


Investigation of serum phoenixin levels in patients with hypertension

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SUMMARY

OBJECTIVE: Hypertension is a major modifiable risk factor for cardiovascular disease and premature death worldwide. Phoenixin is a newly identified neuropeptide with multiple bioactivity. However, there was no published data about phoenixin levels in hypertension. The aim of this study was to evaluate the relationship between phoenixin and hypertension.

METHODS: This study was performed in 36 patients with hypertension and 36 healthy controls. Serum phoenixin-14 and phoenixin-20 levels were determined by Enzyme-Linked ImmunoSorbent Assay method.

RESULTS: Serum phoenixin-14 and phoenixin-20 values were significantly lower in hypertension patients compared with the control group ($p < 0.001$). The levels of phoenixin-14 were negatively correlated with weight ($r = -0.376$; $p < 0.005$), body mass index ($r = -0.407$; $p < 0.001$), systolic blood pressure ($r = -0.586$; $p < 0.001$), and diastolic blood pressure ($r = -0.319$; $p < 0.01$). There was a negative correlation between serum phoenixin-20 and weight ($r = -0.378$; $p < 0.005$), body mass index ($r = -0.383$; $p < 0.005$), systolic blood pressure ($r = -0.551$; $p < 0.001$), and diastolic blood pressure ($r = -0.306$; $p < 0.01$). We used receiver operating characteristic curve analyses to compare the diagnosis value of Phoenixin-14 and Phoenixin-20 levels in hypertensive patients. We found that Phoenixin-14 value is an area under the curve of 0.87 (cutoff value 404.7 ng/L, sensitivity 92%, specificity 72%) and Phoenixin-20 value is an area under the curve of 0.83 (cutoff value 209.9 ng/L, sensitivity 86%, specificity 75%). Phoenixin-14 did nearly show equally compared to phoenixin-20 in predicting hypertension.

CONCLUSION: Serum phoenixin-14 and phoenixin-20 may be related to the pathogenesis of hypertension. Our findings indicated that serum phoenixin-14 and phoenixin-20 may serve as a novel biomarker for the diagnosis of hypertension.

KEYWORDS: Hypertension. Phoenixin-14. Phoenixin-20.

INTRODUCTION

Hypertension (HT) is the most prevalent risk factor for cardiovascular diseases (CVDs). HT affects approximately one-third of the world's adult population and is a major cause of premature death worldwide. Despite technological advances in the diagnosis and treatment of HT, there is still a large population of untreated or inadequately treated HT patients¹. Understanding the mechanisms for HT development is critical for preventing and treating high blood pressure. The etiopathogenesis of HT is multifactorial and complex. Genetic and lifestyle factors, obesity, insulin resistance (IR), activation of sympathetic nervous system, alteration in sodium homeostasis, renin-angiotensin system changes, changes in vascular smooth muscle structure and reactivity, oxidative stress, and inflammation contribute to the development of HT¹⁻³.

Phoenixin (PNX) is a recently discovered neuropeptide produced by proteolytic cleavage of a small integral membrane protein 20 (Smim20), with two active isoforms, phoenixin-14

(PNX-14) and phoenixin-20 (PNX-20). PNX is a neuropeptide that is expressed and secreted not only in the central nervous system (CNS) but also in the peripheral tissues such as heart, adipose tissue, and pancreas^{4,5}. The original study suggests that PNX is a regulator of the reproductive system. Recent research has shown that PNX is involved in food intake, and lipid and glucose metabolism^{6,7}. PNX also exerts cardioprotective, anti-inflammatory, and cell-protective effects^{8,9}. However, the role of PNX in HT remains unknown.

Diseases of energetic imbalance such as obesity and diabetes represent major risk factors for CVD such as HT. There is some evidence that PNX may have some effects on the feeding, body mass regulation, and energy homeostasis^{6,10}. Oxidative stress and inflammation are considered to play a role in HT development^{2,3}. The protective properties of PNX, including antioxidative stress and anti-inflammatory effects, have recently been widely reported^{9,11}. PNX-14 has been reported to regulate proliferation and apoptosis of vascular smooth muscle

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cells (VSMCs)¹². GPR173 agonism by PNX-20 plays a protective role against ox-LDL-induced endothelial dysfunction¹³. Based on these data, this study was designed to evaluate serum concentrations of PNX-14 and PNX-20 in HT patients and healthy controls and the clinical value of these peptides as novel biomarkers for HT.

METHODS

Participants and study design

This study was performed in 36 HT patients and 36 control subjects. The volunteers selected for the patient group are patients with a diagnosis of HT and not having comorbidities. HT patients were selected from those who did not have any additional cardiac complaints such as ischemic or arrhythmic and who were admitted for control for HT. As a rule of exclusion from the research, under the age of 18, having a pregnancy, having comorbidities, having additional complaints, using additional medication other than antihypertensive, and have had COVID-19 in the past 2 years. All subjects provided written informed consent before participating in the study.

Clinical and biochemical assessment

The patients' body mass index (BMI) was calculated by dividing their weight in kilograms by their height in meters squared. BP was measured using an automatic BP monitor in the sitting position, and measurements were performed three times in the participant's right arm with a 2-min interval, after a rest period of at least 5 min. Samples of venous blood were taken after overnight fasting for at least 10 h. Serum biochemical analyte levels were measured immediately by commercially available kits based on routine methods on the Roche Cobas c501 analyzer (Roche Diagnostics, Mannheim, Germany). Blood samples were centrifuged and then serum samples were stored at -80°C for subsequent analysis. The analyses of serum PNX-14 and PNX-20 levels were performed using an enzyme immunoassay method using commercial kits (BT Lab Bioassay Technology Laboratory Human Elisa Kits, Shanghai Korain Biotech, China) in accordance with the manufacturer's guidelines. Absorbance was measured at 450 nm on an ELx800 Absorbance Microplate Reader (Biotek, Winooski, VT, USA).

Statistical analysis

Statistical analyses were done using SPSS v. 22.0 (SPSS Inc., IL, USA). To compare the ratio of categorical variables, we used the chi-squared test [gender (male/female)]. The normality of the variables was evaluated using the one-sample Kolmogorov-Smirnov test.

Differences in the means of variables were tested using both parametric and nonparametric tests depending on the distribution of the variables. For the independent samples, the Student's t-test and Mann-Whitney U test were used for comparing mean and median values, respectively. The correlations between variables were performed by Spearman's correlation test. Differences were considered significant at a probability level of $p < 0.05$.

RESULTS

This study was performed on 36 HT patients and 36 controls. Demographic and clinical characteristics of the HT subjects and controls are shown in Table 1. PNX-14 and PNX-20 values were very significantly lower in HT patients compared with the control group ($p < 0.001$). Spearman's rho correlation analysis was performed (Table 2). In HT group, the levels of PNX-14 were negatively correlated with weight ($r = -0.376$; $p < 0.005$), BMI ($r = -0.407$; $p < 0.001$), systolic BP ($r = -0.586$; $p < 0.001$), and diastolic BP ($r = -0.319$; $p < 0.01$). There was a negative correlation between serum PNX-20 and weight ($r = -0.378$; $p < 0.005$), BMI ($r = -0.383$; $p < 0.005$), systolic BP ($r = -0.551$; $p < 0.001$), and diastolic BP ($r = -0.306$; $p < 0.01$) in HT group.

We used receiver operating characteristic curve (ROC) analyses to compare the diagnosis value of PNX-14 and PNX-20 levels in HT patients (Figure 1). We, therefore, tested whether the predictive value of PNX-14 and PNX-20 were equal or superior by using ROC. We found that PNX-14 value is an area under the curve (AUC) of 0.87 (cutoff value 404.7 ng/L, sensitivity 92%, specificity 72%) and PNX-20 value is an AUC of 0.83 (cutoff value 209.9 ng/L, sensitivity 86%, specificity 75%). PNX-14 nearly showed equally compared to PNX-20 in predicting HT.

DISCUSSION

In this study, we show that in the HT group, serum PNX-14 and PNX-20 levels are significantly lower than that of healthy controls. In HT group, the levels of PNX-14 and PNX-20 were negatively correlated with weight, BMI, systolic BP, and diastolic BP. These findings suggest that serum PNX-14 and PNX-20 may be a potential biomarker in predicting the risk of HT.

Alteration in energy expenditure or metabolism plays important role in onset and course of HT. Central control of feeding behavior plays an essential role in metabolic homeostasis. PNX peptide was detected in the brain areas involved in controlling appetite⁶. Previous studies showed that IR may be involved in the pathogenesis of HT¹⁴. Pancreatic alpha and beta cells produce

and release glucagon and insulin, which differentially modulate the homeostasis of lipids and glucose. There is evidence that the biology of these cells may be modulated by PNX. The results showed that PNX stimulates insulin expression and secretion and promotes proliferation of INS-1E cells. PNX may contribute to the modulation of energy homeostasis and metabolism by controlling the neogenesis and secretion of insulin⁷. Several studies found that the blood PNX level depends on body mass¹⁵. In addition, PNX-14 increases the proliferation and differentiation of preadipocytes and decreases cell death, which indicates its possible

role in the control of body mass regulation¹⁰. In this study, the levels of PNX-14 and PNX-20 were negatively correlated with weight, BMI, systolic BP, and diastolic BP. The PNX-14 and PNX-20 levels are significantly lower in HT patients than control groups and it could be a risk factor for obesity-associated HT. Furthermore, PNX-14 significantly ameliorated HFD-induced obesity and fatty liver¹⁶. Due to the limited numbers of studies concerning the effects of PNX on body mass, these results are difficult to interpret. Therefore, this association should be further investigated to also identify possible confounding factors.

Table 1. Clinical and demographic characteristics of hypertension and control subjects.

| | Hypertension subjects n=36 | Controls n=36 | p |
|-----------------------------|-------------------------------|----------------------|--------|
| Age (years) | 55±10.9 | 53±7.9 | 0.248 |
| Female/male | 14/22 | 18/18 | 0.285 |
| Urea, mg/dL | 29.0 (18–77) | 26.5 (16–45) | 0.073 |
| Creatinine, mg/dL | 0.76±0.16 | 0.82±0.17 | 0.133 |
| AST, U/L | 18.0 (12–56) | 17.5 (9–27) | 0.429 |
| ALT, U/L | 17.0 (8–154) | 20.0 (3–41) | 0.363 |
| Fasting glucose, mg/dL | 95 (78–152) | 94 (71–107) | 0.101 |
| Triglycerides, mg/dL | 162.4±82.9 | 148.7±61.1.9 | 0.425 |
| HDL-C, mg/dL | 51.9±12.0 | 53.1±10.5 | 0.662 |
| LDL-C, mg/dL | 136.8±40.5 | 128.8±26.2 | 0.327 |
| Cholesterol, mg/dL | 217.6±35.9 | 212.1±32.3 | 0.504 |
| Potassium | 4.5±0.4 | 4.4±0.2 | 0.94 |
| Sodium | 141 (137–145) | 140 (136–145) | 0.161 |
| TSH, uIU/mL | 1.4 (0.3–2.9) | 1.7 (0.5–4.2) | 0.157 |
| Free T ₄ , ng/dL | 1.3±0.2 | 1.3±0.1 | 0.393 |
| Weight | 85.5 (57–120) | 66 (52–96) | <0.001 |
| BMI, kg/m ² | 31 (23–53) | 24 (20–29) | <0.001 |
| Systolic BP, mmHg | 148 (120–200) | 120 (110–160) | <0.001 |
| Diastolic BP, mmHg | 90 (70–130) | 80 (70–90) | <0.001 |
| Phoenixin-14, ng/L | 335.4 (242.4–3196) | 876.3 (356.4–3857.1) | <0.001 |
| Phoenixin-20, ng/L | 164.3 (110.4–2198.7) | 285.2 (140.3–2476.8) | <0.001 |

AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; TSH: thyroid-stimulating hormone.

Table 2. Spearman's correlation analyses were performed to investigate the association of biomarkers levels in the hypertension subjects.

| | | Phoenixin-14 | Phoenixin-20 | Weight | BMI | Systolic BP | Diastolic BP |
|--------------|---|------------------|------------------|------------------|------------------|------------------|-----------------|
| Phoenixin-14 | r | 1.0 | 0.905 | -0.376 | -0.407 | -0.586 | -0.319 |
| | p | . | <0.001 | <0.005 | <0.001 | <0.001 | <0.01 |
| Phoenixin-20 | r | 0.905 | 1.0 | -0.378 | -0.383 | -0.551 | -0.306 |
| | p | <0.001 | . | <0.005 | <0.005 | <0.001 | <0.01 |

Bold value indicates statistically significant.

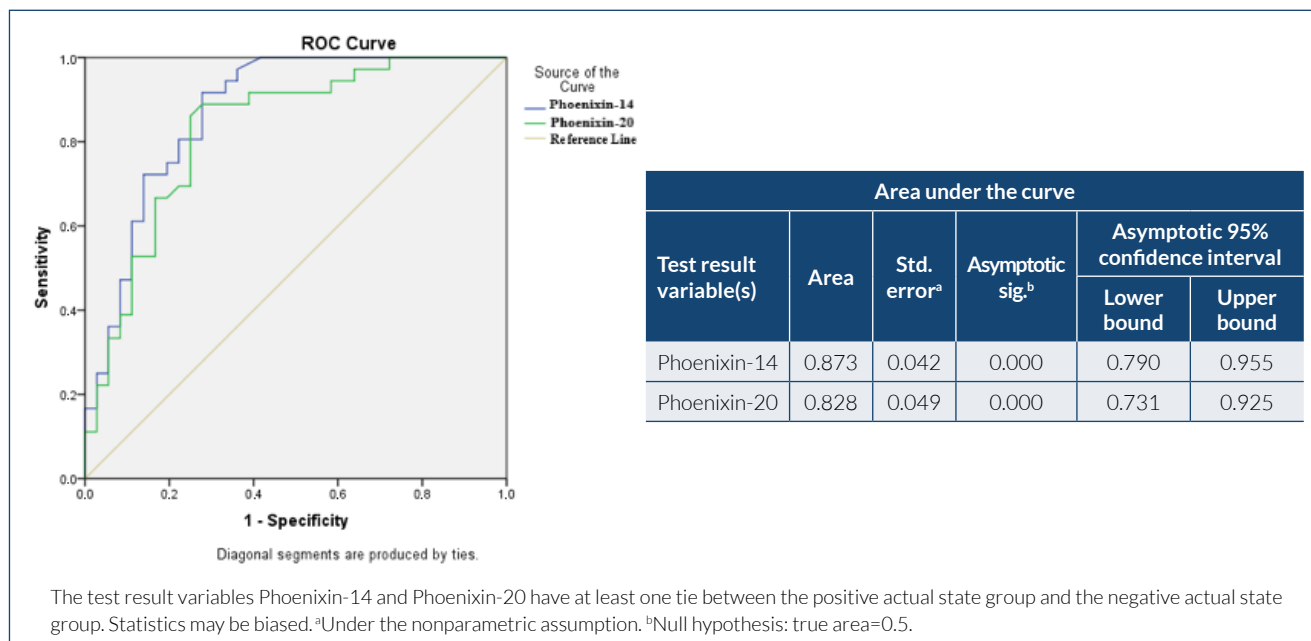


Figure 1. Phoenixin-14 and phoenixin-20 receiver operating characteristic curve.

HT is a multifactorial disorder associated with oxidative stress and inflammation^{2,3}. The inhibitory effects of PNx on inflammation have recently been widely reported. Wang et al. reported that PNx-14 protected against lipopolysaccharide-induced inflammation in astrocytes¹⁷. Zeng et al. found that PNx-20 ameliorated lipopolysaccharide-induced inflammation in microglial cells¹¹. The ability of PNx-14 to protect against oxygen-glucose deprivation/reoxygenation injury was also reported in human brain vascular endothelial cells⁹. Furthermore, since PNx-14 was detected at fairly high concentrations in the heart tissue, an involvement in cardiovascular functions has been hypothesized^{4,8}. The role of PNx in ischemia/reperfusion (I/R) processes in the heart and in microglial cells of the brain was explored^{8,18}. Rocca et al. showed that PNx administered at the reperfusion phase of I/R acted cardioprotectively⁸. PNx-14 inhibits I/R-induced cytotoxicity in microglia¹⁸. Another study showed that the myocardial injury and deteriorated cardiac function in diabetic mice induced by STZ were significantly ameliorated by PNx-14. In addition, the severe oxidative stress and inflammation in diabetic mice were dramatically mitigated by PNx-14¹⁹. The excessively released inflammatory factors and activated oxidative stress in gestational diabetes mellitus mouse model were alleviated by the administration of PNx-20²⁰. These studies show that PNx exerts anti-inflammatory and cell protective effects. The development of HT is closely associated with inflammation and oxidative stress. According to these results, PNx exerts a possible beneficial effect against

inflammation and oxidative stress in HT. In this study, we show that in the HT group, serum PNx-14 and PNx-20 levels are lower than that of healthy controls. Studies investigating the association of PNx with other inflammatory parameters in HT will provide further useful information to define its exact role in the pathogenesis of HT. Endothelium plays an important role in pathogenesis of many diseases and cardiovascular problems such as atherosclerosis and HT²¹. Wei et al. reported that GPR173 agonism by PNx-20 plays a protective role against ox-LDL-induced endothelial dysfunction¹³. VSMCs are major components of the vascular wall and serve to mediate hemodynamic vessel functions, playing a crucial role in regulating blood pressure in physiological conditions and HT. Under pathological conditions, VSMCs undergo differentiation, contractile, proliferative, and migratory alterations. These changes disrupt the function of vessels and can contribute to disease progression²². It was demonstrated that PNx-14 regulated proliferation and apoptosis of ox-LDL-treated VSMCs by modulation of the KCNQ1OT1/miR-183-3p/CTNNB1 axis¹². These anti-inflammatory and antioxidative properties, such as regulatory effect in cell proliferation, apoptosis, and prominent role in controlling energy homeostasis and metabolism, may contribute to the prevention of HT.

These *in vitro* and *in vivo* results support to a potentially significant role for PNx in the control of BP. Our findings are consistent with previous studies, which implicated metabolic and inflammatory changes involved in the pathogenesis of HT.

Our study suggests PNX-14 and PNX-20 can be used as an indicative biomarker of HT.

Limitation of the present study was the small sample size. Further large-scale studies are needed to establish these associations and determine the role of PNX in the pathogenesis of HT. This is the first study presenting data on association between serum PNX-14 and PNX-20 levels and HT. Absence of comorbidities in our patient group increased the power of our study.

CONCLUSION

Phoenixin-14 and phoenixin-20 may be related to the pathogenesis of HT. The results of this study point out the possible role of PNX-14 and PNX-20 as potential novel biomarkers for the prediction the risk of HT. This result will be beneficial for further identification and development of potential drug targets for metabolic diseases.

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INFORMED CONSENT

Informed consent was obtained from all individuals included in this study.

ETHICAL APPROVAL

The study was approved by the Local Ethics Committee Meram School of Medicine, Necmettin Erbakan University, Konya, Turkey (2021/3363).

AUTHORS' CONTRIBUTIONS

SA: Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **UC:** Conceptualization, Formal Analysis, Investigation, Writing – review & editing. **EP:** Conceptualization, Formal Analysis, Writing – review & editing.

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