EVALUATION OF THE RISK OF ATHEROSCLEROSIS IN PATIENTS WITH ADRENAL INCIDENTALOMA USING THE ULTRASOUND BIOMARKERS EFT AND CIMT

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ABSTRACT

Introduction: Adrenal incidentalomas (AIs) are masses frequently observed on radiological examination that have attracted attention due to their coincidence with metabolic disorders. Recently, the relationship between atherosclerosis and adrenal incidentalomas has been noted. Epicardial fat tissue (EFT) and carotid artery intima-media thickness (CIMT) measurements show the development of atherosclerosis (AS). The aim of this study is to evaluate the risk of cardiovascular disease in AI patients using EFT and CIMT readings.

Materials and methods: Our study included 63 AI patients and 48 healthy controls. All patients were evaluated for blood pressure, Body Mass Index (BMI), EFT, CIMT, and lipid parameters. AI patients were also investigated for hormonal secretion. The relationship between risk of atherosclerosis and EFT, CIMT was analyzed.

Results: Control subjects (mean EFT, 3.92 ± 1.14 mm) had lower EFT than patients with subclinical Cushing’s syndrome (SCS) and non-functional adenoma (NFA) (mean EFT, 6.22 ± 1.97 mm and 5.44 ± 1.61 mm, respectively) (both P < 0.001). There was no difference in EFT between SCS and NFA patients. CIMT was significantly lower in control subjects (mean CIMT, 0.65 ± 0.11 mm) than in NFA patients (mean CIMT, 0.75 ± 0.18 mm) (P < 0.01). The EFT of SCS patients was not significantly different from the EFT of either NFA patients or controls.

Conclusion: EFT thickness measurements may be helpful as an early atherosclerosis marker in AI patients.

Key words: adrenal incidentaloma, atherosclerosis, epicardial fat thickness.

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Introduction

Currently, sensitive imaging techniques are being used more frequently. Adrenal incidentalomas (AIs) are masses randomly found during imaging studies conducted for a variety of reasons. As a result of developments in imaging techniques, the detection of AIs is becoming more common1. The prevalence of adrenal tumors when established by computed tomography (CT) varies from 2.5 to 4% for abdomen CT, and 4.2% for thorax CT in adult populations1,2. The prevalence of AI detected at autopsy is less than 1% in patients younger than 30 years of age, increasing to 7% in patients 70 years of age or older3.

These masses should definitely be investigated for the presence of hormone secretion or malignancy4. Recent studies examining the etiology of adrenal masses have found them to be 73.9% non-functioning adenoma (NFA), 7% subclinical Cushing’s syndrome (SCS), 1.2% aldosterone-producing adenoma, 4.7% pheochromocytoma, 4.8% adrenocortical carcinoma (ACC), and 2.3% metastases5.

The relationship between adrenal masses and metabolic disorders has been frequently researched. In patients with AI, metabolic disorders such as insulin resistance are observed and it is proposed that in these patients, the incidence of metabolic syndrome is increased6.
Epicardial fat tissue (EFT), adipose tissue surrounding the heart, is known to be related to atherosclerosis\(^9,10\). EFT measurements are a valuable indicator in evaluating the development of atherosclerosis, and recent studies have emphasized that EFT values are an important marker for the risk of cardiovascular diseases\(^11\). Recent studies have also shown EFT to be closely related to metabolic syndrome-coronary atherosclerosis, insulin resistance, and obesity\(^9,11,12\).

Carotid intima-media thickness (CIMT) has long been used as a marker for the severity of atherosclerosis and to evaluate progression\(^13,14\). In addition, studies have reported increased CIMT to be related to cardiovascular risks\(^15-17\). In the evaluation of cardiovascular risk and atherosclerosis, there is a positive correlation between EFT and CIMT\(^18,19\).

The aim of this study is to evaluate atherosclerosis markers, such as EFT and CIMT, in non-functional and SCS AI patients.

**Materials and methods**

**Patients**

For evaluation of incidental adrenal masses, 63 consecutive patients (32 male, 31 female; age range 37-75 years; mean age 58.51 ± 9.6 years) were admitted to Canakkale Onsekiz Mart University hospital, Department of General Medicine, between 2012 and 2014. In accordance with the Helsinki Declaration, patients signed an informed consent form and the study received approval from the Canakkale Onsekiz Mart University local ethics committee. Incidentalomas were all detected by abdominal Computerized Tomography (CT) and Magnetic Resonans Image(MRI). Scans were performed to evaluate unrelated diseases, such as nephrolithiasis, abdominal pain, or renal diseases. Patients with overt Cushing's syndrome (CS) were excluded from the study. Patients with known extra-adrenal malignancies, suspected endocrine hypertension, or those taking cortisol or medication known to affect cortisol metabolism were removed from the study. Patients did not have any specific signs or symptoms of cortisol excess, such as easy bruising, facial plethora, buffalo hump, moon face, purple striae, skin atrophy, or proximal myopathy.

Clinical evaluation for diabetes mellitus (DM), and/or glucose intolerance, hypertension (HT), and obesity was performed on all patients. Hormonal examination included serum cortisol at midnight, morning serum cortisol after 1 mg dexamethasone (dexamethasone suppression test (DST)), baseline morning plasma ACTH and dehydroepiandosterone sulphate (DHEA-S), and 24 hour urinary excretion of catecholamines. Hypertensive patients with AI were also evaluated in the recumbent position for plasma renin activity and aldosterone.

Exclusion criteria included patients with a history of any cardiovascular disease, end-stage renal disease, or any systemic disease other than DM and HT. Forty-eight control subjects were selected (12 male, 36 female; age range 29-73 years; mean age 55.08 ± 8.22 years) based on age and BMI matching, without AI, cardiovascular disease, or any chronic disease other than HT. Control cases were consecutively recruited from patients referred to our department with non-specific gastric symptoms who were not affected by any relevant pathology.

Diagnosis of subclinical hypercorticolism used no discriminate clinical signs of hormonal overproduction (including easy bruising, facial plethora, proximal myopathy, or reddish-purple striae). Laboratory diagnosis of SCS was made if incomplete suppression of 1 mg DST and two-day 2 mg DST (if both serum cortisols were >1.8 µ/Dl after 1 mg DST) was accompanied by at least one of the following: morning ACTH levels less than 10 pg/mL, midnight serum cortisol levels higher than 7.5 µ/dL, or decreased serum DHEA-S levels (based on age- and gender-matched reference levels)\(^20\).

**Methods**

Epicardial fat and carotid intima-media thickness measurement

A commercially-available ultrasonography device (Toshiba Medical Systems, APLIO XG) with a 2.5 MHz probe was used to measure EFT thickness, defined as the echo-free space between the visceral and parietal pericardium on the anterior wall of...
the right ventricle. EFT thickness was measured over 3 cardiac cycles at end-diastole on parasternal long and short axis views perpendicular to the free wall of the right ventricle. The average of the three measurements was calculated.

The CIMT of the right common carotid artery was evaluated using a linear-array imaging probe (12 MHz) with the same commercially-available ultrasonography device. The probe was manually maneuvered to parallel the common carotid artery. The area 10 mm proximal to the carotid bifurcation was selected and the distance between the lumen-intima and the media-adventitia interfaces of the far wall was calculated to be the intima-media thickness. A still image, magnified to maximize detail, was used to explicitly identify the borders of the CIMT. Using this image, the CIMT was measured at three adjacent sites, 1 mm apart. The average value of the three measurements was calculated for analysis. The same researcher, blind to patient information, performed all measurements.

**Laboratory examination**

Patients were examined for BMI and systolic and diastolic blood pressure.

Electrochemiluminescent immunoassays (ECLIAs) were used to measure serum cortisol levels using a Roche Cobas E601 analyzer (Roche Diagnostics, Indianapolis, USA). A chemiluminescent immunometric assay kit on the IMMULITE 2000 system (Siemens Medical Solutions Diagnostics, Llanberis, United Kingdom) was used to measure plasma ACTH and DHEA-S levels. Routine laboratory procedures were used to identify Fasting Plasma Glucose (FPG), Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein-cholesterol (HDL-C), and Low Density Lipoprotein-Cholesterol (LDL-C) levels.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS) 19.0 (Chicago, IL, USA) computer program was used for statistical analyses. P values of <0.05 were accepted as statistically significant. Mean ± SDs were used to express continuous variables, while percentages were used to express categorical variables. Normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test. Parametric and non-parametric variables in more than two groups were tested using the one-way ANOVA and Kruskal-Wallis tests, respectively. The Tukey test and Mann-Whitney U test with Bonferroni correction were performed for post hoc analysis (for Bonferroni, P<0.017 was considered significant). Pearson and Spearman correlation coefficients were used to analyze the correlation between parameters. Multiple regression analyses assessed the determinants of the dependent EFT thickness variable using the following independent variables: age, gender, BMI, systolic and diastolic blood pressure, FPG, and patient group. An enter method was used for regression analysis.

**Results**

The present study comprised 63 AI patients and 48 control subjects. Among the AI patients, 20.6% had SCS (n=13), with no primary hyperaldosteronism or pheochromocytoma.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (C)</th>
<th>NFA</th>
<th>SCS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>36/12</td>
<td>26/24</td>
<td>5/8</td>
<td>&lt; 0.05a</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.08 ± 8.22</td>
<td>58.12 ± 9.2</td>
<td>60.0 ± 11.28</td>
<td>NSb</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.21 ± 4.89</td>
<td>29.13 ± 4.97</td>
<td>31.42 ± 6.26</td>
<td>NSc</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>9/18.8%</td>
<td>10/20%</td>
<td>3/23.1%</td>
<td>NSd</td>
</tr>
<tr>
<td>Size (mm)</td>
<td>-</td>
<td>21.09 ± 9.72</td>
<td>27.92 ± 13.51</td>
<td>NSa</td>
</tr>
<tr>
<td>ACTH (pg/mL)</td>
<td>-</td>
<td>25.61 ± 32.12</td>
<td>12.35 ± 6.84</td>
<td>&lt; 0.01b</td>
</tr>
<tr>
<td>1 mg DST (µ/dL)</td>
<td>-</td>
<td>1.44 ± 0.49</td>
<td>3.42 ± 2.37</td>
<td>&lt; 0.001d</td>
</tr>
<tr>
<td>MNSC (µ/dL)</td>
<td>-</td>
<td>5.25 ± 2.99</td>
<td>10.03 ± 6.17</td>
<td>&lt; 0.01c</td>
</tr>
<tr>
<td>DHEA-S (µ/dL)</td>
<td>-</td>
<td>103.95 ± 71.49</td>
<td>54.05 ± 25.75</td>
<td>&lt; 0.05d</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>94.31 ± 9.33</td>
<td>100.19 ± 15.14</td>
<td>106.15 ± 33.02</td>
<td>NSb</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>197.11 ± 36.74</td>
<td>213.85 ± 44.32</td>
<td>189.0 ± 41.99</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>126.48 ± 25.09</td>
<td>139.28 ± 32.75</td>
<td>115.62 ± 32.41</td>
<td>&lt; 0.05b</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>46.94 ± 12.62</td>
<td>55.38 ± 13.36</td>
<td>46.0 ± 21.64</td>
<td>&lt; 0.05d</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>166.0 ± 38.6</td>
<td>149.87 ± 67.41</td>
<td>141.33 ± 101.33</td>
<td>NSa</td>
</tr>
<tr>
<td>Systolic BP (mm/Hg)</td>
<td>120.88 ± 22.27</td>
<td>140.5 ± 22.6</td>
<td>134.62 ± 21.06</td>
<td>&lt; 0.001a</td>
</tr>
<tr>
<td>Diastolic BP (mm/Hg)</td>
<td>76.5 ± 9.53</td>
<td>85.12 ± 15.55</td>
<td>84.08 ± 12.52</td>
<td>&lt; 0.01a</td>
</tr>
<tr>
<td>EFT (mm)</td>
<td>3.92 ± 1.14</td>
<td>5.44 ± 1.61</td>
<td>6.22 ± 1.97</td>
<td>&lt; 0.001c</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.65 ± 0.11</td>
<td>0.75 ± 0.18</td>
<td>0.78 ± 0.24</td>
<td>&lt; 0.01b</td>
</tr>
</tbody>
</table>

Table 1: Clinical and laboratory characteristics of study participants. NS: Not significant, C: Control, NFA: Non-functional adenoma; SCS: Subclinical Cushings’ syndrome; BMI: Body mass index, DST: Dexamethasone suppression test; MNSC: Midnight serum cortisol; DHEA-S: Dehydroepiandrosterone-sulphate; FPG: Fasting plasma glucose, TC: Total cholesterol, LDL: Low-density lipoprotein cholesterol, HDL: High-density lipoprotein cholesterol, TG: Triglyceride, BP: Blood pressure, EFT: Epicardial fat tissue, CIMT: Carotid intima-media thickness. *The size of the largest lesion in bilateral incidentomas was used for analysis. a: Chi-Square test; b: Kruskal-Wallis test; c: One-way Anova test; d: Mann-Whitney U test
Table 1 lists the demographic and hormonal characteristics of all subjects. There was no significant difference between the groups in terms of age, BMI, smoking habits, FPG, TC, and TG levels. There was a difference in gender, LDL, HDL, and systolic and diastolic blood pressure between the groups. LDL-C was higher in patients with NFA than in both SCS patients and control subjects (both P<0.05). However, there was no difference between SCS patients and control subjects. There was no difference in systolic and diastolic blood pressure and HDL levels between SCS patients and control subjects. However, systolic and diastolic blood pressures and HDL levels in NFA patients were higher than in the control subjects (P<0.001; <0.01; <0.001, respectively).

There was no difference in EFT between the SCS and NFA patients. CIMT was also higher in NFA patients (mean CIMT, 0.75 ± 0.18 mm) than in control subjects (mean CIMT, 0.65 ± 0.11 mm) (P < 0.01). However, there was no difference between the EFT of SCS patients and either NFA patients or control subjects. EFT and CIMT averages for all groups are shown in Figures 1 and 2, respectively.

The Spearman correlation coefficients of the simple regression analysis of EFT and CIMT with other parameters is shown in Table 2 for the whole population. EFT was significantly positively correlated with age, gender, BMI, and systolic and diastolic blood pressure in the study population, while CIMT was significantly positively correlated with age, gender, and systolic and diastolic blood pressure.

Multiple regression analysis coefficients for the study population are shown in Table 3. EFT thickness was the dependent variable, and age, gender, BMI, systolic and diastolic blood pressure, FPG, and patient group (control, NFA, and SCS) were the independent variables. EFT thickness had an independent positive association with gender, BMI, and patient group (in particular the SCS group).
The effects of the other variables were not significant.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>β</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>0.039</td>
<td>0.016</td>
<td>NS</td>
<td>0.007 - 0.071</td>
</tr>
<tr>
<td>gender</td>
<td>0.588</td>
<td>0.17</td>
<td>&lt; 0.05</td>
<td>0.002 - 1.174</td>
</tr>
<tr>
<td>BMI</td>
<td>0.109</td>
<td>0.328</td>
<td>&lt; 0.05</td>
<td>0.051 - 0.167</td>
</tr>
<tr>
<td>systolic BP</td>
<td>-0.012</td>
<td>-0.173</td>
<td>NS</td>
<td>0.038 - 0.005</td>
</tr>
<tr>
<td>diastolic BP</td>
<td>0.015</td>
<td>0.12</td>
<td>NS</td>
<td>-0.016 - 0.047</td>
</tr>
<tr>
<td>FPG</td>
<td>0.012</td>
<td>0.112</td>
<td>NS</td>
<td>-0.005</td>
</tr>
<tr>
<td>patients group</td>
<td>0.657</td>
<td>0.365</td>
<td>&lt; 0.001</td>
<td>0.347 - 0.907</td>
</tr>
</tbody>
</table>

Table 3: Results of multiple regression analysis for dependent variable epicardial fat thickness of the whole population.
Dependent variable: epicardial fat thickness.

BMI: Body mass index; BP: Blood pressure; FPG: Fasting plasma glucose

Discussion

Our study showed that in AI patients, EFT measurements were significantly higher compared to the control group. In addition, there was no significant difference between the EFT measurements of patients diagnosed with NFA and those with SCS. While there was no difference in CIMT values between NFA and SCS patients, they were significantly higher than those in the control group. These results lead us to believe that the risk of developing atherosclerosis is high for AI patients and close monitoring for cardiovascular disease is necessary.

Currently, AI is frequently observed on thorax and abdominal images. Recent studies have researched the relationship of these masses, not just to hormone secretion and malignancy risk, but also to metabolic changes and cardiovascular diseases. Studies have reported increased incidence of metabolic syndrome and insulin resistance in AI patients. It is emphasized that there is an increased risk of cardiovascular disease parallel to the increase in incidence of metabolic syndrome. In addition, although clinical studies of these masses clearly identify no hormone production, in cases where there is a slight excess release of cortisol (as in SCS), an increased risk of cardiovascular disease has been found. Studies on the increase in metabolic syndrome and insulin resistance in AI have stated this may be due to the effect of the excess release of cortisol or to glucocorticoid receptor polymorphism, increasing sensitivity to cortisol.

In our study, we showed that the risk of developing atherosclerosis is high for AI patients. Our evaluation found similar results for NFA and SCS: this leads us to think that excess cortisol, which cannot be measured, may be released in NFA.

We believe this excess cortisol causes atherosclerosis and is linked to the increased risk of coronary artery disease.

Atherosclerosis is a major etiologic factor in cardiovascular diseases. Clinical studies have tried to measure the presence and severity of atherosclerosis. In a study of 35 NFA patients, Erbil et al. reported that flow-mediated arterial dilation (FMD) measurements reflecting the presence of atherosclerosis showed high risk of cardiovascular diseases. In our study, we evaluated the development of atherosclerosis in AI patients and found the EFT and CIMT to be significantly increased. While the measurement techniques used to evaluate atherosclerosis are different, our study with a larger population supports these results.

EFT is a valuable method for evaluating the formation of atherosclerosis. Meta analyses of EFT measurements have shown that it identifies the presence of risk for metabolic syndrome, similar to cardiovascular diseases. In a study of 46 AI patients (40 NFA, 6 SCS), Iacobellis completed EFT measurements and found them to be significantly high compared to the control group. This study emphasized that EFT was related to left ventricle hypertrophy and that EFT was an early marker for cardiac anomalies in AI. In our study, we evaluated the development of atherosclerosis in patients with adrenal incidentaloma using the ultrasound...
In conclusion, there was an increase in both EFT and CIMT measurements in our patients with non-functional AIs. In SCS patients, this increase is known to be linked to a slight increase in cortisol secretion. While there is a causal relationship, none of our investigations showed increased cortisol release in NFA patients. We believe tests with greater sensitivity tests are required to identify these hormonal changes that may explain cardiometabolic changes in NFA patients. We believe AI cases should be closely monitored for coronary artery disease. To reduce the cardiometabolic risk, especially in NFA patients, prospective studies examining the sites of operations for the treatment of mass removal are required.

References


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