1 (3.3%). Time of onset of atrial fibrillation could not be determined.

Patients' mean systolic blood pressure was 147.33 ± 31.61 mmHg and mean diastolic blood pressure 87.33 ± 28.15 mmHg. Patients' mean GCS was GKS 13.10 ± 2.41 and mean NIHSS 7.50 ± 6.86. In terms of correlation between GCS and NIHSS and 6th and 12th hour SCUBE1 values, and very powerful correlation was observed between GCS and NIHSS scores ($r = -0.873$, $p < 0.001$). NIHSS scores decrease as GCS rises. A very weak positive correlation was seen between GCS and 6th and 12th hour SCUBE1 ($r = 0.214$, $p =0.255$, $r = 0.005$, $p = 0.980$). there was also a very weak negative correlation between NIHSS score and 6th hour SCUBE1 ($r = -0.120$, $p = 0.529$) and a very weak positive correlation with 12th hour SCUBE1 ($r = 0.116$, $p = 0.541$) (Table 1).

In terms of patient group laboratory values, mean platelet value was 245,733.3 ± 78,565.99, mean creatinine 0.97 ± 0.28, mean PT 13.65 ± 1.33, mean PTT 30.56 ± 7.01, mean INR 1.19 ± 0.14 and mean troponin 0.017 ± 0.021. Platelet value was moderately negatively correlated with 6th hour SCUBE1, there was no correlation between PT and 6th hour SCUBE1, while a moderate positive correlation was determined between troponin value and 6th hour SCUBE1 (Table 2).

Patient group mean 6th hour SCUBE1 value was 25.104 ± 15.837 ng/ml, and mean 12th hour SCUBE1 27.395 ± 14.146 ng/ml. Control group mean SCUBE1 value was 35.019 ± 22.310 ng/ml (Figure 1). Control group SCUBE1 value were higher than those of the patient group.

When patient and control group 6th hour SCUBE1 values were compared by age, the age factor was statistically significant (independent samples t test, $p = < 0.001$), while 6th hour SCUBE1 value was statistically insignificant (independent samples t test, $p = 0.052$). In order to eliminate the age factor, age adjusted means were taken with analysis of covariance. Mean adjusted patient group 6th hour SCUBE1 value was 25.921 ng/ml, while control group mean adjusted 6th hour SCUBE1 value was 33.341 ng/ml (Figure 2). When the patient and control groups were compared with age-adjusted values, 6th hour SCUBE1 values were nit statistically significant (analysis of covariance, $p = 0.626$).

Sixth and twelfth hour SCUBE1 values were compared among themselves, but no statistical significance was determined (paired t test, $p = 0.334$). Patient and control group mean SCUBE1 and p values are shown in Table 3.

Brain CT was performed on all patients arriving at the emergency department with suspected ischemic stroke. Diffusion MRI was performed in those with no visible lesions. Five (16.6%) patients were diagnosed with brain CT and 25 (83.4%) cerebral diffusion MRI.

Table 1. Correlation between patients' GCS and NIHSS scores and 6th and 12th hour SCUBE1 values

	Mean ± SD	Pearson correlation analysis, r and p values	
		SCUBE1 6th hour	SCUBE1 12th hour
GCS	13.10 ± 2.41	r = 0.214 p = 0.255	r = 0.005 p = 0.980
NIHSS	7.50 ± 6.86	r = -0.120 p = 0.529	r = 0.116 p = 0.541

Table 2. Correlation between patients' laboratory values and 6th and 12th hour SCUBE1 values

	Mean ± SD	**Pearson correlation analysis, r and p values**	
		SCUBE1 6th hour	**SCUBE1 12th hour**
Platelet	245733±78565	**r =-0.302 p = 0.105**	r = -0.083 p = 0.664
Creatinine	0.97 ± 0.28	r = -0.114 p = 0.556	r = -0.208 p = 0.279
PT	13.6 ± 1.3	r = 0.00 p = 0.999	r = -0.087 p = 0.648
PTT	30.5 ± 7	r = 0.095 p = 0.618	r = -0.034 p = 0.859
INR	1.2 ± 0.14	r = -0.012 p = 0.952	r = -0.057 p = 0.766
Troponin	0.017 ± 0.021	**r = 0.264 p = 0.159**	r= -0.013 p = 0.944

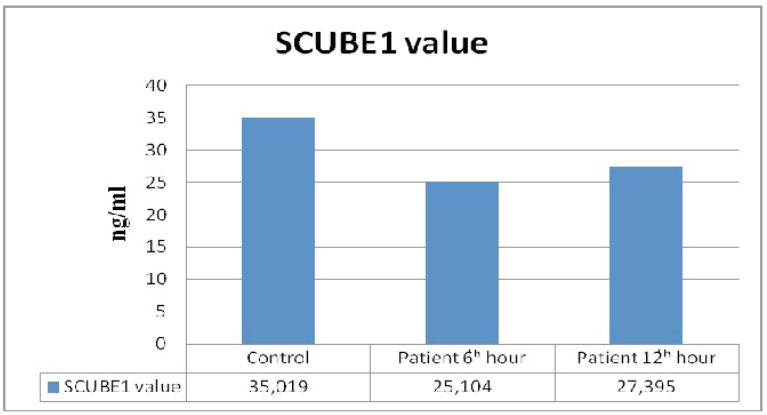

Figure 1. Control and patient group mean 6th and 12th hour SCUBE1 values

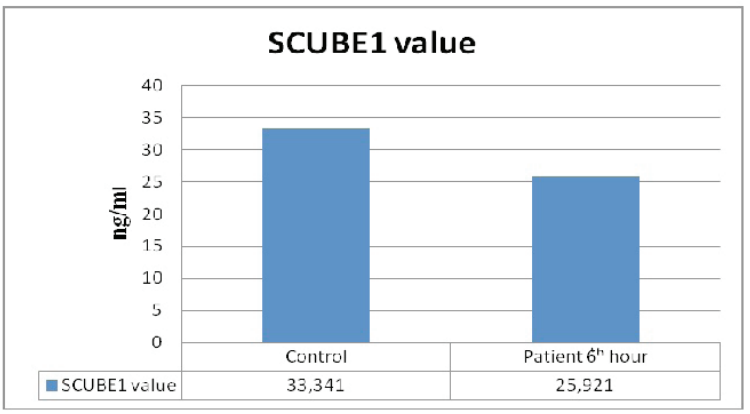

Figure 2. Age-Adjusted control and patient group mean 6th hour SCUBE1 Values

Table 3. Patient and control group mean SCUBE1 and p values

	SCUBE1 value ng/ml (mean ± SD)	p values	
Control	35.019 ± 22.310	0.052*	
Patient 6th hour	25.104 ± 15.837	0.626**	0.334***
Patient 12th hour	27.395 ± 14.146		

* independent samples t test, ** analysis of covariance, *** paired t test

Table 4. Correlation between NIHSS and GCS scores and 6th and 12th hour SCUBE1 values of the patients that died

	Dead patients (n=7) (mean ± SD)	Pearson chi-square p value
NIHSS	12.29 ± 7.15	0.067
GCS	11.86 ± 2.79	0.195
6th hour SCUBE1 value	21.19 ± 11.39	0.373
12th hour SCUBE1 value	28.08 ± 9.89	0.858

Twenty (66.7%) were hospitalized in the emergency department, 8 (26.7%) in intensive care while 2 (6.6) left the emergency department of their own volition. Patients were monitored for 6 months in terms of death, new CVE, MI, CPR requirement and MV requirement. Five (16.6%) patients died on the ward and 2 (6.6%) following discharge in the 2nd month after diagnosis. One (3.3%) patient was hospitalized with a diagnosis of new CVE. No death, new CVE or need for MI or CPR was observed in the other patients.

No statistically significant difference was determined between the NIHSS and GCS scores and 6th and 12th hour SCUBE1 values of the 7 patients that died (Table 4).

Discussion

Stroke is the third most important cause of death after coronary artery disease and cancer, and the most important among those diseases leading to disability [2]. Research has shown that early diagnosis and treatment of patients presenting to the emergency department with stroke can reduce the effect of the disease on mortality and morbidity. [8]. The development of new therapeutic techniques, such as thrombolytics, and the interest in new, reliable biochemical markers showing brain damage in order to exclude conditions imitating stroke, such as complicated migraine and Todd's paralysis, has recently increased [9]. One of these biomarkers, SCUBE1, was evaluated in this study.

Dai et al. reported that SCUBE1 protein can be detected within 6 hours after the onset of ischemic symptoms, that it is not sensitive but can be a good marker in acute thrombotic diseases [7]. In an experimental study, Türkmen et al. reported that SCUBE1 levels rise rapidly in the 2nd hour of ischemia in acute mesenteric ischemia and then continue to rise [10]. Özkan et al. reported higher SCUBE1 levels in hypertensive patients compared to the control group in a clinical study and determined a positive correlation between them. They attributed this to hypertension rising in platelet activation and its association with endothelial dysfunction [11]. Ulusoy et al. found higher SCUBE1 levels in hemodialysis patients than control group. When they compared SCUBE1 and sCD40L levels, they found a positive correlation between them in the same study. This correlation shows a platelet activation associated rise in SCUBE1 levels [12].

Mentese et al. made a study to determine whether SCUBE1 levels were higher in cancer patients. They reported that SCUBE1 levels were higher in gastric cancer patients than the control group's SCUBE1 levels. In this study they suggested that SCUBE1 levels can be used as a marker of gastric cancer and post-treatment recurrence in gastric cancer patients [13].

Dai et al. determined plasma SCUBE1 at the 6th hour after symptom onset at the earliest and the 84th hour at the latest in patients with acute ischemic stroke [7]. We determined and compared patient SCUBE1 levels at the 6th and 12th hours to the control group. Both 6th and 12th hour SCUBE1 levels were lower compared to the control group. When we compared 6th and 12th hour SCUBE1 levels within themselves we determined no statistical significance. Dai et al. found that advanced age tended to increase plasma SCUBE1 levels and cigarettes to reduce them [7]. When we compared our patient and control groups with age-adjusted values, 6th hour SCUBE1 value was statistically significant. Further, 23.3% of our patients smoked, and there was

no significant difference with the control group. Dai et al. concluded that soluble plasma SCUBE1 was obtained from platelets stimulated via proteolytic division and can play pathological roles by facilitating platelet adhesion/agglutination and subsequent thrombus formation [7]. In that respect, when we looked at our patients' platelet levels, mean platelet level was determined as 245,733.3. Platelet value was moderately negatively correlated with 6th hour SCUBE1 value and weakly negatively correlated with 12th hour SCUBE1. A moderate positive correlation was seen between the troponin laboratory parameter and 6th hour SCUBE1 value. Dai et al. observed no correlation between single measurement plasma SCUBE1 value and troponin value in acute coronary syndrome patients. Apart from acute coronary syndrome, elevated troponin shows subclinical myocardial damage [14]. A correlation has been found in studies between elevated troponin and mortality in stroke patients [15].

Our patients were monitored for 6 months after diagnosis, during which time 7 died. Mean NIHSS score in the patients that died was 12.29, mea GCS 11.86, 6th hour SCUBE1 21.19 ng/ml and 12th hour SCUBE1 28.08 ng/ml. No significant correlation was determined between the NIHSS and GCS scores and 6th and 12th hour SCUBE1 values of the 7 patients that died. Dai et al. reported that plasma SCUBE1 concentration was an independent marker for NIHSS and that basal NIHSS value was reliable in the evaluation of stroke severity and well correlated with stroke prognosis [7]. In our study, there was a very weak correlation between NIHSS score and 6th hour SCUBE1 and a very weak correlation with 12th hour SCUBE1, and also a significant correlation between basal NIHSS value and the patients that died.

Limitations

The patients were enrolled at the end of spring and summer. A lengthy period is needed to eliminate seasonal differences. Since our hospital is a tertiary institution, most of our patients are referred and patients with additional problems. Blood collection timing was therefore selected as the 6th hour after onset of symptoms. Another limitation is that the number of cases could not be kept high.

Conclusions

In contrast to the data in the literature, we concluded that SCUBE1 in patients with acute ischemic stroke were not significantly higher than those of the control group, and that they are therefore not useful in the early diagnosis of ischemic stroke. There was a moderate negative correlation between platelet value and 6th hour SCUBE1 value and a moderate positive correlation between troponin value and 6th hour SCUBE1 value. No significant correlation was observed between the NIHSS and GCS scores and 6th and 12th hour SCUBE1 values of the patients that died throughout the 6-month monitoring period. These markers had no effect on mortality.

Conflicts of Interest The authors had not personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process and last twenty four months.

Ethical approval

The study is approved by judgement with 2011-14 reference number of Local Ethical Committee.

References

[1] Ropper AH, Brown RH. Cerebrovascular diseases. Çoban O, Bebek N (Translated). 8th Edition, Ankara: Günes Bookstore, 2006.

[2] Vila N, Castillo J, Davalos A, Chamorro A. Proinflammotory Cytokines and Early Neurological Worsening in Ischemic Stroke. Stroke 2000;31:2325-2329.

[3] Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S et al. Heart disease and stroke statistics 2010 update: a report from the American Heart Association. Circulation 121: e46, 2010. Epub Dec. 17, 2009.

[4] Grotta JC, Helgason C. Ischemic stroke pathophysiology. J Stroke Cerebrovasc Dis. May-Jun 1999;8(3):114-116

[5] Stephen G. Waxman. Correlative Neuroanatomy. Lange Medical Boks, 1999. Sf 168-172.

[6] Tu CF, Yan YT, Wu SY, Djoko B, Tsai MT, et al. J.Biol. Chem. 2008;283:12478-12488.

[7] Dai DF, Thajeb P, Tu CF, Chiang FT, Chen CH, et al. Plasma concentration of SCUBE1, a novel platelet protein, is elevated in patients with acute coronary syndrome and ischemic stroke. J. Am. Coll. Cardiol. 2008;51:2173-80

[8] Phillip AS, Caroline AT. Transient ischemic attack, and other central focal conditions. Judith ET, Gabor DK, Stapczynski JS (editors). Emergency Medicine: a comprehensive study guide. 6th ed., North Caroline: McGraw-Hill, 2006:1382-1390.

[9] Laskowitz DT, Grocott H, Hsia A, et al. Serum markers of cerebral ischemia. J Stroke Cerebrovasc Dis. Jul-Aug 1998;7(4):234-241.

[10] Turkmen S, Mentese S, Mentese A, Sumer AU, Saglam K, et al. The value of signal peptide-CUB-EGF domain-containing protein 1 and oxidative stress parameters in the diagnosis of acute mesenteric ischemia. Acad Emerg Med. 2013; 20 (3):257-64.

[11] Ozkan G, Ulusoy S, Mentese A, Karahan SC, Cansiz M. New Marker of Platelet Activation, SCUBE1, Is Elevated in Hypertensive Patients. Am J Hypertens. 2013; 26 (6):748-53.

[12] Ulusoy S, Ozkan G, Menteşe A, Yavuz A, Karahan SC, Sümer AU. Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1) level in hemodialysis patients and parameters affecting that level. Clin Biochem. 2012; 45(16-17): 1444-9.

[13] Mentese A, Fidan E, Sumer AU, Karahan SC, Sonmez M et al. Is SCUBE 1 a new biomarker for gastric cancer? Cancer Biomark. 2012; 11(5): 191-5.

[14] Elmalı E, Karaeren Z, Özdöl Ç, Akan ÖA. Akut koroner sendrom şüpheli hastalarda kardiyak troponin T ve Tn-I'nın karşılaştırılması. Turkish Journal of Biochemistry. 2005; 30:212-215.

[15] Maliszewska M, Fiszer U, Palasik W, Tadeusiak W, Morton M. Prognostic role of troponin I level in ischemic stroke: preliminary report. Pol Merkuriusz Lek 2005; 19:158-161.