



# Evaluation of the Effects of Adipocytokines on Prognosis in Ischemic Cerebrovascular Events

## *Adipositokinlerin İskemik Serebrovasküler Olaylarda Prognoz Üzerine Etkisinin Değerlendirilmesi*

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### Abstract

**Objective:** Leptin and adiponectin are the most important adipocytokines and recent studies showed that they may have role in the etiology of ischemic stroke. However, the effect of adipocytokines on stroke is not yet well understood, and related studies are confusing. The aim of this study was to investigate the effects of serum leptin and adiponectin levels on patients with acute ischemic stroke who were admitted to hospital within 24 hours of stroke.

**Materials and Methods:** A total of 59 patients with acute ischemic stroke and 24 healthy individuals similar in age and sex to the patients were enrolled in the study. The Rankin disability scale was performed in order to detect the severity of stroke. Levels of serum leptin and adiponectin were detected using an enzyme-linked immunosorbent assay. In addition, the outcomes of the patients were calculated through differences between the first day Rankin score and the 30<sup>th</sup> day Rankin score. Results were compared and the relation with prognosis was investigated. The correlation of leptin and adiponectin levels with stroke severity was analyzed.

**Results:** Thirty-four male and 25 female patients were included in this study. Leptin levels of patients were higher than in the control group. Adiponectin levels did not differ between the patient and control groups. There was no correlation between serum adipocytokine levels and the modified Rankin scale.

**Conclusion:** Serum leptin and adiponectin levels are not associated with the prognosis of ischemic stroke. Even though high levels of leptin may associate with ischemic stroke, this study was not able to show an association with stroke prognosis.

**Keywords:** Leptin, adiponectin, ischemic stroke, prognosis

### Öz

**Amaç:** Leptin ve adiponektin, adipositokinlerin en önemlileridir ve son yıllarda yapılan çalışmalarda iskemik serebrovasküler olay etiolojisinde rolü olduğu düşünülmektedir. Ancak adipositokinlerin inme üzerine etkisi ile ilgili yapılan çalışmaların sonuçları çelişkilidir. Bu çalışmada, iskemik inme tanısı ile ilk 24 saat içerisinde hastanemiz acil servisine başvuran hastaların serum leptin ve adiponektin düzeylerinin prognoz ile ilişkisini değerlendirmeyi amaçladık.

**Gereç ve Yöntem:** Çalışmaya hastanemiz acil servisine başvuran 54 akut iskemik inmeli hasta ile 24 sağlıklı kontrol dahil edildi. İnme sonrası özürürlük skorlarını belirlemek amacıyla 1. ve 30. günlerde modifiye Rankin skalası uygulandı. Hastaneye başvurduktan sonraki ilk 24 saat içerisinde venöz kan numuneleri alınarak ELISA metodu ile serum leptin ve adiponektin düzeyleri belirlendi. Prognoz üzerine etkiyi belirlemek için hastaların 1. ay hesaplanan modifiye Rankin skorları ile ilk modifiye Rankin skorları arasındaki fark hesaplandı.

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## Öz

**Bulgular:** Hastaların serum leptin düzeyleri kontrol grubundan anlamlı olarak yüksek tespit edilirken, adiponektin düzeyleri arasında anlamlı farklılık gözlenmedi. Prognoz üzerine yapılan inceleme sonucunda leptin ve adiponektin düzeyleri ile Rankin skorları arasında anlamlı ilişki saptanmadı. Bu bulgular sonucunda leptin ve adiponektinin iskemik inme prognoz üzerine anlamlı etkisinin olmadığı düşünüldü.

**Sonuç:** Leptin ve adiponektin düzeyi iskemik inme prognozunun belirlenmesinde anlamlı özellik göstermemektedir. Her ne kadar leptin seviyeleri iskemik inme ile ilişkili saptanmış olsa da çalışmamızda inme prognozu ile ilişkisi tespit edilmemiştir.

**Anahtar Kelimeler:** Leptin, adiponektin, iskemik inme, prognoz

## Introduction

Stroke is among the most common causes of mortality worldwide and results in a high rate of morbidity. Studies to determine risk factors associated with disease, its prevention, and treatment are popular nowadays. Thus, determining stroke risk factors and detecting biomarkers that can predict prognosis are important.

Adipose tissue is an important endocrine organ. It has autocrine, paracrine, and endocrine effects mediated by hormones and cytokines it secretes. Leptin and adiponectin are the most important adipocytokines. Reports in recent years suggested that they had important roles in the etiology of cerebrovascular disease (1). Although the association between leptin and adiponectin with stroke etiology was shown previously, studies about their effects on prognosis of stroke are scarce. Obesity is a modifiable risk factor of stroke. Studies have shown that leptin deficiency or resistance causes obesity and diabetes in humans (1).

This study reviewed the roles of adipocytokines in the pathophysiology of stroke and their therapeutic significance, and then investigated their effects in the prognosis of stroke.

## Materials and Methods

This study included 59 patients with ischemic stroke who were admitted to the Emergency Department of the Ministry of Health Medeniyet University Göztepe Training and Research Hospital during the first 24 hours of stroke and followed up in the Neurology Clinic, and 24 healthy individuals whose age and sex values were similar to the study group. Informed consents were obtained from the participants or their relatives. Approval from the Research Evaluation Commission of Istanbul Medeniyet University Göztepe Training and Research Hospital was obtained on 20.03.2012 (decision number: 20/S). The study included patients with a first ischemic stroke. Patients with stroke who presented after the 24 hours of symptoms, history of previous stroke, brain tumors or systemic malignancies, acute head trauma, recent or concurrent heart failure or myocardial infarction, hepatic or renal dysfunction, history of infection within the last 2 weeks, and increased C-reactive protein levels were excluded. Patients were diagnosed by anamnesis, neurologic examination, and neuroradiologic evaluation. Subtypes of ischemic stroke were classified as large artery atherosclerosis (LAA), lacunar stroke, or cardioembolic stroke (CES) in accordance with the Trial of Org 10172 in Acute Stroke Treatment study. For the etiologic evaluation, carotid and vertebral artery color doppler ultrasonography and transthoracic echocardiography were

performed to all patients. Blood pressure was measured after a rest period in all patients and controls, heights and weights were measured, and body mass index (BMI) were calculated. The patients were assessed for stroke risk factors such as hypertension (HT), diabetes, hyperlipidemia (HL), heart disease, and atrial fibrillation. To determine the level of disability due to stroke, the modified Rankin scale was applied by the same neurologist on the 1<sup>st</sup> and 30<sup>th</sup> days of admission. Venous blood samples to detect serum leptin and adiponectin levels were obtained within 24 hours of stroke after 12 hours of fasting, and centrifuged. Serum samples were kept in a -80 °C freezer until analyses were performed. Leptin and adiponectin levels were detected using an ELISA.

### Statistical Analysis

Data were analyzed using the SPSS 20.0 package program. Student's t test was used for the comparison of parametric data. The chi-square test was used to analyze differences between categorical variables. The Kruskal-Wallis test was used to compare more than two continuous variables, the Mann-Whitney U test was used to compare two groups, and Wilcoxon's t-test was used for intragroup comparisons. Spearman and Pearson correlation tests were used for correlation analyses. The difference was accepted as statistically significant if  $p < 0.05$ .

## Results

This study included 59 patients who presented to the emergency department with a diagnosis of ischemic stroke and 24 healthy controls. The mean age was 63.56 years (range, 34-88 years) in the stroke group, and 67.67 years (range, 51-86 years) in the control group. There was no statistically significant difference between the ages of the patient and the control groups ( $p > 0.05$ ). Among the patients 34 (57.6%) were males, and 25 (42.4%) were females. There was no statistically significant difference between the patient and the control groups according to sex distribution ( $p > 0.05$ ).

There was no significant difference between the patient and the control groups according to BMI ( $p > 0.05$ ). The mean BMI of the patient group was mildly obese according to the World Health Organization classification. The BMI values of the females and males were similar ( $p > 0.05$ ).

The mean leptin level of the patient group was 13.35 mg/dL, and the control group was 8.18 mg/dL. The serum leptin level was significantly higher in the patient group ( $p < 0.05$ ) (Table 1). The mean adiponectin level was 3.77 mg/dL in the patient group and 3.65 mg/dL in the control group. There was no significant

difference between the two groups according to adiponectin levels ( $p>0.05$ ).

Correlations between leptin and adiponectin with BMI were evaluated using Pearson's correlation. No statistically significant relation was detected between adipocytokines and BMI ( $p>0.05$ ).

Correlations between leptin, adiponectin levels, and the leptin/adiponectin ratio with ischemia subtype were evaluated but no significant relation was found between these variables and ischemia subtypes ( $p>0.05$ ). The association between patient groups, risk factors, and serum adipocytokines was evaluated and no significant relation was found between serum leptin or adiponectin levels and diabetes, HT or HL ( $p>0.05$ ) (Table 2).

In the patient group, the mean initial value for the modified Rankin scale was 2.73 (range, 1-5), and the mean modified Rankin scale value at the 1<sup>st</sup> month was 2.42 (range, 1-6). The prognosis of patients were evaluated by comparing the initial and 1<sup>st</sup> month modified Rankin scales. The patients were classified as worsened when the Rankin 2 level was higher than Rankin 1 level, improved when Rankin 2 was lower, and stable when the values were equal. The Wilcoxon test was used to evaluate whether the initial and 1<sup>st</sup> month Rankin levels were different. The results demonstrated that Rankin 2 levels were lower than Rankin 1 levels, revealing the improvement in the disability at the first month. The relationships of the clinical courses of the patients determined by changes in

the modified Rankin scale and serum leptin, adiponectin levels, and the leptin/adiponectin ratio were measured and no significant relation was detected ( $p>0.05$ ) (Table 3).

### Discussion

Obesity is one of the most important modifiable risk factors for stroke. Determination of the biologic features of adipocytokines will shed light on obesity research. Leptin was initially thought to be related with satiety and energy balance, but later research suggested that it was an anti-obesity factor due to feedback from adipocytes to the hypothalamus. Increasing evidence suggests an important role for leptin, a crucial hormone for regulating body weight and food intake in both animals and humans (2). Another cause of the inefficiency of leptin in obese people is resistance against it. Higher leptin levels are needed to overcome leptin resistance. Therefore, more leptin is secreted from adipose tissue and leptin causes an increase in the adipose tissue that produces it (3). Although adiponectin is produced only in adipose tissue, unlike many adipocytokines, its level is lower in obese patients than lean patients. Plasma adiponectin levels decrease in obesity forms that

Group		Leptin (ng/mL)	Adiponectin (ng/mL)
Control	n	24	24
	Mean±SD	8.18±9.10	3.65±1.59
Patient	n	59	59
	Mean±SD	13.35±12.89	3.77±1.72
Total	n	83	83
	Mean±SD	11.86±12.10	3.73±1.67
p value		<b>0.027</b>	0.90

Mann-Whitney U test, n: Patient number, SD: Standard deviation

Type of ischemia according to TOAST classification		Leptin (ng/mL)	Adiponectin (ng/mL)
LI	n	14	14
	Mean±SD	15±10	3.7±1.6
LAA	n	36	36
	Mean±SD	12±13	3.7±1.7
CES	n	9	9
	Mean±SD	14±15	3.9±1.7
p value		0.35	0.96

Kruskal-Wallis test, LI: Lacunary infarct, LAA: Large artery atherosclerosis, CES: Cardioembolic stroke, n: Number of cases, SD: Standard deviation, TOAST: Trial of Org 10172 in Acute Stroke Treatment

		n	Mean	Min.	Max.	p
Leptin (ng/mL)	Improved	27	12.50±12.10	1.01	36.60	0.61
	Worsened	12	11.48±12.45	1.01	37.20	
	Stable	20	15.63±14.45	2.23	46.40	
Adiponectin (ng/mL)	Improved	27	3.72±1.83	0.75	6.92	0.96
	Worsened	12	3.88±1.97	1.39	7.42	
	Stable	20	3.76±1.47	1.30	6.62	
Leptin/adiponectin	Improved	27	3.63±2.75	0.15	9.20	0.62
	Worsened	12	4.25±5.83	0.35	19.57	
	Stable	20	4.89±5.19	0.55	20.35	

ANOVA test, n: Case number, SD: Standard deviation, Leptin/adiponectin: Leptin/adiponectin ratio, Min.: Minimum, Max.: Maximum

develop in people consuming high-calorie diets (4). In addition, decreased plasma adiponectin levels are detected in experimental obesity models that develop due to genetic susceptibility and diet.

Many studies demonstrated that leptin was a marker for stroke. The first study between leptin and stroke relation showed that leptin was an independent risk factor for the development of hemorrhagic stroke, but no similar finding was observed in ischemic stroke (5). Later studies demonstrated that leptin was associated with both types of stroke in males, but such a relation was not observed in females (6). In a study on male patients without diabetes, leptin was independently associated with insulin resistance, inflammatory markers, and procoagulant factors, and there was a pathophysiologic link between leptin and stroke (7). The results of our study showed that leptin levels were higher in the patient group, in accordance with the literature, and leptin was suggested to be an important marker of stroke.

The association between adiponectin and stroke is controversial. A study in patients with stroke could not show a predictive value of plasma adiponectin level for stroke (8). A recent study in 145 patients with acute ischemic stroke showed that serum leptin levels increased in the stroke group and adiponectin and ghrelin levels decreased (9). In our study, the comparison of the mean leptin level of patients ( $13.35 \pm 9.10$  ng/mL) with controls ( $8.18 \pm 9.10$  ng/mL) showed a higher leptin level in the patient group. However, the comparison of adiponectin and leptin/adiponectin ratio did not show a statistically significant difference between the patient and control groups. These findings are similar to previous studies. Our failure to demonstrate a significant difference between adiponectin levels was similar to previous studies in the literature that suggested a limited effect of adiponectin on stroke; however, the small sample size might have been a limitation for this evaluation.

The serum leptin level was detected as higher in females than males and this may be explained by increased fat tissue and higher subcutaneous/visceral adiposity rate in females (10). Gynecoid adiposity in females results in the production of higher amounts of leptin than android adiposity in males (10). In contrast to the literature, no significant difference was found in our study between leptin levels and sex. However, we found that mean BMI and leptin ratios were higher in females, although the differences were not significant. We think that females in the study group had non-significantly higher leptin levels and this might be related with the higher BMI values in this group.

Leptin is believed to be a potential marker of HT (11). Vasorelaxation is inadequate in hyperleptinemia. High leptin levels cause endothelial dysfunction due to increased endothelin-1 secretion. In addition, it causes HT due to impaired nitric oxide (NO) secretion and increased reactive oxygen secretion (11). Although the present study did not show a significant relation between HT and serum leptin levels, 43 of the 59 patients had HT. The mean serum leptin level of the patients without HT was 11.46 ng/mL, and the mean leptin level of patients with HT was 14.06 ng/mL. Although there was no statistically significant difference, patients with HT tended to have higher leptin levels. A comparison of vascular risk factors with serum leptin and adiponectin levels revealed no association with important modifiable risk factors. However, we believe that medications used by patients for these defined risk factors might have affected serum leptin and adiponectin levels.

Experimental studies demonstrated potential anti-atherogenic and anti-inflammatory properties of adiponectin (12). *In vivo* studies performed in human aortic endothelial cells showed that adiponectin caused a dose-dependent decrease in adhesion molecules that regulate vascular inflammatory response, and lower adiponectin levels were associated with larger carotid artery intima-media thickness (12,13). However, a cross-sectional study in patients with and without a previous history of ischemic cerebrovascular disease found an association between low adiponectin levels and ischemic cerebrovascular events (14). A prospective study by Hung et al. (15) recorded patients with ischemic heart disease and followed up for a year; unexpectedly, the group with a high adiponectin level was found susceptible to cardiac and cerebral ischemic events. According to the researchers, adipose tissue produces more adiponectin after stroke due to inflammation and insulin resistance (15). In the current study, no significant difference was found between adiponectin levels of the patients and controls. The literature is controversial, but our study suggests that adiponectin is not an important biomarker in the pathophysiology of stroke.

There are a limited number of studies that evaluated the association between stroke subtypes and leptin and adiponectin. However, a study in South Korea in 2012 that included 96 patients with ischemic stroke demonstrated that patients with large artery ischemic stroke had lower levels of serum adiponectin, and a higher leptin/adiponectin ratio (16). Increases in strokes due to augmented atherosclerotic background by low adiponectin is understandable. However, we could not demonstrate an association between ischemia subtypes and leptin, adiponectin or the leptin/adiponectin ratio. The mean values of the variables were not statistically significantly different ( $p > 0.05$ ).

A study by Tang et al. (17) in 2015 demonstrated that leptin receptors regulate human energy homeostasis, and mutations in leptin receptors cause ischemic stroke by increasing cardiovascular risk factors. A study in 2014 also showed a leptin-mediated blood pressure increase in diet-induced obese rodents (18). As these samples demonstrate, adipocytokines generally affect stroke mediated by cardiovascular risk factors.

The mortality rate in our sample was 10.16%. Four of the exitus patients were LAA and 2 were CES. Three patients with stroke were lost during the first week and the other 3 were lost within the first month. No significant relation was found between changes in Rankin scores and serum leptin and adiponectin levels. The results were thought to be limited due to having only one sampling time from the patients for the detection of leptin and adiponectin levels, small sample size, and medications used by the patients. However, no other study was found in the literature that evaluated ischemia subtypes, risk factors, patient disability, and serum leptin and adiponectin levels.

## Conclusion

In conclusion, leptin and adiponectin are important biomarkers in all cardiovascular diseases because of their association with obesity, which is the disease of our time. However, further studies are needed to reveal their effects on disease prognosis. The small sample size is a limitation of this study. These markers should be evaluated in larger sample groups.

### Ethics

Ethics Committee Approval: The study was approved by the Göztepe Training and Research Hospital Local Ethics Committee (Protocol number: 20/S, Date: 20.03.2012), Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

### Authorship Contributions

Concept: M.Ş.A.T.D., N.I., Design: M.Ş.A.T.D., Data Collection or Processing: M.Ş.A.T.D., Analysis or Interpretation: M.Ş.A.T.D., Ö.A.D., Literature Search: M.Ş.A.T.D., Ö.A.D., Writing: M.Ş.A.T.D.

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