

Relationship between Serum Elabela Level and Hypertensive Nephropathy

Yusuf Dogan¹, Erhan Onalan¹, Cundullah Cavli¹, Ebru Etem Onalan², Burak Yakar³ and Emir Donder¹

¹Department of Internal Medicine, Firat University, Medical School, Elazig, Turkey

²Department of Medical Biology, Firat University, Medical School, Elazig, Turkey

³Department of Family Medicine, Firat University, Medical School, Elazig, Turkey

ABSTRACT

Objective: To determine the difference in serum Elabela level in hypertensive patients with and without nephropathy compared to the healthy control group.

Study Design: Cross-sectional descriptive study.

Place and Duration of Study: Firat University Medical School, Elazig, Turkey between December 2018 and November 2020.

Methodology: The cross-sectional descriptive study consisted of 37 patients with hypertensive nephropathy (group 3), 50 hypertensive patients without nephropathy (group 2), and 50 healthy controls (group 1). Hypertensive nephropathy was defined as serum creatinine ≥ 1.8 mg / dl or GFR < 40 ml / minute. Biochemical parameters (Glucose, AST, ALT, urea, creatinine, lipid levels, hemogram, calcium, phosphorus, parathormone) and the levels of serum Elabela were evaluated and compared.

Results: There was no significant difference in age (0.270) and gender (0.951) between groups. The median Elabela levels of the three groups were 40.3 ng/mL (22.5-54.6), 5.1 ng/mL (3.7-8.3), 9.2 ng/mL (6.1-23.1), respectively with a significant difference ($p < 0.001$).

Conclusion: The plasma levels of Elabela were lower in the case of hypertension, independent of nephropathy. However, this decrease is not specific for nephropathy and may be due to other accompanying chronic diseases.

Key Words: Hypertension, Hypertensive nephropathy, Elabela.

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INTRODUCTION

Diabetes-related nephropathy and hypertensive nephropathy are among the most common causes of end-stage renal disease (ESRD). Secondary to hypertension, damage occurs in many systems causing structural and functional changes.¹

Elabela, also known as Apela, is a peptide consisting of 54 amino acids. Elabela is an endogenous binder of the apelin receptor (APJ), expressed in many tissues, mainly kidney. It is secreted and circulated as a hormonal peptide in adults.² Elabela has anti-hypertensive effects. Elabela uses the same receptor as Apelin-13, which shows hypotensive effects in both normotensive and hypertensive rats. Therefore, Elabela is thought to also regulate vascular tone.³

When Elabela was administered to the same groups of rats, blood pressure lowering activity, similar to Apelin-13, was achieved.⁴ Unlike Apelin, Elabela mediated vascular dilation is independent.⁵ Extracellular signal regulated kinase (ERK) can cause excessive vasoconstriction.⁶ High ERK level is blamed in the etiopathogenesis of many hypertension, especially hypertension that develops secondary to obesity.⁷ Elabela has an anti-hypertensive effect by suppressing ERK activation.⁸ This relation needs to be further evaluated for clinical relevance.

The aim of this study was to determine the difference serum Elabela level in the hypertension patients with and without nephropathy compared to the healthy control group.

METHODOLOGY

This single-centre, cross-sectional descriptive study was conducted at Internal Medicine Clinic, of Firat University Medical School, Elazig, Turkey between December 2018 and November 2020. It was designed in accordance with the ethical rules as stated in the Helsinki Declaration. Approval was obtained from the Faculty of Medicine Ethics Committee (25.10.2018, 17/02). The study group was composed of individuals between the ages of 30-75 years. Patient groups consisted of hypertensive patients with and without hypertensive nephropathy. The study group consisted of 87 patients, 37 of whom were diagnosed with

Correspondence to: Dr. Erhan Onalan, Erhan Onalan,
Department of Internal Medicine, Firat University,
Medical School, Elazig, Turkey
E-mail: drakdeniz@msn.com

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HT and had hypertensive nephropathy, and 50 patients, who had hypertension but did not have hypertensive nephropathy. The control group was formed of 50 healthy individuals of similar age and gender, who were non-hypertensive, did not have any comorbidity or lipid metabolism disorder. Individuals with a blood pressure >130/80 mmHg or using antihypertensive drugs and normal blood pressure measurements were considered hypertensive. Hypertensive patients were those with presence of long-term hypertension history with stage I / II retinopathy, failure to show other causes of kidney failure other than HT, serum creatinine ≥ 1.8 mg / dl. Individuals with GFR 40 ml / min were considered as having hypertensive nephropathy. Exclusion criteria were: under age 30 years, coexistence Diabetes mellitus, primary renal disease, non-hypertensive renal pathology, heart failure, liver disease, acute infection, hypothyroidism, malignancy and ESRD patients, who received hemodialysis or peritoneal dialysis.

The anamnesis of the individuals in the patient and control groups were taken, and their systolic and diastolic blood pressures were measured and questioned in terms of family histories. Demographic information (age, gender, body mass index values) were recorded for the entire study group. Glomerular filtration rate was calculated according to the Cockcroft-Gault Formula; as $GFR = (140 - \text{age}) \times \text{Body Weight (kg)} / 72 \times \text{Serum Creatinine (mg/dl)}$, if female $\rightarrow \times 0.85$.

Glucose, AST, ALT, urea, creatinine, lipid levels, complete blood count, calcium, phosphorus, and Parathormone results were obtained from the records of routine examinations. Other blood samples were kept in aprotinin tubes. The blood samples taken into the tubes were centrifuged at 5000 rpm for 5 minutes within half an hour and their serums were separated. Blood samples taken into tubes with aprotinin were stored at -80°C until they were studied after centrifugation. After all samples were collected, serum Elabala levels were measured by enzyme linked-immunosorbent assay (ELISA) method. Serum Elabala levels were determined using an Elabala (human) -EIA kit (Peninsula Laboratories International, Inc., San Carlos, CA, USA) according to manufacturer's instructions. This test has inter- and intra-assay coefficients of variation below 8% and 5%, respectively.

The analyses were evaluated in the SPSS versin 22 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) package programme. Descriptive data in the study were shown as n% values in categorical data, and mean \pm standard deviation, median, interquartilerange (25-75 percentile values) in continuous data. Chi-square analysis (Pearson's Chi-square) was used to compare categorical variables between the groups. The suitability of continuous variables to normal distribution was evaluated with the Kolmogorov-Smirnov test. The Independent samples t-test was used for the comparison of variables conforming to the normal distribution between two groups, the Oneway ANOVA for the comparison between more than two groups, the Mann-Whitney U-test for the comparison of the variables that did not conform to the normal distribution between the two groups, and the Kruskal-Wallis test for the comparison

between more than two groups. The statistical significance level in the analysis was accepted as $p < 0.05$.

RESULTS

The study included 37 patients with hypertensive nephropathy, 50 patients with hypertension but no nephropathy, and 50 healthy controls. The groups were formed of similar age and gender, and no significant difference was found between the groups in terms of age and gender ($p = 0.270$). There was a significant difference between the groups in terms of height, weight and body mass index (BMI, Table I, $p < 0.05$). It was observed that this difference for height was due to the difference between the control group and the hypertensive group and the hypertensive nephropathy group, and for weight and BMI, this difference was between the hypertensive group and the control group and the hypertensive nephropathy group. A significant difference was found between the groups in terms of systolic and diastolic blood pressure ($p < 0.001$). It was seen that this difference was due to the difference between the control and hypertensive groups for systolic blood pressure and the difference between the control and hypertensive nephropathy groups.

It was observed that it was caused by the difference between all groups in terms of diastolic blood pressure. No statistically significant difference was found between the hypertensive group and the hypertensive nephropathy group ($p = 0.191$, Table I).

A significant difference was found between the groups in terms of glucose level (0.007). It was observed that this difference was between the hypertensive group and the hypertensive nephropathy group. AST and calcium levels of the hypertensive group were found to be significantly higher than the hypertensive nephropathy group ($p < 0.05$). Phosphorus and PTH levels in the hypertensive nephropathy group were found to be significantly higher than the hypertensive group ($p < 0.05$). A significant difference was found between the groups in terms of urea, creatinine and GFR ($p < 0.001$). It was seen that this difference was caused by the difference in all three groups. A significant difference was found between the groups in terms of hemoglobin value ($p < 0.001$). It was seen that this difference was due to the difference between the hypertensive nephropathy group and the hypertensive group and the control group. Finally, a significant difference was found between the groups in terms of platelet ($p = 0.022$). It was seen that this difference was caused by the hypertensive nephropathy group and the control group. No significant difference was found between the groups in terms of total cholesterol, LDL, triglyceride and ALT ($p > 0.05$, Table II).

The median Elabala level of the groups was 40.3 ng/mL (22.5-54.6) in the healthy control group, 5.1 ng/mL (3.7-8.3) in the hypertension group, and 9.2 ng/mL (6.1-23.1) in the hypertensive nephropathy group, respectively. A statistically significant difference was found between the groups ($p < 0.001$, Table III).

Table I: Comparison of the groups by age, gender, body measurements and blood pressure measurements.

Variables		Control group	Hypertensive group	Hypertensive nephropathy group	p
		Median value (IQR)	Median value (IQR)	Median value (IQR)	
Gender. n(%)	Male	24 (48.0)	25 (50.0)	19 (51.4)	0.951*
	Female	26 (52.0)	25 (50.0)	18 (48.6)	
Age (year)		59 (52.75-63.25)	61 (49-69)	65 (52-69)	0.270**
Height (m) (Mean±SD)		1.70±.1 ^a	1.655±.1 ^b	1.657±.1 ^b	0.004***
Weight (kg) (Mean±SD)		74.1±14.2 ^a	81.2±13.7 ^b	72.8±13.2 ^a	0.008***
BMI (kg/m ²) (Mean±SD)		25.4±3.4 ^a	29.8±5.5 ^b	26.5±4.6 ^a	<0.001***
Systolic blood pressure (mmHg)		120 (115-125) ^a	135 (130-140) ^b	140 (130-150) ^b	<0.001***
Diastolic blood pressure (mmHg)		70 (65-80) ^a	80 (80-90) ^b	90 (80-90) ^c	<0.001***
Disease duration (years)		-	6 (2-10)	10 (3-15)	0.191****

* Chi-square test, ** Kruskal Wallis test, *** OneWay ANOVA test, **** Mann-Whitney U-test were used. IQR: Interquartilerange. a, b The group from which the difference originates.

Table II: Comparison of blood values of groups.

Variables	Control group	Hypertensive group	Hypertensive nephropathy group	p
	Median value (IQR)	Median value (IQR)	Median value (IQR)	
T. Cholesterol (mg/dl) (Mean±SD)	172.5±30.0	180.4±45.6	180.4±44.7	0.545*
LDL (mg/dl) (Mean±SD)	100.6±25.5	114.4±34.6	108.8±30.5	0.077*
TG (mg/dl)	-	132.0 (100.0-157.0)	126.0 (105.0-157.0)	0.850**
Glucose (mg/dl) (Mean±SD)	90.3±10.2 ^{a,b}	95.2±12.0 ^a	87.6±12.1 ^b	0.007*
AST (mg/dl)	-	23.0 (19.0-26.0)	18.0 (16.0-20.0)	<0.001**
ALT (mg/dl)	-	19.0 (13.0-27.0)	16.0 (12.0-20.0)	0.086**
Urea (mg/dl)	26.0 (22.0-31.0) ^a	38.0 (29.0-51.0) ^b	107.0 (78.0-141.0) ^c	<0.001***
Creatinine (mg/dl)	.7 (.6-.9) ^a	.9 (.7-1.1) ^b	2.9 (2.4-4.0) ^c	<0.001***
Calcium (mg/dl)	-	9.0 (8.8-9.4)	8.7 (8.3-9.4)	0.021**
Phosphorus (mg/dl)	-	3.6 (3.1-4.0)	4.0 (3.2-4.8)	0.023**
GFR (ml/dk)	134.6 (123.3-146.3) ^a	90.5 (67.1-125.4) ^b	23.9 (17.7-31.0) ^c	<0.001***
Hgb (g/dl) (Mean±SD)	14.0±1.4 ^a	13.7±1.7 ^a	11.5±2.4 ^b	<0.001*
PLT (*1000/μl) (Mean±SD)	270.4±64.4 ^a	258.7±67.7 ^{a,b}	224.9±98.8 ^b	0.022*
PTH (pg/ml)	-	66.8 (51.0-104.0)	206.6 (147.2-375.7)	<0.001**

* OneWay ANOVA test, ** Mann-Whitney U-test, *** Kruskal Wallis test, were applied. IQR: Interquartilerange. a, b, c group from which the difference originates.

Table III: Comparison of elabela level according to groups.

Variables	Control group	Hypertensive group	Hypertensive nephropathy group	p
	Median value (IQR)	Median value (IQR)	Median value (IQR)	
Elabela	40.3 (22.5-54.6) ^a	5.1 (3.7-8.3) ^b	9.2 (6.1-23.1) ^c	<0.001*

* Kruskal Wallis test was applied. IQR: Interquartilerange. a,b,c The group from which the difference originates.

DISCUSSION

Hypertension is the major contributing factor to all-cause mortality. It is the main risk factor for stroke (ischemic and hemorrhagic) and coronary artery disease. Moreover, people with hypertension are predisposed to kidney failure, heart failure, peripheral vascular disease, and other medical conditions. It is estimated that around 1.4 billion adults worldwide have hypertension.⁹ Hypertension is the second most common cause of ESRD. ESRD developed due to hypertensive nephropathy in 30% of all patients receiving renal replacement therapy.¹⁰

Elabela, which has many forms, has the highest isoform among vertebrate species, Elabela-11.¹¹ Elabela, which has a high rate in the endothelial structure of the heart and arteries, provides benefits by increasing the contraction of the heart and providing coronary vasodilation.^{12,13} Elabela improves heart function by reducing angiotensin converting enzyme (ACE) expression in the heart during stress.¹⁴ Elabela injection in rats increases diuresis.¹⁵ In addition, elabela is required for mouse embryonic angiogenesis.¹⁶

There are many studies in the literature regarding the function of Elabela and its level in various diseases. In this study, the authors investigated the levels of Elabela in the healthy

group, hypertensive group and hypertensive nephropathy group.

Li *et al* found that the level of Elabela decreased significantly in hypertensive patients compared to healthy individuals ($p = 0.001$).¹⁷

In this study, serum Elabela levels of 50 hypertensive patients who were similar in age and had a significant difference in blood pressure and 50 patients in the control group were examined. Similar to the study by Li *et al.*, serum Elabela level was found to be lower in the hypertensive group with statistical significance.

Chen *et al.* studied ischemia reperfusion injury, one of the causes of acute kidney injury, in mice. Elabela mRNA levels were examined in mice injured by ischemia reperfusion and it was observed that it decreased in nephropathy.¹⁸ In this study, it was found that Elabela was at a statistically lower level in the group with hypertensive nephropathy compared to healthy individuals.

In the study by Xu *et al.* using Anti-ELA antibodies, it was determined that Elabela was mostly found in the apical membrane of the epithelial cells of the collecting tubule.¹⁹ In the same study, salt-sensitive mice were compared with salt-resistant mice. Less apela secretion was observed in the medulla of salt-sensitive mice after the administration of large amounts of salt. Administration of ELA-32 in salt-sensitive mice suppresses hypertension, fibrosis, and inflammation caused by high amounts of salt administration.¹⁹ Again, in this study, blood pressure was measured by radiotelemetry in salt sensitive mice after high salt diet. Exogenous ELA-32 infusion has been shown to significantly suppress hypertension caused by excessive salt administration in salt-sensitive mice.¹⁹

This study, in which Elabela was found low in nephropathy, supports the study of Xu. *et al.* They detected high amounts of Elabela in the renal collecting tubules by RNA scope and immunofluorescence methods. Similarly, low Elabela in hypertension may indicate that Elabela has protective effects on hypertension.

Zhang *et al.* reported that serum Elabela level decreased with the increase of diabetic kidney damage and serum Elabela level had a positive correlation with GFR.²⁰ They also experimented on mice, on the basis that Elabela may have a protective role in diabetic nephropathy. Injections of Elabela were administered after diabetes was established in mice. As a result, they found that kidney fibrosis and renal inflammation were reduced, and kidney damage was improved.²¹ In this study, Elabela level was found to be lower in the healthy group compared to the hypertension and hypertensive nephropathy group. This result leads to the conclusion that Elabela may decrease with kidney damage or decrease in Elabela level may cause kidney damage. The fact that

Elabela is low in conditions such as hypertension and nephropathy and that exogenous Elabela application improves existing pathology in other studies shows the positive effects of Elabela on these systems.

The most important limitations of this study are that it is a single-centre study and the number of patients is low.

CONCLUSION

In this study, the level of Elabela were decreased in hypertensive nephropathy, is consistent with the studies in the current literature. However, these findings should be supported by large-scale studies conducted in several different centres.

ETHICAL APPROVAL:

The study protocol was approved by the Ethics Committee of Firat University (No. 17-02, Date: 25.10.2018). The study was conducted in accordance with the principles of the Declaration of Helsinki.

PATIENTS' CONSENT:

Written informed consents were obtained from all patients.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

YD, EO: Data collection, design, compiling, and discussion, Led and conceived the project, and authored the manuscript. CC: Data collection, design.

EEO: Study of biochemical data.

BY: Collecting and analysing data, discussion.

ED: Collecting, statistics and analysing data.

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