Adropin and circadian variation of blood pressure

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Abstract

Background: Nocturnal hypertension and non-dipping pattern are often associated with endothelial dysfunction. Previous studies suggested that adropin, a novel secreted energy homeostasis protein, has the unique ability to regulate endothelial cell function.

Aim: This study aims to investigate the association between absolute night-time blood pressure (BP) and circadian BP pattern with serum adropin and high-sensitivity C-reactive protein (hsCRP) levels in patients with newly diagnosed untreated arterial hypertension.

Methods: Twenty-four-hour ambulatory BP monitoring was recorded in 100 hypertensives (50 dippers, 50 non-dippers) and 50 healthy controls. Serum levels of adropin and hsCRP were measured and recorded.

Results: A strong correlation was found between night-time BP levels with adropin and hsCRP levels (p < 0.001). On the other hand, the non-dipper group demonstrated lower adropin levels compared to the dipper and normotensive groups: non dipper group, 2580 ± 457 pg/mL; dipper group, 3298 ± 530 pg/mL; normotensive group, 3681 ± 411 pg/mL; p < 0.001). HsCRP levels were significantly higher in the non-dipper group than in the two other groups (p = 0.017). In a multivariate logistic regression analysis, adropin (p = 0.012) and hsCRP (p = 0.039) were independently associated with a non-dipping pattern.

Conclusions: Decreased adropin levels were found in the nocturnal hypertensive and non-dipper groups. Adropin and hsCRP were found to be independently associated with a non-dipping pattern. We suggest that decreased levels of adropin in non-dipper hypertensive patients might be associated with a longer duration of exposure to high BP. These results point to a possible future role of adropin in identifying hypertensive patients at higher risk of target organ damage.

Key words: adropin, high-sensitivity C-reactive protein, dipper, non-dipper hypertension

INTRODUCTION

Hypertension (HT) is one of the most prominent risk factors for adverse cardiovascular (CV) events [1]. High blood pressure (BP) is frequently associated with endothelial dysfunction and vascular complications [2]. Twenty-four-hour ambulatory BP monitoring (ABPM) is useful in the diagnosis of HT and assessment of BP regulation and variability. The definition of HT by using ABPM is based on the averaged 24-h systolic BP (SBP) or diastolic BP (DBP) > 130 mmHg and > 80 mmHg. There is increasing research to establish the relative impact of each of the components delivered by APBM (i.e. daytime BP, night-time BP, dipping status pattern) on heart and intermediate CV and renal outcomes [3, 4]. Increased night-time BP (nocturnal HT) and non-dipping of BP during sleep are distinct entities that often occur together and are important harbingers of left ventricular hypertrophy, microalbuminuria, arterial stiffness, and poor CV prognosis [5]. The guidelines for nocturnal BP according to the American Heart Association Council on High Blood Pressure Research are as follows: nocturnal BP lower than 115/65 mmHg is optimal, lower than 120/70 mmHg is normal, and higher than 125/75 mmHg is abnormal. Elevated night-time BP was associated with reduced endothelial function [6]. A non-dipping BP profile is usually defined as a nocturnal BP fall of less than 10% [7]. Release of endothelial nitric oxide is diminished in patients with HT, resulting in decreased endothelium-dependent vasodilation, which constitutes the first step in the development of atherosclerosis. Furthermore, endothelium-dependent vasodilation is impaired in non-dippers [7].
Adropin is a peptide hormone that is encoded by the energy homeostasis-associated (ENHO) gene [8]. The gene is highly conserved across mammalian species. Adropin is abundant in the liver and secreted into the circulation [8]. Circulating adropin concentrations are highly regulated by energy intake as well as being involved in CV function, particularly in endothelial function [9].

In this study, we investigated the association of absolute night-time BP and circadian BP pattern with serum adropin and high-sensitivity C-reactive protein (hsCRP) levels in patients with newly diagnosed untreated arterial hypertension.

**METHODS**

**Study population and design**

Prospectively, 119 subjects were screened from the cardiology polyclinic. One hundred hypertensive patients aged between 18 and 75 years were included in the study. Nineteen subjects were excluded from the study (one patient suspicious for adrenal adenoma, 11 patients with accompanying diseases, four patients with obstructive sleep apnoea, and three patients taking medications with side effects). After a 24-h ABPM assessment, the patients were divided into two groups, i.e. a dipper group and a non-dipper group. All hypertensive patients enrolled in the study had office BP ≥ 140 mmHg and/or ≥ 90 mmHg (the average of two proper readings or more measured on at least two visits). All hypertensive patients were newly diagnosed with no previous antihypertensive therapy. In addition, 50 healthy control subjects with normotensive ABPM were enrolled in the study.

Exclusion criteria were as follows: patients who refused to participate in the study, and patients with secondary HT, diabetes, systolic dysfunction of the left ventricle (ejection fraction < 50%), atrial fibrillation, history of coronary artery disease or angina, moderate to severe valvular diseases, malignancy, known chronic obstructive pulmonary disease and obstructive sleep apnoea (obstructive sleep apnoea was excluded with absence of obesity, daytime insomnia and loud snoring; if any of these conditions existed, the patient was directed to the department of chest diseases in order to exclude obstructive sleep apnoea), moderate to severe renal (estimated glomerular filtration rate of < 60 mL/min) and hepatic dysfunction (presence of cirrhosis or alanine aminotransferase and/or aspartate aminotransferase > 3 × ULN and total bilirubin > 2 × ULN), active inflammatory diseases, chronic pharmacological treatment (e.g. lipid reducing and anti-inflammatory drugs), and alcohol or substance addiction.

This study was approved by the Institutional Ethics Committee of Sivas Cumhuriyet University, and written informed consent was obtained from each participant. The investigation conformed with the principles outlined in the Declaration of Helsinki.

**Measurement of ambulatory blood pressure**

Following an office BP measurement, a 24-h ABPM (Bravo HR ABP Sun Tech Medical Inc., Morrisville, NC, USA) was applied to all study subjects. Appropriate cuff sizes were selected for all patients. BP was measured with intervals of 15 min during the daytime (6:00 am to 10:00 pm) and with 30 min intervals during the night-time (10:00 pm to 6:00 am). If less than 80% of the measurements were valid, the patients were excluded. The diagnosis of HT was made if one the following occurred: (1) an average of 24-h SBP > 130 mmHg and/or DBP > 80 mmHg, (2) an average daytime SBP > 135 mmHg and/or DBP > 85 mmHg, or (3) an average night-time SBP > 120 mmHg and/or DBP > 70 mmHg. A non-dipper pattern was defined as less than 10% decrease in SBP between the daytime and night-time hours.

**Collection of blood samples and biochemical analysis**

Peripheral venous blood was collected from the antecubital vein on admission. Baseline creatinine concentration, white blood cell count, platelet count, and haemoglobin level were measured. On the first morning after admission, lipid profile, hsCRP, and other biochemical parameters were measured using standard methods. Blood collected for adropin was immediately centrifuged, and serum was stored at −80°C until analysis.

Serum adropin was measured with a human adropin enzyme-linked immunosorbent assay (Sunred Biological Technology Co., Shanghai, PRC) as recommended by the manufacturer’s instructions.

**Statistical analysis**

Statistical analyses were conducted using the Statistical Package for the Social Sciences for Windows 21.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine whether the continuous variables were normally distributed. Normally distributed variables were shown as mean and standard deviation, while non-normally distributed variables were shown as median (minimum–maximum) values. Descriptive statistics are given as a percentage and absolute values. Basal characteristics were compared with an χ² test. Where appropriate, a one-way analysis of variance (ANOVA) or Kruskal-Wallis test was used for the comparison of the three groups for continuous variables. Differences between subgroups were revealed using Dunn’s procedure (for data without normal distribution). Data were analysed to identify whether adropin was independently associated with the risk of non-dipper HT by using univariate logistic and multivariate logistic regression models. Univariate analyses considered the following variables: haemoglobin, body mass index, hsCRP, age, sex, LV mass index (LVMI), smoking, fasting blood glucose, and creatinine. Covariates with p < 0.1 from
univariate logistic regression were included for multivariate analysis. We performed receiver operating characteristic (ROC) analysis to find the most sensitive adropin cutoff level for identifying patients with non-dipper HT. P < 0.05 was accepted to be statistically significant. We performed post hoc power analysis based on adropin results (effect size: 0.60, α: 0.05), which revealed a study power of 86% by assuming the parent distribution as a Laplace distribution.

RESULTS

Demographic, laboratory, and echocardiographic characteristics of the study population are shown in Table 1. Hypertensive patients had higher serum triglycerides than control subjects, but the difference was not significant (p > 0.05; Table 1). Demographic parameters, complete blood count, lipid profile, and creatinine were not different between the groups (p > 0.05).

Echocardiographic assessments revealed that the LV wall was thicker in the hypertensive group when compared to the control group. Furthermore, the subjects with HT had also higher LVMI than controls (p = 0.001). No significant difference was found in terms of daytime and 24-h average SBP and DBP readings between the dipper and non-dipper groups (Table 2). The control group had normal daytime and night-time BP levels and normal nocturnal dip (average daytime BP 125 ± 9/82 ± 5 mmHg, average night-time BP 108 ± 4/72 mmHg, average nocturnal dip 13%). Statistically significant high nocturnal BP was accompanied by non-dipping pattern (p < 0.001).

Adropin levels were significantly different among the three groups (p < 0.001). Average systolic night-time BP levels showed significant negative correlation with adropin (r = 0.442, p < 0.001) and positive correlation with hsCRP.

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### Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 50)</th>
<th>Dipper (n = 50)</th>
<th>Non-dipper (n = 50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>52 ± 7.4</td>
<td>48.2 ± 8.1</td>
<td>48.4 ± 5.9</td>
<td>0.165</td>
</tr>
<tr>
<td>Men</td>
<td>15 (42%)</td>
<td>21 (60%)</td>
<td>16 (46%)</td>
<td>0.307</td>
</tr>
<tr>
<td>Body mass index [kg/m²]</td>
<td>22.8 (20.3–24.4)</td>
<td>22.1 (19.9–25.8)</td>
<td>21.7 (19.3–25.7)</td>
<td>0.105</td>
</tr>
<tr>
<td>Smokers</td>
<td>8 (22.9%)</td>
<td>4 (11.4%)</td>
<td>4 (11.4%)</td>
<td>0.307</td>
</tr>
<tr>
<td>Total cholesterol [mg/dL]</td>
<td>184.9 ± 39.4</td>
<td>197.4 ± 48.6</td>
<td>192.3 ± 39.4</td>
<td>0.469</td>
</tr>
<tr>
<td>LDL cholesterol [mg/dL]</td>
<td>130.2 ± 39.4</td>
<td>132.2 ± 40.8</td>
<td>135.8 ± 35.7</td>
<td>0.826</td>
</tr>
<tr>
<td>HDL cholesterol [mg/dL]</td>
<td>46 (30–76)</td>
<td>44 (30–72)</td>
<td>44 (30–95)</td>
<td>0.863</td>
</tr>
<tr>
<td>Triglycerides [mg/dL]</td>
<td>108 (48–294)</td>
<td>152 (46–253)</td>
<td>129 (55–300)</td>
<td>0.055</td>
</tr>
<tr>
<td>Creatinine [mg/dL]</td>
<td>0.7 (0.5–1.4)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.7 (0.4–1.1)</td>
<td>0.441</td>
</tr>
<tr>
<td>Fasting glucose [mg/dL]</td>
<td>89 (77–116)</td>
<td>85 (69–109)</td>
<td>85 (51–112)</td>
<td>0.188</td>
</tr>
<tr>
<td>Haemoglobin [g/dL]</td>
<td>14.2 ± 1.5</td>
<td>14.8 ± 1.1</td>
<td>14.2 ± 1.7</td>
<td>0.135</td>
</tr>
<tr>
<td>White blood cell count [10³/mm³]</td>
<td>8.2 (4.8–11.4)</td>
<td>8.1 (4.8–10.6)</td>
<td>8.1 (5.0–14.4)</td>
<td>0.830</td>
</tr>
<tr>
<td>Adropin [pg/mL]</td>
<td>3681.90 ± 411.25</td>
<td>3298.00 ± 529.65</td>
<td>2580.10 ± 456.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HsCRP [mg/L]</td>
<td>2.9 (0.1–15.0)</td>
<td>4.1 (0.1–11.0)</td>
<td>6.0 (1.0–13.84)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

**Table 2.** 24-hour ambulatory blood pressure values of dipper and non-dipper groups

<table>
<thead>
<tr>
<th></th>
<th>Dipper (n = 50)</th>
<th>Non-dipper (n = 50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime SBP [mmHg]</td>
<td>150 ± 7</td>
<td>152 ± 8</td>
<td>0.934</td>
</tr>
<tr>
<td>Daytime DBP [mmHg]</td>
<td>91 ± 7</td>
<td>93 ± 4</td>
<td>0.599</td>
</tr>
<tr>
<td>Night-time SBP [mmHg]</td>
<td>130 ± 7</td>
<td>144 ± 8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Night-time DBP [mmHg]</td>
<td>81 ± 7</td>
<td>88 ± 4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24-h SBP [mmHg]</td>
<td>147 ± 8</td>
<td>150 ± 9</td>
<td>0.120</td>
</tr>
<tr>
<td>24-h DBP [mmHg]</td>
<td>92 ± 5</td>
<td>93 ± 9</td>
<td>0.703</td>
</tr>
<tr>
<td>Nocturnal dip</td>
<td>19%</td>
<td>6%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

All values are presented as mean and standard deviation or percentage; SBP — systolic blood pressure, DBP — diastolic blood pressure for dipper and non-dipper groups.
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In a pairwise comparison, while the non-dipping group had lower adropin levels compared to both dipper HT and control groups, there was no significant difference between adropin levels of the dipper HT and control groups (p > 0.05; Fig. 2). Serum hsCRP levels were significantly higher in the non-dipper group than in the other groups (Fig. 3). In a pairwise comparison, hsCRP levels were significantly higher in the non-dipper group than in the dipper group but no significant differences were found between dippers and the control group (p > 0.05). In patients with HT, plasma adropin levels correlated negatively with hsCRP (r = 0.247, p = 0.039). The multivariate analysis with adjustment for potential confounding variables revealed that lower adropin and higher hsCRP levels were independently associated with a non-dipping pattern (Table 3). ROC analysis showed that adropin levels lower than 2959 pg/mL can predict non-dipping status (p < 0.001) as shown in Figure 4 (sensitivity: 75%; specificity: 67%; area under the curve: 0.731; 95% confidence interval: 0.582–0.819).

**DISCUSSION**

This study showed that there was a negative correlation between absolute night-time BP and adropin levels. On the other hand, serum adropin levels were lower in non-dipper
hypertensive patients than in dipper hypertensives and normotensives. Hence, serum levels of adropin were independently associated with the non-dipping status. To the best of our knowledge, this study is the first to investigate the relationship between blood adropin levels with absolute night-time BP levels and daily circadian BP patterns.

The endothelium plays a crucial role in the maintenance of vascular homeostasis, and endothelial dysfunction contributes to the development and progression of CV diseases [10]. Endothelial nitric oxide (NO) synthase (eNOS) releases NO which is a potent endogenous vasodilator. NO is released in response to shear stress and plays an important role in flow-mediated dilatation and in maintaining endothelial homeostasis [11]. Adropin is a newly identified protein that plays a role in the protection and regulation of endothelial cells [12]. Lovren et al. [9] demonstrated that adropin could enhance the expression of eNOS in the endothelium via activation of vascular endothelial growth factor receptor (EGFR) 2, phosphatidylinositol 3-kinase Akt, and EGFR 2 extracellular signal-regulated kinase 1/2 pathways. Because eNOS is responsible for the production of vascular NO, adropin deficiency is associated with reduced NO bioavailability in the endothelium [13]. Ignarro et al. [13] showed that serum adropin leads to increased expression of eNOS in the endothelium and decreased serum adropin level is associated with reduced NO bioavailability in the endothelium. Reduced NO bioavailability is a cardinal feature of endothelial dysfunction, which is a predictor of atherosclerosis [9]. Gözal et al. [14] showed that the adropin concentration is reduced in children with obstructive sleep apnoea and endothelial dysfunction. Also, Topuz et al. [15] showed low levels of adropin in subjects with endothelial dysfunction. Based on this, it has been suggested that adropin may be a new and effective marker for noninvasive evaluation of endothelial function. Interestingly, low levels of serum adropin are associated with cardiac syndrome X, a pathogenesis linked to endothelial dysfunction [16]. Furthermore, adropin also plays a role in HT via endothelial dysfunction. Aydn et al. [17] revealed that adropin is an independent predictor of essential hypertension.

Nocturnal HT and nocturnal non-dipping pattern are associated with inflammation and endothelial dysfunction [6, 18, 19]. The clinical relevance of nocturnal HT and non-dipping status in hypertensive patients lies in its proven association with more severe target organ damage and increased risk for CV events [20, 21]. Many studies have shown that LV hypertrophy, carotid intima–media thickness, increased pulse wave velocity, and microalbuminuria are more prevalent in patients with nocturnal HT and a non-dipping BP pattern.
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[22, 23]. Adropin levels were independently associated with non-dipping pattern in our study. Because of high inflammatory activity, hsCRP levels were significantly higher in the non-dipper group and were independently associated with the non-dipping pattern. On the other hand, a positive and graded relationship between hsCRP and average night-time BP levels was found. Decreased adropin levels in nocturnal and non-dipper HT patients are considered as markers of inflammatory response and endothelial dysfunction. In the present study, the effect of higher night-time BP levels on adropin could be significant in the non-dipper group. Despite no difference in average 24-h BP between the dipper group and the non-dipper group, elevated hsCRP and decreased adropin levels might be related to increased sympathetic tone. Endothelial dysfunction and autonomic nervous system imbalance often co-exist in the development of HT [24]. Previous studies have shown that activation of the sympathetic system precludes a decrease in nocturnal BP and seems to play an important role in endothelial activation [25]. In our study, patients with nocturnal HT and/or non-dipper pattern were exposed to more endothelial damage due to an overall hypertensive state throughout the day and night, which could explain the decreased adropin levels. This and previous findings demonstrate that adropin might be a dynamic biomarker of endothelial activation due to a hypertensive state.

Although patients with dipper HT had lower adropin levels than the control group in our study, it was not shown to have statistical significance. This finding would appear to conflict with previous studies. However, we think that the reason of this situation might be due to the relatively small number of patients in our study and the lack of classification of hypertensive patients in terms of circadian BP pattern in previous studies.

The major limitation in this study is the small sample size. Prospective studies with a larger number of patients can clearly state whether adropin predicts high-risk hypertensive patients. In addition, the results of our study would be much more robust if endothelial dysfunction was quantified. The fact that pro-inflammatory markers were not measured can be presented as another limitation. On the other hand, the definition of day and night periods was constant and it was not modified in each patient based on their diary.

In conclusion, absolute night-time BP levels showed a significantly negative correlation with adropin levels. Furthermore, circulatory levels of adropin were lower in the non-dipper group when compared to both the dipper group and normotensives. Adropin and hsCRP were found to be independently associated with a non-dipping pattern. Based on these findings, we suggest that decreased levels of adropin in non-dipper hypertensive patients might be associated with a longer duration of exposure to high BP throughout the day and night. Therefore, adropin might be a potential marker to quantify endothelial activation in hypertensive patients and could play future role in the selection of hypertensive patients at higher risk or target organ damage.

Conflict of interest: none declared

References


