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Serum adropin levels in patients with acute ischemic stroke

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Abstract
Stroke is one of the principal diseases leading to death and permanent disability worldwide. One of the main causes of stroke is atherosclerosis. A decrease in Adropin levels has been identified as an independent predictor of atherosclerosis development. The purpose of this study was to determine serum Adropin levels in patients with acute ischemic stroke (AIS) and to investigate the relation between Adropin levels and stroke. Fifty-nine patients presenting to the emergency department with symptoms of stroke and diagnosed with AIS, and 51 healthy individuals representing the control group, were included in the study. Patient and control group demographic data and clinical characteristics were recorded. Serum Adropin levels were studied in blood specimens collected from both groups. The results were then compared. Patient group serum Adropin levels were significantly lower than those of the control group (2.16±0.76, and 2.72±1.01, respectively p=0.002). Multivariate logistic regression analysis identified a low serum Adropin level as an independent predictor of stroke (OR=0.420, 95% Cl=0.221-0.801, p=0.008). We also determined significant inverse correlation between serum Adropin levels and glucose levels (r= -0.345, p=0.013). Our study suggested that, low serum Adropin levels are associated with AIS. Adropin may be used as a screening tool for AIS.

Keywords: Adropin, ischemic stroke, atherosclerosis, endothelial dysfunction, biomarker

Introduction

Stroke is the second most common cause of death worldwide, and one of the principal diseases leading to disability. Approximately 85% of all strokes are ischemic, while 15% are hemorrhagic in origin [1]. Ischemic stroke occurs as the result of a thrombotic or embolic event causing a decrease in cerebral blood flow. Thrombosis involves an obstructive process that prevents blood flow to regions of the brain. Thrombosis generally derives from clot formation in ulcerated atherosclerotic plaques [2]. The development of atherosclerosis is a complex, multifactorial event involving endothelial dysfunction, vascular inflammation, vascular smooth muscle cell proliferation, thrombus formation, and monocyte infiltration [3]. Atherosclerosis is one of the main modifiable risk factors associated with approximately two-thirds of cases of acute ischemic stroke (AIS) [4]. Intracranial atherosclerotic disease may be isolated or else occur concurrently with systemic atherosclerosis involving extracranial, coronary or peripheral arteries or other arterial beds [5].

Adropin is a novel bioactive protein released from the liver and brain and coded by the energy homeostasis gene (ENHO). Adropin has been shown to contribute to atherogenesis development and energy homeostasis closely linked to its progression, and to maintenance of insulin response [6]. Recent studies have determined that it is also released from coronary artery endothelial cells and plays a potential endothelial-protective role [7]. Endothelial dysfunction plays a key role in the early period of atherogenesis and is essential in the onset of coronary artery disease [8]. A decreased serum Adropin level is an independent predictor clinically associated with coronary atherosclerosis [9]. Yu et al. determined significantly lower serum Adropin levels in patients with acute myocardial infarction (AMI) than in patients with stable angina pectoris [10]. Similarly in their study of non-ST AMI patients, Ertem et al. reported that low Adropin can be used as an alternative for estimating the severity of coronary artery disease [11].

We think that levels of serum Adropin, closely linked to the development of atherosclerosis in previous studies, and determined to be significantly low in myocardial infarction patients, may occupy

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an important place in the pathogenesis of AIS, in the etiology of which atherosclerosis plays a significant role. The purpose of this study was to determine serum Adropin levels in patients with AIS and to investigate the relation between Adropin levels and AIS.

Material and Methods

Study design

The research was performed as a single-center, prospective and clinical study in the emergency department between October 2017 and April 2018. The local ethical committee approved the study (No. KAEK-41). Inform consent was obtained from each study patient and control group.

Patients and methods

Patients presenting to the emergency department with symptoms of stroke in the six-month period and diagnosed with AIS were included in the study. Patients aged under 18, pregnant women, or subjects with hemorrhagic stroke, active infection, acute pulmonary edema, severe hepatic and renal disorder, autoimmune disorders, acute coronary syndrome, pulmonary embolism, mesenteric ischemia, peripheral artery disease or cardiopulmonary arrest were excluded. A control group was consisting of volunteers was also established. In order for these to be similar to the patient group in terms of age, they were generally selected from individuals accompanying patients presenting to the emergency department and with no active symptoms in the presence of chronic disease. The study population was divided into patient and control groups, whose results were finally compared with one another.

Patients' vital signs at time of presentation, Glasgow coma scores, electrocardiogram findings, demographic data, symptoms, histories of disease, and physical examination findings were recorded onto study forms. Body mass index (BMI) was calculated by measuring height and weight at time of presentation to the emergency department for all patients. Brain computed tomography (CT), and diffusion-weighted magnetic resonance imaging (DW-MRI) were performed on all patients at time of presentation. AIS was diagnosed based on brain CT and DW-MRI evaluation by a radiologist.

Sample collection and measurement of Adropin levels

Blood samples were collected from all patients and control groups at time of presentation for routine tests, and complete blood count, biochemical parameters, lipid profile, and cardiac enzyme level values were recorded onto the study forms. In addition, 5 cc venous blood specimens collected from the patient and control groups for Adropin level measurement were placed into gel-containing biochemistry tubes and subjected to rapid centrifugation, after which the resulting serum specimens were stored at -80 C until being used for analysis. Serum Adropin levels were analyzed using an enzyme-linked immunosorbent assay reagent kit (SunRed Biological Technology Co., Shanghai, PRC, Catalogue No. 201-12-3107), and the results were expressed as ng/mL.

Statistical analysis

Statistical analysis was performed on SPSS (Statistical Package for Social Sciences for Windows v.17.0) software. The Kolmogorov-Smirnov test was used to determine whether data complied with normal distribution. The independent samples T test was used to compare normally distributed data between two groups, and the Mann Whitney U test was employed for non-normally distributed variables. Correlation analysis was performed using Pearson's correlation test for normally distributed data and Spearman's correlation test for non-normally distributed data. Multivariate logistic regression analysis was employed to determine independent disease predictors. P values < 0.05 were regarded as statistically significant.

Results

Fifty-nine patients and a control group of 51 volunteers were included in the study. Forty-nine patients (82.1%) were admitted to the ward, and 10 (16.9%) to the intensive care unit. The study population baseline demographic and clinical characteristics are shown in Table 1, and laboratory findings are given in Table 2. No significant difference in age was determined between the patient and control groups (p = 0.134). BMI, and systolic and diastolic blood pressures were significantly higher in patient group than control group (p=0.014, p=<0.001, and p=0.003, respectively). In laboratory findings; white blood cells, platelet, glukose, urea, creatine, total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and troponin T levels significantly higher and high density lipoprotein cholesterol (HDL-c) levels were significantly lower in patient group than control group.

Table 1. Baseline demographic and clinical characteristics of the patient and control groups

Variables	Patient group (n=59)	Control group (n=51)	p value
Sex; n (%)			
• Male	25 (42.4)	18(35.3)	
• Female	34 (57.6)	33(64.7)	
Age (years); mean ± SD	73.26 ± 11.47	69.88 ± 11.83	0.134
BMI (kg/m2); mean ± SD	28.46 ± 3.67	26.37 ± 5.05	**0.014**
Blood pressure; median (min-max)			
• SBP (mm Hg)	160 (100-220)	130 (90-160)	**< 0.001**
• DBP (mm Hg)	80 (50-110)	80 (50-100)	0.003
Comorbid disease; n (%)			
• CAD	18 (30.5)	11 (21.6)	
• HT	40 (67.8)	25 (49)	
• HL	5 (8.5)		
• DM	19 (32.2)	8 (15.7)	
• TIA	4 (6.8)		
• Stroke	17 (28.8)		
• Smoking	10 (16.9)	2 (3.9)	
Symptoms start time (hour); median (min-max)	5 (0.5-72)		
Symptoms ; n (%)			
• Left hemiparesis	17 (28.8)		
• Right hemiparesis	12 (20.3)		
• Syncope	9 (15.3)		
• Aphasia	11 (18.6)		
• Dizziness	8 (13.6)		
• Visual deficit	2 (3.4)		
Glasgow Coma Score at admission; n(%)			
• 11	3 (5.1)		
• 12	2 (3.4		
• 13	4 (6.8)		
• 14	8 (13.6)		
• 15	42 (71.2)		
ECG findings at admission; n (%)			
• Normal sinus rhythm	41 (69.5)		
• Atrial fibrillation	18 (30.5)		

Table 2. Laboratory findings of the patient and control groups

Variables	Patient group (n=59)	Control group (n=51)	p value
Complete blood count			
• WBC (U/L)	9824 ± 3765	6002 (4690-11000)	**<0.001**
• Hemoglobin (g/dL)	13.360 ± 1.730	13 (11.3-16)	0.652
• Platelet (U/L)	272000 (126000-482000)	242000 (153000-308000)	**0.006**
Biochemical analysis			
• Glucose (mg/dL)	140 (63-417)	99 (83-157)	**<0.001**
• Urea (mg/dL)	42.24 ± 19.72	12 (10-42)	**<0.001**
• Creatine (mg/dL)	0.99 ± 0.32	0.85 (0.58-1.19)	**0.015**
Lipid profile			
• TC (mg/dL)	187.25 ± 44.7	153 (120-180)	**<0.001**
• TG (mg/dL)	151.7 ± 67.08	136 (71-149)	0.243
• LDL-c (mg/dL)	113.71 ± 36.49	93 (62-99)	**<0.001**
• HDL-c (mg/dL)	40,75 ± 10,76	48 (36-63)	**0.003**
Cardiac markers			
• CK-MB (ng/mL)	2.34 (0.1-35.8)	2.8 (1-4)	0.525
• Troponin T (µg/L)	0.014 (0.001-0.32)	0.001 (0.001-0.049)	**<0.001**
Serum Adropin Levels (ng/mL)	2.16 ± 0.76	2.72 ± 1.01	**0.002**

All values are presented as mean ± SD or median value (min-max). WBC = White blood cells, TC = total cholesterol, TG = triglycerides, LDL-c = low density lipoprotein cholesterol, HDL-c = high density lipoprotein cholesterol, CK-MB = creatine kinase-MB

Table 3. Multivariate logistic regression analysis for predictor of AIS

Variables	OR (95% Cl)	p value
Age	1.013 (0.966-1.062)	0.597
Female (yes)	0.640 (0.226-1.814)	0.401
BMI	1.070 (0.959-1.194)	0.225
Adropin (ng/ml)	0.420 (0.221-0.801)	**0.008**
Smoking (yes)	1.801 (0.244-13.268)	0.564
Presence of DM	1.196 (0.354-4.041)	0.773
Presence of HT	1.044 (0.328-3.325)	0.942
Presence of CAD	1.232 (0.379-4.005)	0.729
Presence of Stroke	18.437 (2.024-167.892)	**0.010**

OR = odds ratio, CI = confidence interval, other abbreviations are as in Table 1.

Table 4. Correlation between adropin and clinical characteristics and laboratory findings in patient group

Variables	Correlation coeffient	p value
Age	0.260	0.063
BMI	-0.217	0.123
SBP	-0.094	0.509
DBP	0.204	0.147
TC	-0.106	0.480
TG	-0.128	0.397
LDL	-0.018	0.904
HDL	-0.111	0.465
WBC	-0.093	0.514
Hemoglobin	-0.151	0.286
Platelet	-0.250	0.073
Glukoz	-0.345	**0.013**
Troponin	-0.056	0.722

All abbreviations are as in Table 1 and Table2.

Serum Adropin levels in our patient group were significantly lower than those in the control group (2.16 ± 0.76, and 2.72 ± 1.01, respectively, p=0.002) (Table 2).

We applied multivariate logistic regression analysis to the variables shown in Table 3 in order to determine independent predictors for AIS. Our findings identified a low serum Adropin level and previous history of stroke as independent predictors of AIS (OR=0.420, 95% Cl=0.221-0.801, p=0.008 and OR=18.437, 95% Cl=2.024-167.892, p=0.010).

Correlations between serum Adropin levels and clinical characteristics and laboratory findings are shown in Table 4. The only statistically significant correlation was inverse correlation between serum Adropin levels and glucose levels (r= -0.345, p=0.013).

Discussion

Recent studies have determined that low Adropin levels are associated with atherosclerosis and cardiovascular diseases. In the present study, we investigated the relation between Adropin and AIS, in the etiology of which atherosclerosis occupies a very important place. We observed significantly lower serum Adropin levels in AIS patients than in the control group. In addition, multivariate logistic regression analysis identified Adropin as an independent predictor for AIS. Moreover, we determined significant inverse correlation between serum Adropin levels and glucose levels in AIS patients. Based on our review of the available databases, ours is the first clinical study to investigate the relation between serum Adropin levels and AIS.

Adropin is released by numerous tissues and organs, including the central nervous system. This peptide has been determined to play an important role in the development of stroke, schizophrenia and bipolar disorder and various diseases of the central nervous system,

such as Alzheimer's, Parkinson's, and Huntington's diseases [12]. Adropin regulates the expression of endothelial nitric oxide synthase (eNOS), responsible for the synthesis of vascular nitric oxide (NO). Adropin deficiency is associated with decreased NO bioavailability in the endothelium [5]. Decreased bioavailability of NO is a cardinal characteristic of endothelial dysfunction, which leads to the development of atherosclerosis [13]. Adropin acts as a potential protective regulator in atherogenesis and cardiovascular diseases [7]. Serum Adropin levels have been determined to exhibit inverse correlation with serum levels of homocysteine-a, a potential risk factor for atherosclerosis and cardiovascular diseases, and severity of atherosclerosis [14].

Serum Adropin levels were lower in our AIS patients than in the control group. Yu et al. observed low serum Adropin levels in AMI patients, and Ertem et al. in patients with non-ST AMI [10,11]. Our findings support the results of these studies involving AMI patients, in the etiology of which atherosclerosis plays an important role, similarly to AIS. In addition, Yang et al. reported that synthetically administered Adropin exhibited a protective effect against endothelial barrier dysfunctions under ischemic conditions in the brain in a rat model [15]. It may therefore be deduced that low serum Adropin levels may result in endothelial barrier dysfunction in ischemic stroke. In support of this hypothesis, we determined, for the first time in the literature, lower serum Adropin levels in AIS patients compared to a control group. Considering our findings together with those of Yang et al., it may be concluded that low Adropin levels in AIS patients are associated with vasogenic edema and microvascular damage occurring in the brain resulting from an increase in blood-brain barrier permeability developing in association with endothelial barrier dysfunction.

Our multivariate logistic regression analysis results showed that Adropin is an independent predictor of stroke. Although there are generally recognized criteria for the diagnosis and treatment of stroke, approximately 25% of all diagnosed patients actually have diseases that mimic ischemic stroke. Biomarkers may therefore be of assistance in differentiating ischemic stroke from hemorrhagic stroke and other diseases mimicking ischemic stroke [16]. While there have been many studies of potential blood-based protein biomarkers, brain natriuretic peptide (BNP) and S100B have been shown to differentiate ischemic stroke patients from healthy controls, hemorrhagic stroke, and ischemic stroke mimics [17]. On the basis of our findings, we think that Adropin may be used as a potential biomarker in the diagnosis of ischemic stroke such as BNP and S100B.

We also investigated correlations between Adropin and other clinical characteristics and laboratory findings in patients with ischemic stroke. Our results showed significant inverse correlation between serum Adropin levels and serum glucose levels. This result supports previous studies reporting an inverse correlation between serum Adropin levels and glucose [7,18]. From that perspective, Adropin may indicate damage in the vascular system before visible complications emerge in patients with diabetes mellitus. BMI, TC, LDL-c levels were significantly higher in our patient group than in the controls. However, while serum Adropin levels were inversely correlated with BMI and lipid parameters, this was not statistically significant. While Yu et al. determined significant inverse correlation between serum Adropin levels and BMI and triglyceride values in patients with acute myocardial infarction, and Butler et al. in obese patients undergoing gastric bypass surgery, Lian et al. reported positive correlation between serum Adropin and BMI in patients with heart failure [9,19,20]. Although our finding was not statistically significant, it is compatible with Yu et al. and Butler et al. in terms of determining an inverse relation between serum Adropin and BMI and triglyceride.

The principal limitation of our study is that the patient number was not particularly high. Another limitation is that we did not investigate serum Adropin levels and prognosis. We therefore think that further studies with larger patient populations are now needed to investigate the relation between serum Adropin levels and stroke and the prognosis thereof.

Conclusions

Serum Adropin levels were lower in our AIS patients then in the control group, and Adropin emerged as an independent predictor of AIS. We therefore think that Adropin may be used as a screening tool for AIS.

Conflict of interest
The authors declare that there are no conflicts of interest.

Financial Disclosure
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Ethical approval
The local ethical committee approved the study (No. KAEK-41)

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