Levels of Serum and Urine Catecholaminergic and Apelinergic System Members in Acute Ischemic Stroke Patients

Özlem Güler[1], Cemile Buket Tuğan Yıldız[1], Hakan Hakkoymaz[1], Süleyman Aydın[2], and Meltem Yardım[2]

[1]Kahramanmaras Sutcu Imam University Faculty of Medicine
[2]Firat University School of Medicine

August 23, 2020

Abstract

Objectives: To compare levels of catecholaminergic system members, renalase, cerebellin, and their subtrates, epinephrine, norepinephrine and dopamine and apelinergic system members, apelin, elabela and nitric oxide in the blood and urine of patients with acute ischemic stroke and healthy controls. Materials and Methods: 42 patients with acute ischemic stroke and 42 age and sex matched healthy controls were included. Patients had first ischemic stroke attack and aged older than 18 years old. Blood and urine samples were collected simultaneously and within first 24 hours after the onset of acute stroke clinical manifestations and were measured using enzyme-linked immunosorbent assay method. Results: The levels of serum and urine cerebellin, renalase, epinephrine, norperinephrine, dopamine, apelin, elebela and nitric oxide were similar in ischemic stroke and in control groups (P$>$ 0.05). Strong correlations were found between renalase, cerebellin and catecholamine levels in serum and urine (p $<$0.001) both in stroke patients and controls. There were also strong correlations between apelin, elabela and NO levels in serum and urine (p $<$0.001) in two groups. Conclusions: Serum and urine cerebellin, renalase, epinephrine, norperinephrine, dopamine, apelin, elebela and nitric oxide levels do not significantly change in the acute phase of ischemic stroke. Strong correlations among renalase, cerebellin and catecholamines emphasize that these substances act together in healthy individuals and ischemic stroke patients. Similarly, strong correlations between apelin, elabela and NO indicate that these agents act together in healthy subjects and patients with ischemic stroke.

INTRODUCTION

Stroke is the second leading cause of death in the world and an important cause of disability. The incidence of stroke increases with prolonged human life. In generally stroke is divided into two types as ischemic and hemorrhagic stroke. Ischemic stroke accounts for 80-85% of all strokes and hemorrhagic stroke accounts for 15-20%. Stroke is divided into stages according to the duration of the current clinic. It is called hyperacute between 0-6 hours after the onset of the event, late hyperacute between 6-24 hours, acute between 24 hours and 7 days, subacute between 1-3 weeks, chronic phase after 3 weeks.[1] Today, 3-4% of health expenditures in western countries constitute stroke related treatment. [2]

Currently acute treatment in ischemic stroke targets reperfusion of brain tissue. Thrombolytic drugs and/or endovascular intervention is applied for this purpose. However, only 25% of patients with ischemic stroke are eligible for medical thrombolysis and 10-12% for endovascular treatment.[3] Therefore, there is a demanding need to further explore the underlying pathophysiologic mechanisms of ischemic stroke in order to develop novel treatments. The current approach to ischemic stroke focuses on seeking new treatment and improving rehabilitation in patients.[4] The main goals in new treatment strategies are to protect neurons in the ischemic penumbra area, to prevent further cell damage during reperfusion, to regulate local inflammatory response to ischemia.[5]

Mechanisms to respond to stressors in humans and animals are important for living and protection from hazards. Dopamine, norepinephrine and epinephrine are the catecholamines which rapidly release into the blood circulation in response to sympathetic activation, have stimulating effects on the cardiovascular system and energy-producing systems.[6] Catecholamines play a key role in regulating many physiological processes and are found to be associated with a constantly expanding range of neurological, psychiatric, endocrine and cardiovascular disorders.[7] Cerebellin is derived from precerebellin and has neuromodulatory functions such as maintaining of synaptic structures and modulating of their functions.[8] Previously cerebellin genes were thought to exclusively expressed in the brain. However, it has been determined that cerebellin is secreted from adrenal gland, neuroendocrine system and pancreas.[8-10] Cerebellin mRNA was shown to be expressed in the tumour tissues of phaeochromocytoma, cortisol-producing adrenocortical adenoma, ganglioneuroblastoma and neuroblastoma.[11] Cerebellin has a stimulating effect on the secretion of aldosterone, cortisol and catecholamine from the adrenal glands.[12] Renalase is a flavin adenine dinucleotide (FAD) dependent monoamine oxidase enzyme originating mainly from renal tissues directly degrades circulating catecholamines, (noradrenaline, adrenaline and dopamine).[13] Although renalase is predominantly expressed in the kidney, it was also detected in other tissues such as the skeletal muscle, cardiac muscle, blood, liver, and brain. In addition to the metabolization of catecholamines, anti-apoptotic and anti-inflammatory, cell survival, and protective effects of renalase were reported.[14]

The apelinergic pathway is of interest as a potential therapeutic target for cardiovascular and metabolic disorders. The Apelin/APJ system is involved in a wide range of biological functions. Apelin was found to be secreted in lung, stomach, skeletal muscle, adrenal gland, intestine, kidney and central nervous system.[15] Until recently, apelin was thought to be the sole ligand for the apelinergic pathway. However, a novel peptide, Elabela (Apela), which acts via the apelinergic pathway, has been identified. Various roles of Elabela in cardiovascular system, fluid balance, metabolism, diabetes, preeclampsia have been identified.[16]

There are studies about renalase and apelin in patients with ischemic stroke in the literature. However, there are no studies comparing elabela and cerebellin levels in patients with ischemic stroke and healthy subjects. In this study, we aimed to compare renalase, cerebellin, and their subtrates epinephrine, norepinephrine and dopamine in the blood and urine of patients with acute ischemic stroke and healthy individuals, which we think that they may have roles in the pathophysiology of ischemic stroke. In addition, we aimed to compare the levels of apelinergic system members apelin, elabela and nitric oxide in the blood and urine of patients with acute ischemic stroke and healthy individuals. Thus, we aimed to provide a broad perspective for understanding the pathophysiology of acute ischemic stroke

MATERIALS AND METHODS

Our study was carried out in accordance with the Declaration of Helsinki and approved by Kahramanmaraş Sütçü İmam University Faculty of Medicine Clinical Trials Ethics Committee (decision date: 03.01.2018 and number: 2018.01.01). Informed consent was obtained from the patients or their relatives when cooperation was not provided. The study group consisted of patients with acute ischemic stroke who applied to Kahramanmaraş Sütçü İmam University Emergency Medicine Clinic. The diagnosis of acute ischemic stroke was confirmed by the neurologist with cranial CT and/or MRI consistent with clinical findings. 42 patients with acute ischemic stroke and 42 age and sex matched healthy controls were included in the study. Inclusion criteria were: 1) patients with acute ischemic stroke clinic within 24 hours of symptom onset 2) aged older than 18 years old 3) first stroke attack. Patients with hemorrhagic stroke, brain tumors, chronic inflammation, chronic renal failure, increased creatinine levels, trauma were excluded. Blood and urine samples were collected simultaneously and within first 24 hours after the onset of acute stroke clinical manifestations. Serum and urine samples were centrifuged at 4000 g for 10 minutes and stored at -80 °C until required for analysis.

Biochemical Analysis

Serum and urine cerebellin, renalase, epinephrine, norepinephrine, dopamine, apelin, elabela and nitric oxide were measured using enzyme-linked immunosorbent assay method according to the manufacturer's

protocol.[17] All of the kits used in this study were supplied from Sunred Biological Technology (Shanghai, CHINA). The catologue numbers, intra-assay coefficient of variances (CV), inter-assay CVs, detection ranges and sensitivities of the kits used were presented in **Table 1.**

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS ver.20) and P values of <0.05 were considered statistically significant.Previously, the suitability of the data for normal distribution were evaluated by Kolmogorov Simirnov and Shapiro Wilk tests. Independent samples t test was used to compare normal distributed parameters and Mann Whitney-U test was used to compare non-normally distributed parameters. Pearson correlation analysis was used to compare normal distributed parameters and Spearman correlation analysis was used to compare non-normally distributed parameters for correlations.

RESULTS:

The ages of patients with ischemic stroke and control subjects were similar (p = 0.357). The mean age was 69.37 ± 13.37 (35-91) in the stroke group and 66.70 ± 12.87 (35-89) in the control group. The sexes of the participants were similar in both groups (p = 0.818). There were 15 females, 27 males in the stroke group and 14 females and 28 males in the control group. Systolic blood pressure was higher in the stroke group (p <0.001). The mean systolic blood pressure was 155.56 ± 3.04 mmHg (100-216) in the stroke group and 121.26 ± 9.57 mmHg (100-130) in the control group. Mean diastolic blood pressure was higher in the stroke group than in the control group, but the difference was statistically insignificant (P = 0.056). Mean diastolic blood pressure was 82.41 ± 19.86 mmHg (50-170) in the stroke group and 74.37 ± 6.29 mmHg (60-80) in the control group. Other clinical parameters and comparison results of the stroke patients and controls were given in**Table 2.** Serum cerebellin, renalase, epinephrine, norepinephrine, dopamine, apelin, elabela, nitric oxide levels and comparison results between the groups were given in **Table 3.**The levels of serum cerebellin, renalase, epinephrine, norperinephrine, dopamine, apelin, elebela and nitric oxide were similar in patients with ischemic stroke and in the controls (P> 0.05). Urine cerebellin, renalase, epinephrine, norepinephrine, dopamine, apelin, elabela, nitric oxide levels in stroke patients and controls and comparison results of these parameters between groups were presented in**Table 4.** There was no difference in terms of urine cerebellin, renalase, epinephrine, norperinephrine, dopamine apelin, elebela and nitric oxide levels between stroke patients and control subjects (p> 0.05). Strong correlations were found between renalase, cerebellin and catecholamines levels in serum and urine (p <0.001). The correlation analysis results between renalase, cerebellin and catecholamines were given in **Table 5.** There were also strong correlations between apelin, elabela and NO levels in serum and urine (p <0.001). The correlation analysis results among apelin, elabela and NO levels were given in **Table 6.**

DISCUSSION

Oxygen and nutrient deficiencies caused by ischemic stroke cause complex pathophysiological events. Current acceptance is that acute ischemic stroke activates hypothalamic-pituitary-adrenal axis to eliminate ischemic status and increases circulating cortisol and catecholamine levels.[18] Thus, systemic blood pressure increases and urgent brain perfusion is tried to be increased.[19] It has been reported that elevated plasma and urine cortisol and catecholamine levels are associated with high mortality and poor functional outcome in stroke patients.[20,21] However, different results have been reported in more recent studies. Stress hormones have been reported to increase in large hemispheric strokes, on the other hand moderate cortical infarcts have not been reported to affect the levels of stress hormones.[22,23] Oto et al found no relationship between clinical outcomes and plasma catecholamines in patients with ischemic stroke. Also, there was no correlation between plasma catecholamine (epinephrine, norepinephrine) levels and proinflammatory cytokine concentration in this study. This may be due to the short half-life of catecholamines and pathological conditions such as emotional factors and heart failure, which affect the release of catecholamines.[24]Renin-angiotensin-aldosterone system is also activated in patients with ischemic stroke. Activation of this system contributes to vasoconstriction and fluid retention.[25] However, the increment of blood pressure in patients with ischemic stroke usually tends to return to normal after 7 days in adults and after 2 days in young people. In addition, blood pressures tend to

be higher in people with previous hypertension than in those without hypertension. These findings suggest that other sources, besides central autonomic systems, may contribute to the high blood pressure in stroke patients.[19] Dopamine is the predominant catecholamine in the central nervous system and is associated with the management of motor functions, learning, consciousness and immunity.[26] Treatment with L-dopa, a precursor of dopamine, has improved motor abilities in patients with ischemic stroke.[27,28] In an animal study it was found that large amounts of dopamine were released rapidly from the striatum to the environment after ischemia.[29] Dopamine was determined to be decreased in ischemic stroke in another study.[30]Like other catecholamines, dopamine has a very short plasma half-life. All these studies and the results of our study suggest that the release of catecholamines in acute ischemic stroke may be affected by many factors and that there is no continuous catecholamine release in this process.

Renalase is a FAD-dependent amine oxidase enzyme that metabolizes catecholamines in the bloodstream. Renalase's substrat preference has been reported as dopamine, epinephrine and norepinephrine, respectively.[31] The level of renalase secreted into the blood is determined by renal function, renal perfusion and circulating catecholamine levels.[32] In patients with primary hypertension, serum renalase level was associated with elevated blood pressure.[33] However, no association has been reported between plasma renalase level and blood pressure in healthy subjects.[34] In elderly hypertensive patients, increased renalase levels were found to be associated with renal function and cardiovascular diseases rather than age effect.[35] There are publications investigating the relationship between renalase and ischemic stroke. A relationship between single-nucleotide polymorphisms of the renalase gene and ischemic stroke was reported.[36] Another study reported that renalase may be associated with stroke in hemodialysis patients. It has been suggested that the cause of the relationship may be due to increased sympathetic nervous system activation in these patients. Serum renalase levels were found to be lower in patients with ischemic stroke history than patients without stroke history in the same study. However, serum catecholamine levels were not measured in this study.[37] Hennebry et al. found that renalase exists in the brain and peripheral nerves. The authors reported that renalase could potentially contribute to the regulation of monoamine neurotransmitters.[38] Cerebellin is involved in the formation and function of synapses, regulation of motor and non-motor functions.[39,40] The adrenocortical secretagogue activity of cerebellin is regulated as paracrine manner with catecholamines that are locally released and affect the cortex.[41] Cerebellin strongly stimulates catecholamine release by rat adrenal medulla through adenylate-cyclase/PKA coupled receptors.[42] There is only one publication in the literature about cerebellin and stroke. In a human study, cerebellin did not show any difference in ischemic and hemorrhagic stroke patients in bloods taken within the first 24 hours. However, the control group was not present in this study.[43]

Apelin has been found to inhibit cell death, facilitate angiogenesis and enhance healing in ischemic stroke.[44] Apelin 13 and Apelin 36 have been reported to have neuroprotective effects in ischemic stroke.[45,46] Apelin acts against the vasoconstrictor effect of angiotensin II by NO-dependent mechanisms. Apelin increases NO secretion from vascular endothelial cells.[47] Plasma Apelin 17 and apelin 36 levels were found to be higher in patients with good collateral circulation compared to patients without good circulation and healthy subjects in ischemic stroke. NO levels in eyes with good collateral circulation were found to be higher than healthy controls, but similar to those with poor collateral circulation.[48] Elabela is involved in regulating blood pressure, fluid hemostasis, cardiac contraction and vasodilatation. Elabela performs vascular relaxation independently of NO. Vasodilation provided by Elabela may persist even when blood vessels are exposed to NO inhibitor L-NAME.[49] Like apelin, Elabela increases cardiac contraction and induces coronary vasodilatation. This effect is achieved by activating extracellular signal-regulated kinase (ERK), but the mechanism is independent of protein kinase C (PKC) activation. Elabela reduces secretion of angiotensin converting enzyme (ACE) and improves cardiac function In the event of stress.[50]Elabela levels were found to decrease in essential hypertension patients. It has been suggested that decrease of the endogenous Elabela level may be important in the pathogenesis of essential hypertension.[51] Regulating microcirculation, providing vascular resistance and neurotransmission tasks of NO has been identified in brain. It also regulates synaptic transmission, induces synaptogenesis and synaptic remodeling and is involved in the protection of cerebral blood flow.[52] NO was found to be released from endothelial cells, neurons, glial cells and neutrophils. The beneficial

or detrimental effects of NO have been reported in ischemic stroke depending on the time of release, the cell type and concentration in which it is secreted, and the condition of the ischemic region. Immediately after stroke, the amount of NO in the medium decreases rapidly. Three types of NO synthetases have been reported in ischemic stroke: inducible NOS (iNOS)-derived NO and neuronal NOS (nNOS)-derived NO play neurotoxicity, but endothelial NOS (eNOS)-derived NO plays a neuroprotective role in acute ischemic stroke. The toxic effects of iNOS and nNOS are due to that they have role in the formation of nitrates and free radicals, which show direct toxic effects to mitochondrial enzymes and genetic materials. The neuroprotective effect of eNOS is achieved by regulating vascular blood flow.[53] The indicated forms of NO are secreted in different amounts at different times in ischemic stroke. NNOS levels were the same as controls on the first day of ischemic stroke, lower than controls on day 3 and higher than controls on day 14. INOS begins to increase 12 hours after the onset of ischemic stroke and persists at high levels for one week. ENOS starts to rise 30 minutes after ischemia and is elevated 6 hours after ischemia. ENOS starts to rise 4 hours after reperfusion and significantly decreases 24 hours after the onset of reperfusion.[54]

In this study, urinary renalase, cerebellin, apelin and elabela and serum elabela were investigated for the first time in acute ischemic stroke patients. Additionally this is the first study in which renalase-cerebellin-catecholamines and apelin-elabela-NO are studied as a whole in acute stroke patients. We found very strong correlations among renalase, cerebellin and catecholamines in stroke and control groups in serum and urine. We also determined very strong correlations among apelin, elabela and NO in stroke and control groups in serum and urine. All these findings emphasize that renalase, cerebellin and catecholamines act together in healthy individuals and stroke patients. Similarly, strong correlations between apelin, elabela and NO indicate that these agents act together in healthy subjects and patients with stroke. Contrary to our expectations serum and urine cerebellin, renalase, epinephrine, norperinephrine, dopamine apelin, elebela and nitric oxide levels in the ischemic stroke group were similar to the control group in this study. Mracsko et al.[22] found that stress hormones were increased only in large infarcts in the animal model in which they produced large and moderate-mild infarcts. Liesz et al.[23] found that only large strokes increased plasma catecholamine metabolites and cortisol levels. A quantitative analysis of infarct volume (infarct volume measurement at diffusion MRI, NIHS scores) was not performed in our study. This may be one of the limitations of our study. Other limitation of our study was that samples were taken only in the acute phase of ischemic stroke. Therefore, the results of our study cannot be generalized to all stages of ischemic stroke.

In conclusion serum and urine cerebellin, renalase, epinephrine, norperinephrine, dopamine apelin, elebela and nitric oxide levels do not significantly change in the acute phase of ischemic stroke. Large multicenter studies are needed to explore the exact roles of these molecules in the pathophysiology of ischemic stroke.

Financial support: There are no financial supports

Conflict of interest: The authors report no conflict of interest.

Ethical approval : This study was carried out in accordance with the Declaration of Helsinki and approved by Kahramanmaraş Sütçü İmam University Faculty of Medicine Clinical Trials Ethics Committee (decision date: 03.01.2018 and number: 2018.01.01).

REFERENCES

1- Katan M, Luft A. Global Burden of Stroke. *Semin Neurol.*2018;38:208-211.

2-Struijs JN, van Genugten ML, Evers SM, Ament AJ, Baan CA, van den Bos GA. Future costs of strokein the Netherlands: theimpact of stroke services. *Int J Technol Assess Health Care.* 2006;22:518-524.

3- Zerna C, Thomalla G, Campbell BCV, Rha JH, Hill MD. Current practice and future directions in the diagnosis and acute treatment of ischaemic stroke. *Lancet.* 2018;392:1247-1256.

4- Rabinstein AA. Treatment of Acute Ischemic Stroke. *Continuum (Minneap Minn).* 2017;23:62-81.

5-Patel RAG, McMullen PW. Neuroprotection in the Treatment of Acute Ischemic Stroke. *Prog Cardiovasc Dis.* 2017;59:542-548.

6-Lundberg U. Stress hormones in health and illness: the roles of work and gender. *Psychoneuroendocrinology.* 2005;30:1017-1021.

7-Goldstein DS, Eisenhofer G, Kopin IJ. Clinical catecholamine neurochemistry: a legacy of Julius Axelrod. *Cell Mol Neurobiol.*2006;26:695-702.

8-Mazzocchi G, Andreis PG, De Caro R, Aragona F, Gottardo L, Nussdorfer GG. Cerebellin enhances in vitro secretory activity of human adrenal gland. *J Clin Endocrinol Metab.* 1999;84:632-635.

9-Rucinski M, Malendowicz LK. Precerebellin-related genes and precerebellin 1 peptide in endocrine glands of the rat-pattern of their expression. *Int J Mol Med.* 2009;23:113-119.

10- Strowski MZ, Kaczmarek P, Mergler S, et al. Insulinostatic activity of cerebellin-evidence from in vivo and in vitro studies in rats.*Regul Pept.* 2009;157:19-24.

11-Satoh F, Takahashi K, Murakami O, et al. Cerebellin and cerebellin mRNA in the human brain, adrenal glands and the tumour tissues of adrenal tumour, ganglioneuroblastoma and neuroblastoma. *J Endocrinol.* 1997;154:27-34.

12- Aydın S. Can cerebellin and renalase measurements contribute to the elimination of false positive results in pheochromocytoma and paraganglioma diagnoses? *Med Hypotheses.* 2017;107:64.

13-Tokinoya K, Shiromoto J, Sugasawa T, Yoshida Y, Aoki K, Nakagawa Y, Ohmori H, Takekoshi K. Influence of acute exercise on renalase and its regulatory mechanism. *Life Sci.* 2018;210:235 242.

14- Wang F, Xing T, Li J, et al. Renalase's expression and distribution in renal tissue and cells. *PLoS One.* 2012;7:e46442.

15- Zhang Y, Wang Y, Lou Y,et al. Elabela, a newly discovered APJ ligand: Similarities and differences with Apelin. *Peptides.*2018;109:23-32.

16- Marsault E, Llorens-Cortes C, Iturrioz X, et al. The apelinergic system: a perspective on challenges and opportunities in cardiovascular and metabolic disorders. *Ann N Y Acad Sci.* 2019;1455:12-33.

17- Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA.*Peptides.* 2015;72:4-15.

18- Fassbender K, Schmidt R, Mössner R, Daffertshofer M, Hennerici M. Pattern of activation of the hypothalamic-pituitary-adrenal axis in acute stroke. Relation to acute confusional state, extent of brain damage, and clinical outcome. *Stroke.* 1994;25:1105-1108.

19-Sternberg Z, Schaller B. Central Noradrenergic Agonists in the Treatment of Ischemic Stroke-an Overview. *Transl Stroke Res.*2020;11:165-184.

20- Feibel JH, Hardy PM, Campbell RG, Goldstein MN, Joynt RJ. Prognostic value of the stress response following stroke. *JAMA.*1977;238:1374-1376.

21- Olsson T. Urinary free cortisol excretion shortly after ischaemic stroke. *J Intern Med.* 1990;228:177-181.

22-Mracsko E, Liesz A, Karcher S, Zorn M, Bari F, Veltkamp R. Differential effects of sympathetic nervous system and hypothalamic-pituitary-adrenal axis on systemic immune cells after severe experimental stroke. *Brain Behav Immun.* 2014;41:200-209.

23-Liesz A, Rüger H, Purrucker J, et al. Stress mediators and immune dysfunction in patients with acute cerebrovascular diseases. *PLoS One.* 2013;8:e74839.

24-Oto J, Suzue A, Inui D, et al. Plasma proinflammatory and anti-inflammatory cytokine and catecholamine concentrations as predictors of neurological outcome in acute stroke patients. *J Anesth.* 2008;22:207-212.

25- Sharma JC, Ross I, Vassallo M. Cardio-protection in acute stroke.*Int J Stroke.* 2007;2:299-301.

26-Huck JH, Freyer D, Böttcher C, et al. De novo expression of dopamine D2 receptors on microglia after stroke. *J Cereb Blood Flow Metab.*2015;35:1804-1811.

27- Scheidtmann K, Fries W, Müller F, Koenig E. Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomised, double-blind study. *Lancet.*2001;358:787-790.

28- Rösser N, Heuschmann P, Wersching H, Breitenstein C, Knecht S, Flöel A. Levodopa improves procedural motor learning in chronic stroke patients. *Arch Phys Med Rehabil.* 2008;89:1633-1641.

29- Toner CC, Stamford JA. 'Real time' measurement of dopamine release in an in vitro model of neostriatal ischaemia. *J Neurosci Methods.* 1996;67:133-140

30- Lin L, Sun D, Chang J, et al. Cocaine- and amphetamine-regulated transcript (CART) is associated with dopamine and is protective against ischemic stroke. *Mol Med Rep.* 2018;18:3298-3304.

31-Malyszko J, Bachorzewska-Gajewska H, Dobrzycki S. Renalase, kidney and cardiovascular disease: are they related or just coincidentally associated? *Adv Med Sci.* 2015;60:41-49.

32-Wang Y, Safirstein R, Velazquez H, Guo XJ, Hollander L, Chang J, Chen TM, Mu JJ, Desir GV. Extracellular renalase protects cells and organs by outside-in signalling. *J Cell Mol Med.* 2017;21:1260-1265.

33-Lemiesz M, Tenderenda-Banasiuk E, Sosnowska D, Taranta-Janusz K, Wasilewska A. Serum Renalase Levels in Adolescents with Primary Hypertension. *Pediatr Cardiol.* 2018;39:1258-1264.

34-Wang Y, Lv YB, Chu C, et al. Plasma Renalase is Not Associated with Blood Pressure and Brachial-Ankle Pulse Wave Velocity in Chinese Adults With Normal Renal Function. *Kidney Blood Press Res.*2016;41:837-847.

35-Zbroch E, Musialowska D, Koc-Zorawska E, Malyszko J. Age influence on renalase and catecholamines concentration in hypertensive patients, including maintained dialysis. *Clin Interv Aging.*2016;11:1545-1550.

36-Zhang R, Li X, Liu N, et al. An association study on renalase polymorphisms and ischemic stroke in a Chinese population.*Neuromolecular Med.* 2013;15:396-404.

37-Malyszko J, Koc-Zorawska E, Malyszko JS, Kozminski P, Zbroch E, Mysliwiec M. Renalase, stroke, and hypertension in hemodialyzed patients. *Ren Fail.* 2012;34:727-731.

38-Hennebry SC, Eikelis N, Socratous F, Desir G, Lambert G, Schlaich M. Renalase, a novel soluble FAD-dependent protein, is synthesized in the brain and peripheral nerves. *Mol Psychiatry.* 2010;15:234-236.

39-Lee SJ, Uemura T, Yoshida T, Mishina M. GluRδ2 assembles four neurexins into trans-synaptic triad to trigger synapse formation.*J Neurosci.* 2012 Mar 28;32:4688-4701.

40-Otsuka S, Konno K, Abe M, et al. Roles of Cbln1 in Non-Motor Functions of Mice. *J Neurosci.* 2016;36:11801-11816.

41- Hochól A, Neri G, Majchrzak M, Ziolkowska A, Nussdorfer GG, Malendowicz LK. Prolonged cerebellin administration inhibits the growth, but enhances steroidogenic capacity of rat adrenal cortex. *Endocr Res.* 2001;27(1-2):11-17.

42-Albertin G, Malendowicz LK, Macchi C, Markowska A, Nussdorfer GG. Cerebellin stimulates the secretory activity of the rat adrenal gland: in vitro and in vivo studies. *Neuropeptides.* 2000;34:7-11.

43-Montaner J, Mendioroz M, Delgado P, et al. Differentiating ischemic from hemorrhagic stroke using plasma biomarkers: the S100B/RAGE pathway.*J Proteomics.* 2012;75:4758-4765.

44- Wu Y, Wang X, Zhou X, Cheng B, Li G, Bai B. Temporal Expression of Apelin/Apelin Receptor in Ischemic Stroke and its Therapeutic Potential.*Front Mol Neurosci.* 2017;10:1.

45- Wu F, Qiu J, Fan Y,et al. Apelin-13 attenuates ER stress-mediated neuronal apoptosis by activating Gα(i)/Gα(q)-CK2 signaling in ischemic stroke. *Exp Neurol.* 2018;302:136-144.

46- Gu Q, Zhai L, Feng X, et al. Apelin-36, a potent peptide, protects against ischemic brain injury by activating the PI3K/Akt pathway.*Neurochem Int.* 2013;63:535-540.

47- Yang P, Maguire JJ, Davenport AP. Apelin, Elabela/Toddler, and biased agonists as novel therapeutic agents in the cardiovascular system. *Trends Pharmacol Sci.* 2015;36:560-567.

48- Jiang W, Hu W, Ye L, et al. Contribution of Apelin-17 to Collateral Circulation Following Cerebral Ischemic Stroke. Transl Stroke Res. 2019;10:298-307.

49- Shin K, Kenward C, Rainey JK. Apelinergic System Structure and Function. *Compr Physiol.* 2017;8:407-450.

50-Marsault E, Llorens-Cortes C, Iturrioz X, et al. The apelinergic system: a perspective on challenges and opportunities in cardiovascular and metabolic disorders. Ann N Y Acad Sci. 2019 ;1455:12-33.

51- Li Y, Yang X, Ouyang S, et al. Declined circulating Elabela levels in patients with essential hypertension and its association with impaired vascular function: A preliminary study. *Clin Exp Hypertens.* 2020;42(3):239-243.

52- Narne P, Pandey V, Phanithi PB. Role of Nitric Oxide and Hydrogen Sulfide in Ischemic Stroke and the Emergent Epigenetic Underpinnings.*Mol Neurobiol.* 2019;56(3):1749-1769. 53- Chen ZQ, Mou RT, Feng DX, Wang Z, Chen G. The role of nitric oxide in stroke. *Med Gas Res.* 2017;7:194-203.

54-Liu H, Li J, Zhao F, et al. Nitric oxide synthase in hypoxic or ischemic brain injury. *Rev Neurosci.* 2015;26(1):105-17.

TABLES

Table 1: The catologue numbers, intra-assay coefficient of variances (CV), inter-assay CV, detection ranges and sensitivities of the kits

Kit Name	Catalog Number	Intra assay CV	Inter assay CV	Detection Range	Ser
Human Cerebellin-1	201-12-3438	<9%	<10%	10ng/L-1500 ng/L	8.6
Human Renalase (MAO-C)	201-12-5282	<10%	<12%	3ng/ml-600ng/ml	2.0
Human Epinephrine	201-12-1039	<10%	<12%	0.3ng/ml-60ng/ml	0.2
Human Norepinephrine	201-12-0987	<10%	<12%	10ng/L-300ng/L	8.6
Human Dopamine	201-12-1302	<10%	<12%	8nmol/L-2000nmol/L	7.0
Human Apelin	201-12-2015	<10%	<12%	1ng/L-200ng/L	0.7
Human Elabela	201-12-7693	<9%	<10%	15ng/L-3000ng/L	12.0
Human Nitric Oxide	201-12-1511	<10%	<12%	4μmol/L-600μmol/L	2.0

Table 2: Clinical parameters and comparison results of the patient and control groups**.** WBC: White Blood Cell, NEU: Neutrophil, RBC: Red Blood Cell, HB: Hemoglobin, HCT: Hematocrit, MCV: mean cell volume, RDW: Red cell distribution volume, PLT: Platelet, MPV: Mean platelet volume, BUN: Blood Urea Nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, LDL: Low density lipoprotein, HDL: High density lipoprotein, CRP: C reactive protein

	ISCHEMIC STROKE	CONTROL	P VALUE
WBC (x10^3 cells/mcL)	9.58±3.55 (4.37-24.30)	8.17±2.61 (3.44-16.55)	0.062
NEU (x10^3 cells/mcL)	6.55±3.18 (2.31-20.11)	5.59±3.56 (1.44-18.90)	0.064
LYMPHOCYTE (x10^3 cells/mcL)	2.07±1.01 (0.44-5.87)	2.31±0.63 (1.37-4.02)	0.064
MONOCYTE (x10^3 cells/mcL)	0.74±3.69 (0.05-2.25)	0.63±0.26 (0.27-1.27)	0.145

	ISCHEMIC STROKE	CONTROL	P VALUE
EOSINOPHIL (x10^3 cells/mcL)	0.17±0.14 (0-0.72)	0.20±0.14 (0.14-0.67)	0.185
BASOPHIL (x10^3 cells/mcL)	0.03±0.18 (0.01-0.08)	0.04±0.016 (0.01-0.06)	**0.026**
RBC (x10^6 cells/mcL)	4.85±0.72 (3.58-6.84)	4.64±1.14 (0.43-5.77)	0.804
HB (g/dL)	13.18±1.98 (8.40-17.60)	13.64±1.91 (8.90-17.50)	0.355
HCT (%)	40.02±5.20 (27.00-51.60)	40.65±5.18 (24.30-51.40)	0.627
MCV (fL)	83.03±6.67 (63.30-92.10)	85.79±8.68 (70.30-114.00)	0.424
RDW (%)	43.35±6.06 (35.20-70.70)	41.71±11.33 (11.30-66.20)	0.916
PLT (x10^5 cells/mcL)	271.02±94.78 (148-629)	244.52±93.73 (78.00-555)	0.496
PDW (%)	12.15±2.06 (9.10-17.20)	12.18±1.94 (9.00-17.10)	0.957
MPV (fL)	10.26±0.93 (8.60-12.50)	10.37±0.99 (8.80-12.20)	0.634
TOTAL PROTEIN (g/dL)	6.68±0.64 (4.60-7.80)	7.11±0.40 (6.50-8.15)	**0.006**
ALBUMIN (g/dL)	4.00±0.35 (2.90-4.80)	4.17±0.21 (3.80-4.52)	**0.029**
CLOR (mEq/L)	104.78±3.98 (96-116)	105.24±2.11 (103-108)	0.802
CALCIUM (mg/dL)	9.00±0.50 (7.60-10.30)	9.40±0.46 (8.60-10.60)	**0.004**
POTASSIUM (mEq/L)	4.45±0.66 (3.40-7.40)	4.42±0.36 (3.80-5.20)	0.598
BUN (mg/dL)	17,90±5.61 (10-38)	14.33±2.61 (10-21)	**0.002**
AST (U/L)	27.11±9.78 (14-64)	24.71±6.67 (16.00-43.00)	0.313
ALT (U/L)	18.90±7.09 (9.00-40.00)	24.23±13.44 (11.00-61.00)	0.141
SODIUM (mEq/L)	139.97±2.83.(134.00-145.00)	141.22±2.08 (137.00-146.00)	**0.029**
GLUCOSE (mg/dL)	138.90±52.96 (64.00-317.00)	107.43±29.36 (67.00-199.00)	**0.002**
CREATININE (mg/dL)	0.88±0.20 (0.46-1.30)	0.79±0.22 (0.30-1.10)	0.063
LDH (U/L)	251.00±91.43 (159-595)	LDH 233.25±35.86 (201-233)	0.861
CHOLESTEROL (mg/dL)	177.62±43.36 (94-276)	199.40±36.90 (162.20-236)	0.316
LDL (mg/dL)	109.97±38.23 (48.00-197.00)	103.80±32.19 (51.00-133.00)	0.966
TRIGLYCERIDE (mg/dL)	134.97±51.28 (58.00-256.00)	148.60±92.37 (36.00-232.00)	0.689
HDL (mg/dL)	38.88±11.97 (23-74)	51.50±6.13 (43.00-56.00)	**0.032**
CRP (mg/L)	18.55±27,63 (3.13-121)	3.74±0.90 (3.02-6.14)	**0.042**

Table 3: Serum cerebellin, renalase, epinephrine, norepinephrine, dopamine, apelin, elabela, nitric oxide, levels in ischemic stroke and control groups and comparison results between groups.

	ISCHEMIC STROKE	CONTROL	P VALUE
CEREBELLIN (ng/L)	253.13±165.42 (114.19-778.84)	252.29±148.93(137.53623.43)	0.775
RENALASE (ng/mL)	115.57±202.80 (6.33-795.14)	103.77±178.00 (3.61-885.83)	0.847
EPINEPHRINE (ng/mL)	10.97±25.71 (0.57-143.85)	17.48±39,17 (0.69-172)	0.312
NOREPINEPHRINE (ng/L)	389.10±901.49 (0-5144.62)	362.08±955.80 (0-4947.69)	0.241
DOPAMINE (nmol/L)	364.22±698.62 (3.13-3231.72)	424.67±860.96 (9.37-3378.75)	0.876
APELIN (ng/L)	54.72±102.83 (3.80-423.58)	36.53±68.95 (3.80-342.32)	0.403
ELABELA (ng/L)	932.09±1598.79 (80-6307)	662.502±502 (85.71-5254.44)	0.439
NITPIˆ OΞIΔE (μμoλ/Λ)	29.39±46.83 (0-190.81)	26.87±41.74 (0-185.70)	0.696

Table 4: Urine cerebellin, renalase, epinephrine, norepinephrine, dopamine, apelin, elabela, nitric oxide levels in stroke patients and controls and comparison results of these parameters between groups

	ISCHEMIC STROKE	**CONTROL**	
CEREBELLIN (ng/L)	266.66±98.40 (124.03-608.77)	243.31±86.27 (141.13-486.84)	0.223
RENALASE (ng/mL)	151.44±45.03 (81.13-335.53)	154.19±55,50 (82.26-326.84)	0.568
EPINEPHRINE (ng/mL)	2.13±0.79 (0.93-4.19)	2.01±0.85 (0.63-4.13)	0.464
NOREPINEPHRINE (ng/L)	104.30±26.99 (63.08-173.85)	100.36±28.08 (52.31-170.77)	0.487
DOPAMINE (nmol/L)	453.96±138.07 (242.37-1022.31)	461.36±169.77 (245.95-995.22)	0.507
APELIN (ng/L)	4.07±3.12 (0.26-15.00)	3.60±3.50 (0.18-14.92)	0.256
ELABELA (ng/L)	122.41±63.54 (40.50-300.00)	107.50±64.39 (22.50-302.06)	0.308
NITPIˆ OΞIΔE (μμoλ/Λ)	15.41±7.81 (4.74-40.09)	13.67±8.51 (2.47-40.82)	0.288

Table 5: Results of correlation analysis among renalase, cerebellin and catecholamines.

CORRELATIONS	**SERUM CONTROL**	**SERUM STROKE**	**URINE CONTROL**	**URINE STROKE**
CEREBELLIN-RENALASE	r=0.769, **p<0.001**	r=0.864, **p<0.001**	r=0.565, **p<0.001**	r=0.522, **p<0.001**
CEREBELLIN-EPINEPHRINE	r=0.539, **p<0.001**	r=0.817, **p<0.001**	r=0.637, **p<0.001**	r=0.723, **p<0.001**
CEREBELLIN-NOREPINEPHRINE	r=0.661, **p<0.001**	r=0.761, **p<0.001**	r=0.658, **p<0.001**	r=0.728, **p<0.001**
CEREBELLIN-DOPAMINE	r=0.820, **p<0.001**	r=0.872, **p<0.001**	r=0.563, **p<0.001**	r=0.522, **p<0.001**
RENALASE-EPINEPHRINE	r=0.509, p=0.001	r=0.758, **p<0.001**	r=0.652, **p<0.001**	r=0.691, **p<0.001**
RENALASE-NOREPINEPHRINE	r=0.605, **p<0.001**	r=0.693, **p<0.001**	r=0.657, **p<0.001**	r=0.693, **p<0.001**
RENALASE-DOPAMINE	r=0.820, **p<0.001**	r=0.889, **p<0.001**	r=1.000, **p<0.001**	r=1.000, **p<0.001**
EPINEPHRINE-NOREPINEPHRINE	r=689, **p<0.001**	r=0.946, **p<0.001**	r=0.995, **p<0.001**	r=0.999, **p<0.001**
EPINEPHRINE-DOPAMIN	r=0.703, **p<0.001**	r=0.953, **p<0.001**	r=0.651, **p<0.001**	r=0.690, **p<0.001**
NOREPINEPHRINE-DOPAMIN	r=0.825, **p<0.001**	r=0.858, **p<0.001**	r=0.656, **p<0.001**	r=0.692, **p<0.001**

Table 6: Results of correlation analysis between apelin, elabela and NO.

CORRELATIONS	**SERUM CONTROL**	**SERUM STROKE**	**URINE CONTROL**	**URINE STROKE**
APELIN-ELABELA	r=0.998, **p<0.001**	r=0.992, **p<0.001**	r=0.705, **p<0.001**	r=0.545, **p<0.001**
APELIN-NO	r=0.725, **p<0.001**	r=0.653, **p<0.001**	r=0.715, **p<0.001**	r=0.582, **p<0.001**
ELABELA-NO	r=0.736, **p<0.001**	r=0.677, **p<0.001**	r=0.994, **p<0.001**	r=0.994, **p<0.001**