EURASIAN JOURNAL OF EMERGENCY MEDICINE
DOI: 10.4274/eajem.galenos.2023.03780
Original Article
Eurasian J Emerg Med. 2023;22(3): 190-5

Importance of Serum Adropin Levels in Ischemic Stroke: A Prospective Clinical Study

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Abstract

Aim: Stroke should be diagnosed quickly and accurately in emergency settings. This study investigated serum adropin levels as a novel biomarker in the diagnostic value of ischemic stroke.

Materials and Methods: A prospective cross-sectional study was conducted in a tertiary university hospital. Serum adropin levels were measured at the time of the first arrival in ischemic stroke patients (n=46) and healthy control (n=45). In the ischemic stroke group, blood samples were retaken 72 h after arrival.

Results: There was a significant difference between the ischemic stroke group and the control group regarding adropin values at arrival (2.67+0.63 vs. 2.34+0.69, respectively p=0.032). There was no significant difference between the other groups in terms of arrival values (p>0.05). Logistic regression analysis revealed a statistically significant difference between the ischemic stroke and control groups (odds ratio: 2.23; 95% confidence interval: 1.140-4.360, p=0.019). In the ischemic stroke group, the adropin level was statistically significantly decreased on the third day compared with the arrival (p=0.041). The adropin levels predicted ischemic stroke patients with 58.7% sensitivity and 59.4% specificity with the 2.49 ng/mL cut-off value (area under the curve=0.635).

Conclusion: In this study, high serum adropin levels can be thought to be supportive in the diagnostic value of ischemic stroke.

Keywords: Adropin, ischemic stroke, emergency department, biomarker

Introduction

Stroke is a clinical manifestation resulting from the inability of blood flow to a particular area of the brain due to occlusion or hemorrhage (1). Ischemic stroke is defined as neurological dysfunction caused by focal cerebral, spinal, or retinal infarction (2).

In ischemic stroke, an inflammatory process is activated due to endothelial dysfunction involving large and medium-sized arteries, monocyte migration, and the release of cytokines and growth factors that can lead to endothelial damage (3). One of the growth factors associated with ischemic stroke is vascular endothelial growth factor (VEGF) (4). Studies investigating VEGF and ischemic stroke relationships have shown that VEGF activation provides a smaller infarction volume through neuroprotection and is involved in neurogenesis, angiogenesis, endothelial cell proliferation, migration, and vascular permeability (5).

Adropin is a peptide hormone discovered in 2008 by Kumar et al. (6) and is associated with carbohydrate-lipid metabolism, metabolic diseases, central nervous system function, endothelial

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Received: 03.04.2023
Accepted: 20.04.2023

Presented in: Presented as an oral presentation at the 58th National Neurology Congress.

Cite this article as: Tekin E, Koçak MN, Bayraktar M, Özlü İ, Çelik M, Kurt E, Halıcı Z. Importance of Serum Adropin Levels in Ischemic Stroke: A Prospective Clinical Study. Eurasian J Emerg Med. 2023;22(3): 190-5.

function, and cardiovascular diseases (7). Due to the complex effects of adropin in chemical reactions, it is considered to affect neurogenesis, neurotoxicity, increased vascular wall permeability, locomotor coordination, apoptosis, and especially angiogenesis and neuroprotection (8). Adropin activates the VEGF receptor and plays a role in regulating endothelial nitric oxide synthase (9). Thus, adropin improves vascular endothelial cell function and shows anti-inflammatory properties by increasing the proliferation of endothelial cells and capillary-like structures (10).

The management of ischemic stroke is crucial for rapid diagnosis and immediate thrombolytic and endovascular intervention (1). Easy, fast, and inexpensive differential diagnostic techniques for ischemic stroke management are still under investigation for more practical perspectives. In this study, serum adropin levels were investigated as a biomarker in the diagnosis of ischemic stroke.

Materials and Methods

Study Design

This prospective clinical study was conducted in a tertiary university hospital between November 2019 and May 2020. The hospital where the study was conducted is accepted as a regional reference healthcare center located in a province with a 770,000 population. Annually, 135,662 patients receive emergency medical services from the hospital.

Atatürk University Faculty of Medicine Local Ethics Committee approval was received (date: 26.09.2019, numbered: 06/53). Informed consent was obtained from the patients or their relatives. The study was conducted following the Good Clinical Practices criteria of the Declaration of Helsinki. It should also be noted that the revised final version of the Helsinki Declaration has been complied with.

Patients

Patients over 18 who presented to the third-level emergency department with stroke symptoms were included in the study. Patients who applied to the emergency department due to stroke symptoms such as loss of strength in the extremities, speech disorder, and facial asymmetry were included in the study. The control group consisted of volunteers over the age of 18.

Patients who presented to the emergency department more than 24 h after the onset of ischemic stroke signs and symptoms, and those who did not give informed consent were excluded from the study. Patients receiving ongoing stroke treatment started in another center, diagnosed as hemoragic stroke patients, pregnant and lactating women, and patients presenting with traumatic cerebrovascular events were also excluded. The patients who died or were transferred to another hospital during follow-up were excluded from the study. In addition, patients whose blood samples could not be obtained on the third day were not included in the evaluation.

Study Groups and Protocol

Brain computed tomography (CT) imaging was performed within the first 20 min after the admission of patients with a prediagnosis of stroke. Patients with hemorrhagic stroke findings on brain CT were excluded. At the time of arrival, the demographic features, smoking status, chronic diseases, atrial fibrillation, vital signs, National Institutes of Health Stroke Scale, Modified Rankin Scale, and Glasgow Coma Scale scores, complete blood count, lipid profile, biochemical test parameters, and outcomes were recorded for the patients.

Blood Sample Collection

In the study, 5 cc venous blood samples were taken in the emergency department to determine the serum adropin level in the ischemic stroke and control groups. These blood samples were recorded as arrival adropins. For serum adropin levels, venous blood samples were taken once from the patients in the control group and twice from the patients with ischemic stroke. For the ischemic stroke patients, a second venous blood sample was obtained at 72 h of arrival in the neurology stroke unit or neurology stroke care unit, where their treatment was ongoing. These blood samples were recorded as third-day adropins.

Biochemical Analysis

Serum glucose, total cholesterol, triglyceride, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol levels were analyzed using a Beckman Coulter AU 5800 chemistry analyzer (Beckman Coulter, Japan) according to the manufacturer's instructions.

Serum Adropin Level Measurement

The 5 cc blood samples taken into serum tubes for adropin measurements were kept at room temperature for 20 min to be coagulated; then, they were centrifuged at 3,000 rpm for 15 min, alquoted, and stored at -80 °C until analysis. All samples were first kept at -20 °C for one night and at +4 °C for a further night during biochemical analysis. The serum samples and study kits were maintained at room temperature for approximately 2 h before analysis. According to the manufacturer's instructions, serum adropin levels were measured using commercially available ELISA kits (Human Adropin ELISA kits; Shanghai Coon Koon Biotech Co. Ltd.). The intra- and inter-assay coefficients of variation of the kits were <8% and <10%, respectively. The assay range for adropin was 0.05-15 ng/mL.

Statistical Analysis

In this study, statistical analyzes were performed using the IBM Statistical Package for the Social Sciences package program v. 25.0. The Kolmogorov-Smirnov test was used for assessing the normal distribution. Categorical data were presented as frequency and percentages, while numerical data were presented as mean and standard deviation, if normally distributed, or median and interquartile ranges if not normally distributed. Pearson's chi-square and Fisher's exact tests were used to compare categorical data. For comparing two groups, Student's t-test was used if the data were normally distributed, and the Mann-Whitney U test was conducted for the data without normal distribution. For comparing two dependent groups, the dependent t-test was used if there was a normal distribution and the Wilcoxon test if the distribution was skewed. Regression analysis of the arrival adropin level was performed for the groups. The area under receiver operating characteristic (ROC) curves (AUC) of the adropin levels was calculated. The Youden J index was used to estimate the best cutoff points. Sensitivity and specificity were calculated with 95% confidence interval (CI). For all analyzes $p<0.05$ was considered statistically significant.

Results

The study included 58 patients with ischemic stroke and 45 control patients. During the follow-up of ischemic stroke patients, 12 patients were excluded from the study for various reasons. The flowchart of the study is shown in Figure 1.

When the age, gender, smoking and chronic disease existence of the patients participating in the study were compared, there was no statistically significant difference between the groups ($p>0.05$). However, the presence of atrial fibrillation, systolic and diastolic blood pressure in arrival vital signs, and triglyceride and HbA1c values in laboratory findings were statistically different between the groups ($p>0.05$). The demographic and clinical characteristics and laboratory findings of the groups are given in Table 1.

In the ischemic stroke group, the arrival and third-day serum adropin levelscomparison revealed a statistically significant decrease (2.67 ± 0.63 vs. 2.41 ± 0.87, respectively; $p=0.041$) (Figure 2).

The logistic regression analysis of the arrival adropin level between the groups showed that the result was statistically significant for the analysis between the ischemic stroke and control group (OR: 2.23; 95% CI: 1.140-4.360, $p=0.019$) (Table 2).

The adropin levels were found to be predicting ischemic stroke patients with 58.7% sensitivity and 59.4% specificity with the 2.49 ng/mL cut-off value (AUC=0.635). The ROC curve analysis showed that the serum adropin level of ischemic stroke patients was statistically significant compared with the control group ($p=0.016$, 95% CI: 0.531-0.739) (Figure 3).

Discussion

In this study, the comparison between the ischemic stroke and control groups in terms of the serum adropin level was significantly higher in patients with ischemic stroke than in the control group. Thus, it could be used as an essential parameter in the diagnostic value of ischemic stroke.

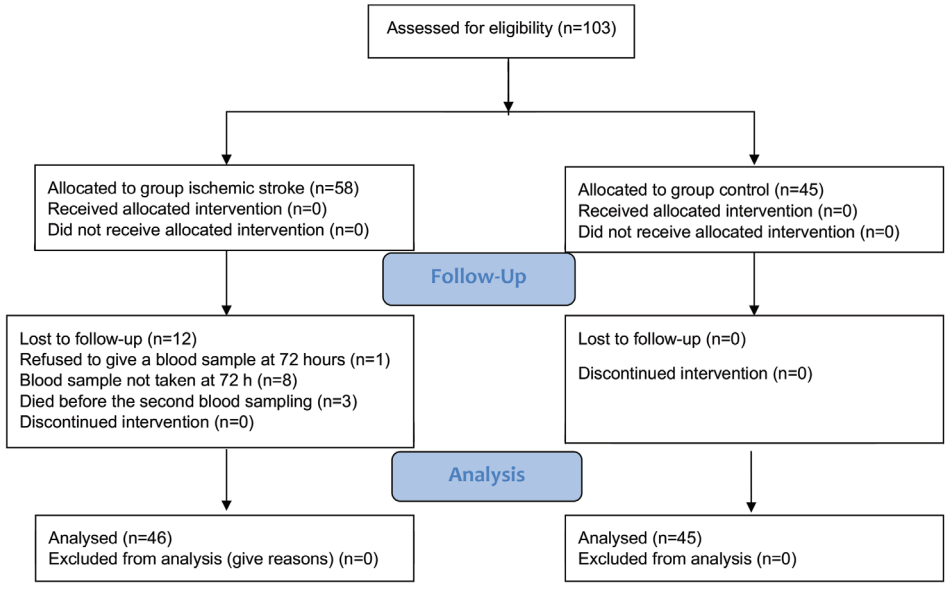

Figure 1. The flow chart of the study

Table 1. Demographic and clinical characteristics and laboratory findings of the groups			
	Ischemic stroke (n=46)	**Controls (n=45)**	**p value**
Age (years), mean±SD	78.65±8.88	79.53±10.30	0.663[a]
Gender, n (%) Male Female	 22 (47.8%) 24 (52.2%)	 25 (55.6%) 20 (44.4%)	0.461[b]
Smoking, n (%)	22 (47.8%)	21 (46.7%)	0.912[b]
Chronic diseases, n (%) Hypertension CAD Diabetes mellitus The history of CVE Chronic renal failure Chronic obstructive pulmonary disease Hypertension + CAD Hypertension + diabetes mellitus Diabetes mellitus + CAD Hypertension + diabetes mellitus + CAD None	 16 (34.8%) 5 (10.9%) 1 (2.2%) 2 (4.3%) 1 (2.2%) 3 (6.5%) 8 (17.4%) 2 (4.3%) 1 (2.2%) 3 (6.5%) 4 (8.7%)	 16 (35.6%) 7 (15.6%) 3 (6.7%) - - 4 (8.9%) 7 (15.6%) 3 (6.7%) - 2 (4.4%) 3 (6.7%)	0.661[c]
Presence of AF, n (%)	22 (47.8%)	12 (26.7%)	**0.037[b]**
Mortality and morbidity scales, mean±SD NIHSS mRS GCS	 9.4±6.8 2.9±1.7 13.5±2.3	 0±0 1±0 15 (0)	 **<0.001[a]** **<0.001[a]** **<0.001[a]**
Arrival vital signs, mean (SD) Systolic blood pressure (mmHg) Diastolic blood pressure (mmHg) The respiratory rate (min) Fever, (°C)	 149.0±27.4 88.1±18.4 15.5±2.5 36.5±0.3	 138.1±13.4 78.9±9.6 14.9±1.7 36.5±0.3	 **0.018[a]** **0.004[a]** 0.165[a] 0.321[a]
Arrival laboratory findings, mean±SD WBC count, ($\times10^9$/L) Hemoglobin, (g/dL) Platelet, ($\times10^9$/L) INR Lactate, (mmol/L) Creatinine, (mg/dL) Troponin, (ng/L) CRP, (mg/L) Glucose, (mg/dL) HbA1c, (%) Triglyceride, (mg/dL) Cholesterol, (mg/dL) HDL, (mg/dL) LDL, (mg/dL)	 8.23±3.77 13.3±2.8 258.6±74.8 1.18±0.27 1.64±0.64 0.94±0.59 4.5±1.9 3.4±3.2 125.9±56.0 6.1±1.6 122.42±62.59 187.8±42.54 44.64±13.23 121.62±27.84	 8.05±3.55 14.1±2.7 273.1±91.7 1.16±0.60 1.91±0.86 0.91±0.49 4.1±2.0 2.5±1.7 142.4±80.0 7.2±2.6 157.28±67.15 183.88±43.76 42.85±10.73 116.83±28.8	 0.596[a] 0.178[a] 0.311[a] 0.865[a] 0.173[a] 0.765[a] 0.345[a] 0.476[a] 0.260[a] **0.039[a]** **0.023[a]** 0.985[a] 0.791[a] 0.684[a]
Outcome, mortality, n (%)	6 (13%)	-	**0.026[c]**
Arrival adropin mean±SD	2.67±0.63	2.34±0.69	**0.019[a]**

[a]Student's t-test.
[b]Pearson's chi-square test.
[c]Fisher's exact test.
SD: Standard deviation, CAD: Coronary artery disease, CVE: Cerebrovascular event, AF: Atrial fibrillation, NIHSS: National Institutes of Health Stroke Scale, mRS: Modified rankin scale, GCS: Glasgow Coma Scale, INR: International normalized ratio, CRP: C-reactive protein, WBC: White blood cell, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Table 2. Logistic regression analysis of the arrival serum adropin level between the groups					
	Mean±standard deviation	**B±standard error**	**OR**	**95% CI**	**p value**
Ischemic stroke vs. controls	2.67±0.63 vs. 2.34±0.69	0.80±0.34	2.23	1.140-4.360	**0.019**
Cox and Snell: 062; Nagelkerke: 071; McFadden: 031. B: Regression coefficient, CI: Confidence interval, OR: Odds ratio					

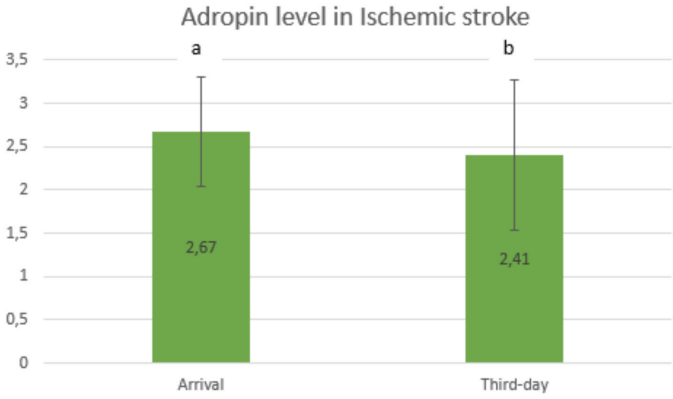

Figure 2. A comparison of the arrival and third-day serum adropin levels of the ischemic stroke group (dependent t-test, p=0.041)

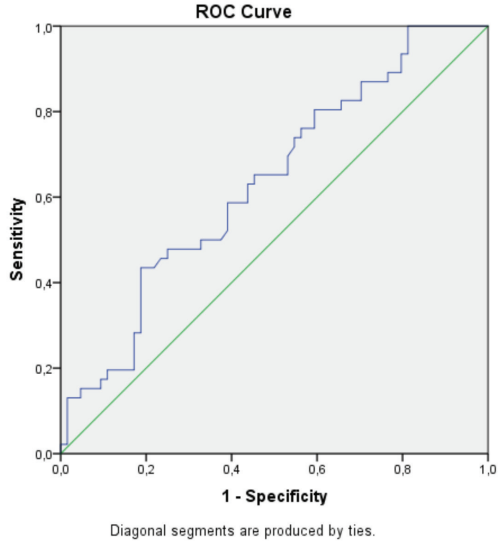

Figure 3. ROC curves of adropon levels in the ischemic stroke and control groups

As it is known that adropin increases neovascularization (7), an increase in the serum adropin level should be expected in patients with ischemic stroke. Also, a study in the literature revealed that the serum adropin level was elevated in acute hypoxia (11). Another study performed by exogenous adropin administration in rats emphasized that adropin protects against endothelial barrier dysfunction during ischemic conditions (12). Reliably, in our study, the serum adropin level was significantly higher in patients with ischemic stroke compared to the control group. Besides, regression analysis indicated that the serum adropin level could be used as an independent biomarker for ischemic stroke.

In our study, when the change in arrival and third-day serum adropin levels was investigated in the ischemic stroke group, a statistically significant decrease was observed. This shows that the serum adropin level can also be used to differentiate acute and subacute phases in ischemic stroke. The temporal changes in the samples taken for measuring adropin levels conducted should be considered in studies conducted in the area.

In the literature, an ischemic preconditioning study conducted on experimental diabetic ratsreported that increased adropin levels inside the tissue after ischemia could provide neuroprotection and could be a novel biomarker in ischemic stroke (13). In this study, ischemic preconditioning involved three cycles of 10 min of reperfusion and 10 min of occlusion of the unilateral left proximal internal carotid artery. Ischemic preconditioning was performed 72 h before the ischemic stroke. The serum adropin level was found to be significantly lower in preconditioned rats (13). This result is parallel to the decrease in the adropin level observed on the third day of our study.

It has been suggested in many studies in the literature that adropin plays a role in protecting energy metabolism (10,14,15). It is stated that in atherosclerotic heart diseases, adropin plays an essential role in maintaining the nutrition of the myocardium by regulating energy metabolism (6). A study investigating adropin changes in an experimentally induced myocardial infarction (MI) model in rats found that adropin levels increased statistically after MI events (16). However, some studies also claim between adropin and MI (17,18). In a study, it was found that low adropin levels could be used for diagnostic purposes in acute coronary events, and in a meta-analysis, there was a relationship between low adropin levels and acute coronary syndrome (17,18). The adropin levels in MI patients should be investigated in further studies to eliminate these conflicts.

The serum adropin level being inversely proportional to mortality and morbidity in this patient group can be attributed to the association between adropin and nitric oxide release, its antioxidant effect (19) and the prevention of monocyte adhesion to endothelial cells through its anti-atherosclerotic effects (20). In particular, the antioxidant activity of adropin is increased in the early stage of ischemic stroke through an acute compensation mechanism. Thus, it can prevent the expansion of the necrotic area by providing blood flow to the penumbra via the microvascular collateral circulation. Thus, elevated serum adropin levels suggest that ischemic stroke damage will be limited by known neuroprotection and angiogenesis mechanisms, and thus it can be used to predict lower morbidity and mortality.

Study Limitations

Our study has some limitations. First, it was performed in a single center with a relatively small number of patients. Multicenter studies with a large group of patients to determine serum adropin levels are needed. Also, there is no known normal reference range for adropin levels in a healthy population, and

confounding factors affecting serum adropin levels are unknown. Future studies are required to measure the serum adropin levels of ischemic stroke patients hourly or at frequent intervals to monitor the changes.

Conclusion

In this study, serum adropin levels of ischemic stroke patients were significantly higher than those of the control group. Thus, it could be used as an independent biomarker and a critical parameter in the diagnostic value for ischemic stroke. Besides, the serum adropin level of ischemic stroke decreased from the first day to the third day.

Ethics

Ethics Committee Approval: Atatürk University Faculty of Medicine Local Ethics Committee approval was received (date: 26.09.2019, numbered: 06/53).

Informed Consent: Informed consent was obtained from the patients or their relatives.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.T., M.N.K., İ.Ö., E.K., Z.H., Concept: E.T., M.N.K., M.B., M.Ç., Z.H., Design: E.T., M.N.K., M.B., İ.Ö., M.Ç., E.K., Z.H., Data Collection or Processing: E.T., M.N.K., M.B., İ.Ö., Analysis or Interpretation: M.Ç., E.K., Z.H., Literature Search: E.T., M.N.K., M.B., Z.H., Writing: E.T., M.B., İ.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was financially supported by the Scientific Research Project (BAP) unit of Atatürk University with project number THD-2019-7544.

References

1. Simon R, Aminoff M, Greenberg D. Stroke. Clinical neurology. McGraw-Hill Education; 2018.
2. From the American Association of Neurological Surgeons (AANS), American Society of Neuroradiology (ASNR), Cardiovascular and Interventional Radiology Society of Europe (CIRSE), Canadian Interventional Radiology Association (CIRA), Congress of Neurological Surgeons (CNS), European Society of Minimally Invasive Neurological Therapy (ESMINT), et al. Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke. Int J Stroke. 2018;13:612-32.
3. Sato K, Yamashita T, Shirai R, Shibata K, Okano T, Yamaguchi M, et al. Adropin Contributes to Anti-Atherosclerosis by Suppressing Monocyte-Endothelial Cell Adhesion and Smooth Muscle Cell Proliferation. Int J Mol Sci. 2018;19:1293.
4. Matsuo R, Ago T, Kamouchi M, Kuroda J, Kuwashiro T, Hata J, et al. Clinical significance of plasma VEGF value in ischemic stroke - research for biomarkers in ischemic stroke (REBIOS) study. BMC Neurol. 2013;13:32.
5. Shimotake J, Derugin N, Wendland M, Vexler ZS, Ferriero DM. Vascular endothelial growth factor receptor-2 inhibition promotes cell death and limits endothelial cell proliferation in a neonatal rodent model of stroke. Stroke. 2010;41:343-9.
6. Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab. 2008;8:468-81.
7. Jasaszwili M, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Adropin as A Fat-Burning Hormone with Multiple Functions-Review of a Decade of Research. Molecules. 2020;25:549.
8. Shahjouei S, Ansari S, Pourmotabbed T, Zand R. Potential Roles of Adropin in Central Nervous System: Review of Current Literature. Front Mol Biosci. 2016;3:25.
9. Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta M, et al. Adropin is a novel regulator of endothelial function. Circulation. 2010;122(11 Suppl):185-92.
10. Ganesh Kumar K, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. Obesity (Silver Spring). 2012;20:1394-402.
11. Yang F, Zhou L, Qian X, Wang D, He WJ, Tang ZW, et al. Adropin is a key mediator of hypoxia induced anti-dipsogenic effects via trpv4-camkk-ampk signaling in the circumventricular organs of rats. Front Mol Neurosci. 2017;10:105.
12. Yang C, DeMars KM, Hawkins KE, Candelario-Jalil E. Adropin reduces paracellular permeability of rat brain endothelial cells exposed to ischemia-like conditions. Peptides. 2016;81:29-37.
13. Altintas O, Kumas M, Altintas MO. Neuroprotective effect of ischemic preconditioning via modulating the expression of adropin and oxidative markers against transient cerebral ischemia in diabetic rats. Peptides. 2016;79:31-8.
14. Yu HY, Zhao P, Wu MC, Liu J, Yin W. Serum adropin levels are decreased in patients with acute myocardial infarction. Regul Pept. 2014;191:46-9.
15. Jasaszwili M, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Adropin as A Fat-Burning Hormone with Multiple Functions-Review of a Decade of Research. Molecules. 2020;25:549.
16. Aydin S, Kuloglu T, Aydin S, Kalayci M, Yilmaz M, Çakmak T, et al. Elevated adropin: A candidate diagnostic marker for myocardial infarction in conjunction with troponin-I. Peptides. 2014;58:91-7.
17. Ertem AG, Ünal S, Efe TH, Açar B, Yayla Ç, Kuyumcu MS, et al. Association between serum adropin level and burden of coronary artery disease in patients with non-st elevation myocardial infarction. Anatol J Cardiol. 2017;17:119-24.
18. Zheng J, Liu M, Chen L, Yin F, Zhu X, Gou J, et al. Association between serum adropin level and coronary artery disease: A systematic review and meta-analysis. Cardiovasc Diagn Ther. 2019;9:1-7.
19. Kuloglu T, Aydin S. Immunohistochemical expressions of adropin and ınducible nitric oxide synthase in renal tissues of rats with streptozotocin-induced experimental diabetes. Biotech Histochem. 2014;89:104-10.
20. Sato K, Yamashita T, Shirai R, Shibata K, Okano T, Yamaguchi M, et al. Adropin contributes to anti-atherosclerosis by suppressing monocyte-endothelial cell adhesion and smooth muscle cell proliferation. Int J Mol Sci. 2018;19:1293.