Visfatin levels in hormonally inactive adrenal adenoma and their association with metabolic parameters

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1. Introduction

The increase in the use of imaging qualities and frequencies has led to an increase in the detection of adrenal adenomas. However, despite the increase in incidence, the factors that play roles in its development have not been clearly defined until now. Cytokines or growth factors have been employed to play roles in the deterioration of signal transduction. The gross portions of adrenal adenomas demonstrate long-term inactive hormonal processes. Unfortunately, the questions related to when and how the adenomas gain hormonal activation are still left unanswered.

Visfatin is primarily defined as a pre-B-cell colony-enhancing factor and affects the immune system (1,2). Visfatin also plays a role in the biosynthesis of NAD in the form of nicotinamide phosphoribosyl transferase (Nampt), which is the rate-limiting enzyme that converts nicotinamide into nicotinamide mononucleotide (3). This process acts as a control point for the growth, apoptosis, and angiogenesis of mammalian cells. Therefore, studies determining the effect of visfatin on tumor cells have shown an increase in recent years. Nevertheless, the possible role of visfatin in the tumorigenesis process of adrenal adenomas has not yet been investigated.

Visfatin levels and cardiometabolic risk factors have also not been clearly defined yet. Very few studies showing the association between cardiovascular disease and adrenal adenoma are available.

Therefore, the present study was performed with an aim of evaluating visfatin levels and cardiometabolic risk factors in patients with adrenal adenoma.
adenoma and in a healthy control group, and also to determine whether or not visfatin levels show any variations in the adrenal adenoma group. We were looking for the responses so that visfatin levels could be used as a marker in the follow-up period of hormonally inactive adrenal adenoma.

2. Materials and methods
Thirty patients with hormonally inactive adrenal adenoma (17 females, 13 males; mean age 53.6 ± 8.9 years) along with 30 healthy participants (20 females, 10 males; mean age 47.2 ± 9.3 years) were studied in a tertiary healthcare center. The patient had been referred to the Endocrinology Outpatient Clinic at the Haseki Training and Research Hospital during the period between October 2014 and June 2015. The protocol was approved by the local ethics committee. All patients gave written informed consent.

All patients underwent hormonal excess scanning. Tests of 24-h excretion of urinary free cortisol (UFC) and the overnight 1-mg dexamethasone test (DST) were performed. The cut-off point was taken as 1.8 µg/dL for DST. The excretion of urinary metanephrine and normetanephrine and the upright plasma aldosterone to plasma renin activity ratio were measured to exclude the presence of pheochromocytoma and primary hyperaldosteronism. In control subjects, 24-h UFC excretion and overnight low-dose DST were performed.

According to the blood pressure levels reported in the Joint National Committee (JNC 7) guidelines (4), patients with systolic blood pressure of ≥140 mmHg and diastolic blood pressure of ≥90 mmHg were defined as hypertensive. Current smokers were recorded by a yes/no questionnaire.

Severe hepatic failure and renal failure, anemia, unstable cardiovascular conditions (congestive heart failure or a history of myocardial infarction or stroke), or past incidences of cerebrovascular conditions within 6 months before the study enrollment were exclusion criteria. Patients with systemic infection, collagenous tissue disease, malignancy, thyroid disease, severe depression, or dementia were excluded. Women who were pregnant or breastfeeding were also excluded from the study.

The control group (n = 30) consisted of participants who had negative adrenal gland scan results.

Weight and height were measured in light clothing without shoes. Body mass index (BMI) was calculated by dividing the weight by the square of height (kg/m²).

A 24-h ambulatory blood pressure monitoring was performed for each participant (PhysioQuant tension Holter system). Minimum, maximum, and mean values of systolic and diastolic blood pressure were recorded. Delta blood pressures calculated as a result of the difference between the maximum and minimum blood pressure values were obtained.

2.1. Measurement of carotid intima-media thickness
Carotid intima-media thickness (CIMT) was derived from a noninvasive ultrasound of the common carotid arteries using a high-resolution ultrasound machine (GE Logiq 200 PRO) with a 7.5-MHz linear probe. The intima-media thickness was defined as the distance between the blood-intima and media-adventitia boundaries on B-mode imaging.

2.2. Biochemical evaluation
Blood samples were drawn from each patient after a 12-h overnight fast for the determination of glucose, insulin, lipids, hormone profile, and visfatin level.

Fasting glucose levels were determined with the glucose oxidase/peroxidase method. Levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were determined with enzymatic colorimetric assays by spectrophotometry. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.

Cortisol and fasting insulin levels were measured by the chemiluminescence method (Unicel Dxl 800 Immunoassay System, Beckman-Coulter Inc., USA). Aldosterone and renin levels were determined by the chemiluminescence enzyme immunoassay method. The 24-h urine catecholamines were determined by liquid chromatography/tandem mass spectrometry.

Visfatin levels were measured by a human enzyme-linked immunosorbent assay (ELISA) kit obtained from Shanghai Sunred Biological Technology Co. (China). Visfatin’s measurable range was 0.04 ng/mL to 10 ng/mL. The measurable minimum level was 0.032 ng/mL.

2.3. Statistical analyses
Collected data were entered into SPSS 17 (SPSS Inc., USA). Continuous data were shown as mean ± SD. Fisher’s exact and chi-square tests were used to compare differences in rates. Frequencies of the variables were given with the percentage rates as appropriate. Normally distributed variables were compared by using Student’s t-test, whereas nonnormally distributed data were compared by the Mann–Whitney U test. The degree of association between continuous variables was calculated by Pearson’s correlation coefficient. The multiple linear regression stepwise method was used to determine the independent predictors. Univariate analyses were used to adjust visfatin with respect to age, BMI, presence of diabetes mellitus, and hypertension. P < 0.05 was accepted as statistically significant.

3. Results
Clinical, biochemical, and hormonal parameters screened in patients with adrenal adenoma and in healthy control subjects are shown in Table 1. Thirty patients with adrenal adenoma (53.6 ± 8.9 mean age, range 34-69 years; BMI: 25.32 ± 4.33, range 19.96-38.10 kg/m²) and 30 age- and
BMI-matched healthy individuals constituting the control group with negative imaging scans (47.27 ± 9.37 mean age, range 33-67 years, BMI: 25.36 ± 5.36, range 21.23-37.11 kg/m²) were studied. The groups were also similar in terms of sex ratio (P = 0.426). The mean size of adrenal adenoma was 23 ± 7.8 mm (range 10-40 mm).

The frequency of diabetes mellitus and hypertension was found to be statistically higher in the adrenal adenoma group (P < 0.05) (Table 1). Mean fasting TG, total cholesterol, LDL-C, and HDL-C levels were determined to be similar between the groups. Although mean fasting glucose, insulin, HOMA-IR, triglyceride, and uric acid levels were higher in the adrenal adenoma group as compared to the control group, the differences did not reach levels of statistical significance. Mean high-sensitive C-reactive protein (hsCRP) and visfatin levels were significantly higher in the adenoma group (Table 2).

The mean visfatin level was established to be 4.40 ± 3.05 ng/mL in the adrenal adenoma group, while the mean visfatin level was 3.06 ± 1.44 ng/mL in the healthy control group (P = 0.035). Even after adjustment for age, BMI, the presence of diabetes mellitus, and hypertension, the levels of visfatin still remained significantly higher in the adenoma group (P = 0.011). After correction, the estimated mean visfatin level in the adenoma group was 4.58, while it was 2.87 in the control group. A significant positive correlation was found between visfatin and insulin levels (r = 0.350; P = 0.006). In multiple linear regression analysis visfatin was found to be significantly associated with fasting insulin level (beta coefficient = 0.350, P = 0.006) (age, BMI, and fasting glucose were included in the model).

The maximum systolic and diastolic, minimum systolic and diastolic

### Table 1. Clinical features of the patients and healthy participants.

<table>
<thead>
<tr>
<th></th>
<th>Adrenal adenoma (n = 30)</th>
<th>Controls (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53.66 ± 8.99</td>
<td>47.27 ± 9.37</td>
<td>0.075</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.32 ± 4.33</td>
<td>25.36 ± 5.36</td>
<td>0.314</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>13 (43.3%)</td>
<td>5 (16.7%)</td>
<td>0.024</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>13 (43.3%)</td>
<td>4 (13.3%)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

BMI: Body mass index. Continuous variables are given as mean ± standard deviation; categorical variables are given as %.

### Table 2. Biochemical parameters of the groups.

<table>
<thead>
<tr>
<th></th>
<th>Adrenal adenoma (n = 30)</th>
<th>Controls (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>109.50 ± 36.36</td>
<td>96.76 ± 15.02</td>
<td>0.084</td>
</tr>
<tr>
<td>Insulin, µIU/mL</td>
<td>10.76 ± 6.38</td>
<td>8.71 ± 4.91</td>
<td>0.170</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.95 ± 2.00</td>
<td>2.12 ± 1.35</td>
<td>0.065</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>201.26 ± 36.34</td>
<td>216.80 ± 47.89</td>
<td>0.163</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>134.13 ± 57.00</td>
<td>124.06 ± 50.47</td>
<td>0.472</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>47.93 ± 9.29</td>
<td>49.26 ± 10.35</td>
<td>0.602</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>126.33 ± 35.45</td>
<td>142.60 ± 39.49</td>
<td>0.099</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>4.83 ± 3.52</td>
<td>2.92 ± 2.48</td>
<td>0.019</td>
</tr>
<tr>
<td>Visfatin, ng/mL</td>
<td>4.40 ± 3.05</td>
<td>3.06 ± 1.44</td>
<td>0.035</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>5.76 ± 0.91</td>
<td>5.05 ± 1.23</td>
<td>0.060</td>
</tr>
</tbody>
</table>

HOMA-IR: Homeostasis Model Assessment Insulin Resistance Index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; hsCRP: high-sensitive C-reactive protein.
blood pressures were higher in the adrenal adenoma group. Delta systolic blood pressures calculated as a result of the difference between maximum and minimum systolic blood pressure values were also found to be higher in the adenoma group as compared to the control group. However, these values did not differ significantly between the groups (P > 0.05; Table 3).

4. Discussion
The underlying etiology of adrenal adenoma is not clearly defined and there are still few studies that investigate its association with cardiovascular disease and metabolic dysfunction.

The prevalence of diabetes mellitus was found to be higher in the group composed of patients with adrenal adenoma, which is consistent with the results obtained in previous research (5). Additionally, the patients in the adenoma group showed higher systolic and diastolic blood pressure (6,7). In the present study, although statistically significant differences were not obtained, the ambulatory mean systolic and diastolic blood pressures were found to be higher. Furthermore, blood pressures, except the delta diastolic pressures, were also observed to be higher in the adenoma group, although significant differences were not obtained. This might be due to the small sample size. In the adenoma group, these differences may be of significant importance in the future.

In a recent study, the prevalence of diabetes mellitus and hypertension was reported to be 54.9% and 59.6%, respectively, in the adrenal adenoma group (8). In the present study diabetes mellitus and hypertension were observed to be seen in almost half of the patients with adrenal adenoma, which is consistent with these results (43.3% and 43.3%, respectively). Additionally, the frequency of hypertension was higher in the adenoma group. Consistent with our study, in a recent study the frequency of hypertension was higher in patients with nonfunctioning adrenal adenoma compared to the control subjects (9).

There are limited studies evaluating the risk of cardiovascular disease in patients with nonfunctioning adrenal adenoma. Atherosclerosis risk evaluated by carotid Doppler was also found to be higher in a nonfunctioning adrenal adenoma group, compatible with our results (5,7,10). In recent studies HOMA-IR scores were higher in the nonfunctioning adrenal adenoma group (7,10). In the present study fasting glucose, insulin, uric acid levels, and HOMA-IR, predictors for metabolic dysfunction, were found to be higher in the nonfunctioning adrenal adenoma group; however, these differences were not statistically significant. In the studies of Yener et al., uric acid levels were also higher in the nonfunctioning adrenal adenoma group, consistent with our results (7,11).

The mechanism of tumorigenesis and the underlying etiology of adrenal adenoma have not yet been clearly defined.

Recent studies reported growing evidence on the correlation between visfatin and cancers (12-14). It has recently been demonstrated that visfatin-mediated Notch1 upregulation contributes to NF-κB signaling in human breast cell tumorigenesis (15). Visfatin also increases the proliferation and the rate of the synthesis of DNA in breast cancer cells (13), and it is associated with a number of other human malignancies, including gastric, brain, pancreas, liver, and prostate cancers (14,16). In the present study the patients with adrenal adenoma showed higher visfatin levels. The possible role of visfatin in adrenal adenomas needs to be investigated further.

Visfatin levels were found to be higher in type 2 diabetic patients (17,18) and also in patients with metabolic syndrome, especially in those with carotid plaques and in patients with polycystic ovary syndrome (19-21). The

<table>
<thead>
<tr>
<th></th>
<th>Adrenal adenoma</th>
<th>Controls</th>
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<tbody>
<tr>
<td>(n = 30)</td>
<td>(n = 30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean systolic blood pressure</td>
<td>125.00 ± 12.83</td>
<td>123.13 ± 9.74</td>
<td>0.529</td>
</tr>
<tr>
<td>Mean diastolic blood pressure</td>
<td>80.36 ± 10.34</td>
<td>77.93 ± 6.59</td>
<td>0.282</td>
</tr>
<tr>
<td>Delta systolic blood pressure</td>
<td>59.76 ± 27.97</td>
<td>57.23 ± 24.36</td>
<td>0.710</td>
</tr>
<tr>
<td>Delta diastolic blood pressure</td>
<td>45.06 ± 30.58</td>
<td>46.53 ± 23.21</td>
<td>0.835</td>
</tr>
<tr>
<td>Maximum systolic blood pressure</td>
<td>157.90 ± 26.15</td>
<td>153.50 ± 18.11</td>
<td>0.452</td>
</tr>
<tr>
<td>Maximum diastolic blood pressure</td>
<td>106.73 ± 27.80</td>
<td>103.50 ± 19.86</td>
<td>0.606</td>
</tr>
<tr>
<td>Minimum systolic blood pressure</td>
<td>98.13 ± 15.82</td>
<td>96.26 ± 13.35</td>
<td>0.623</td>
</tr>
<tr>
<td>Minimum diastolic blood pressure</td>
<td>61.66 ± 11.06</td>
<td>56.96 ± 10.85</td>
<td>0.102</td>
</tr>
</tbody>
</table>
visfatin levels still remained high in metabolic syndrome patients even after adjustment for age, sex, and BMI.

Visfatin was strongly correlated with proinflammatory gene expression, including CD68 and the tumor necrosis factor-alpha gene; it was also found to have a proinflammatory effect (22). Visfatin is also found to be associated with systemic insulin resistance, fasting glucose, fasting triglyceride, and total cholesterol levels (23). Additionally, the role of visfatin in NAD biosynthesis as Nampt is critical for energy homeostasis and cell viability. Nampt binds to the insulin receptor and exhibits insulin-mimetic effects, suppresses neutrophil apoptosis, and affects the immune and inflammatory functions (24). Its role in the inflammatory pathway and immunogenic system also affects cell differentiation and metabolic functions. A positive correlation between visfatin and insulin levels was determined in the present study. The inhibition of visfatin production with specific inhibitors might provide clinical benefits in cases of adrenal adenoma enlargement and hormonal activation. Further studies are required to confirm this relationship in other related pathways.

In view of this evidence, visfatin may be assumed to have an insulin-like effect and may exhibit reduced sensitivity in inflammatory situations causing metabolic deterioration. Metabolic deterioration, including higher blood glucose and higher blood pressure, may cause higher visfatin levels. Its role in the tumorigenesis pathway may lead to the development of adrenal adenoma. In the future, visfatin may prove to be a useful marker for the follow-up of patients with adrenal adenoma in terms of size, progression, hormonal activation, metabolic dysfunction, and cardiovascular disease.

References

10. Androulakis I, Kaltsas GA, Kollias GE, Markou AC, Gouli AK, Thomas DA, Alexandraki KI, Papamichael CM, Hadjidakis DJ, Piaditis GP. Patients with apparently nonfunctioning adrenal incidentalomas may be at increased cardiovascular risk due to excessive cortisol secretion. J Clin Endocrinol Metab 2014; 99: 2754-2762.


