Non-Alcoholic Fatty Liver Disease in Subjects with Non-functioning Adrenal Adenomas

Fonksiyon Göstermeyen Adrenal Adenomlu Kişilerde Non-Alkolik Yağlı Karaciğer Hastalığı

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Abstract

Objectives: The relation between non-functioning adrenal adenoma and unfavorable metabolic status has been a debate so far. We aimed to demonstrate the prevalence of non-alcoholic fatty liver disease (NAFLD) in subjects with silent adrenal adenomas.

Materials and Methods: 130 consecutive subjects with non-functioning adrenal adenomas, 170 age-, gender- and BMI-matched individuals without adrenal gland disorders, and 20 patients with Cushing’s syndrome were included in the study. Fatty liver disease was diagnosed by ultrasonography and the severity was scored semiquantitatively. Liver function tests were performed. Cushing’s syndrome and non-functioning adrenal adenoma were diagnosed using appropriate tests of hypothalamus-pituitary-adrenal function.

Results: The prevalence of NAFLD was 30.7%, 65.0% and 39.4% in adenoma group, Cushing’s syndrome group and control group, respectively. There was no significant difference in terms of Type 2 diabetes mellitus, hypertension and NAFLD prevalence between adenoma group and controls. NAFLD was not only more common in subjects with Cushing’s syndrome but was also more severe. Hypercortisolemia strongly predicted the development of metabolic syndrome (OR: 10.571, p=0.004). When age, gender, hypercortisolemia and metabolic syndrome were assessed, metabolic syndrome remained as the sole independent predictor of fatty liver development (OR: 9.162, p<0.001).

Conclusion: Comparable prevalence between adenoma and control group was likely to be associated with similar rates of metabolic derangements and similar BMI. Cortisol excess seemed to be related with fatty liver development mainly through its unfavorable metabolic effects. Türk Jem 2011; 15: 116-20

Key words: NAFLD, adrenal adenoma, Cushing Syndrome
Introduction

Subsequent to the recognition of increased prevalence of obesity, glucose intolerance and hypertension in subjects with adrenal adenomas, a variety of metabolic and cardiovascular disturbances have been identified in those individuals [1-4]. Previously, we showed several aspects of unfavorable metabolic and cardiovascular outcome in terms of elevated uric acid, the homeostasis model assessment (HOMA) and D-dimer levels and increased carotid intima media thickness in individuals with silent adrenal adenomas mainly in older ages [5,6].

Non-alcoholic fatty liver disease is a clinical situation between simple steatosis and steatohepatitis. Several aetiological factors have been described including Type 2 diabetes mellitus (Type 2 DM), obesity and metabolic syndrome as the leading causes [7]. Recent studies have provided several lines of evidence that could be attributable to the hypothalamus-pituitary-adrenal (HPA) axis alterations in fatty liver disease and non-alcoholic steatohepatitis (NASH) [8-11].

Current literature has suggested that clinically non-functioning adrenal adenomas may not be completely “silent” in terms of cortisol secretion. In vivo data demonstrated that steroid production in inactive adrenocortical tumors was not overtly different from those of subclinical Cushing’s syndrome [12]. We also showed that, subjects with non-functioning adrenal adenomas featured subtle cortisol autonomy in terms of elevated post dexamethasone cortisol and reduced dehydroepiandrosterone sulfate levels when compared to healthy subjects [5,6].

In this study, we aimed to investigate NAFLD prevalence in 130 consecutive individuals with non-functioning adrenal adenomas. For this purpose, we included 20 subjects with Cushing’s syndrome and 170 age-, gender- and BMI-matched individuals without adrenal gland disorders as control subjects.

Materials and Methods

This retrospective study was conducted in Dokuz Eylul University, Division of Endocrinology. Data of 200 consecutive subjects, who were referred to our institute due to incidentally discovered adrenal tumors between January 2007 and January 2009, were evaluated. Among those, 130 individuals, who had non-functioning adrenal adenomas, were included in the study. Control group was generated from the patients of our obesity and diabetes units of the division. Data of control subjects were achieved consecutively from their medical records. Age, gender and BMI were matched between non-functioning adrenal adenoma and control groups. The essential criterion for the inclusion of control subjects was the presence of an abdominal ultrasonography providing adequate data regarding liver parenchyma and adrenal glands. We also included 20 consecutive subjects with Cushing’s syndrome (Cushing Disease n=10, ACTH independent Cushing’s syndrome due to an adrenal adenoma n=10) in order to investigate the prevalence of hepatosteatosis in hypercortisolemic individuals.

Exclusion criteria for all participants were: chronic liver disease, chronic kidney disease, malignancy, and lack of ultrasonography examination or inappropriate ultrasonography findings.

Liver steatosis was assessed on a three-point scale (mild, moderate or severe), based on ultrasonographic characteristics. Mild steatosis was defined as a slight augmentation in liver echogenicity and a weak exaggeration of echogenic discrepancy between liver and kidney. Moderate steatosis was defined as a loss of echo line in the portal vein wall, particularly from the peripheral branches, resulting in a featureless appearance of the liver. Severe steatosis was defined as much greater reduction in echo penetration and a large echo discrepancy between liver and kidney [13-15].

Non-alcoholic fatty liver disease was diagnosed in subjects with fatty liver who met the following criteria: alcohol consumption <20 g/day, no history of chronic viral hepatitis, autoimmune hepatitis or liver cirrhosis, and no use of toxins or drugs associated with steatosis [16].

Alcohol consumption was assessed by detailed history. Viral hepatitis and autoimmune hepatitis markers were measured in subjects who had elevations in either ALT or GGT, without a definite underlying etiology, more than 3 months. Four subjects in control group were further evaluated by liver biopsy due to the persistently elevated ALT levels and were diagnosed with non-alcoholic steatohepatitis.

Initial radiological examination was computed tomography (CT) in all subjects with incidentally discovered adrenal tumors. Malignancy was excluded if the following criteria were met in CT evaluations: (i) regular shape with well-defined margins and homogenous (ii) attenuation value of 10 or less Hounsfield units on unenhanced CT scan, and (iii) 30 or less Hounsfield units on enhanced CT scan. Radiological examination depended on the etiology of hypercortisolism in subjects with Cushing’s syndrome. The definitive diagnosis was Cushing Disease in 10 subjects and ACTH-independent Cushing’s syndrome due to an adrenal adenoma in 10 subjects. Adrenal imaging was not performed in subjects with Cushing Disease. In control subjects, adrenal glands were evaluated by ultrasonography.

Hormonal evaluation was performed in all subjects with incidentally discovered adrenal tumors and Cushing’s syndrome. Morning cortisol, dehydroepiandrosterone sulfate (DHEA-S), adrenocorticotropic hormone (ACTH), and, in hypertensive subjects, plasma renin activity and serum aldosterone were measured. Subsequently, urinary free cortisol (UFC) (normal range: 110 µg/day), urinary normetanephrine (normal range: 88-444 µg/day) and urinary metanephrine (normal range: 52-341 µg/day) were measured and the mg overnight dexamethasone suppression test (DST) was performed. In subjects with non-suppressed cortisol levels, diurnal rhythm of cortisol was also evaluated (normal: midnight cortisol<7.5 µg/dl).

The suppression in overnight DST was adequate when morning cortisol fell below 1.8 µg/dl. When post DST cortisol was over 1.8 µg/dl, 2-day 2 mg dexamethasone suppression test, involving the administration of 0.5 mg oral dexamethasone given every 6 hours for 48 hours, was performed. The patients with suppressed post DST cortisol levels were considered to have non-functional adenoma, if they additionally had at least one of the following criteria: (i) morning dehydroepiandrosterone sulfate (DHEA-S) levels>40 µg/dl, (ii) non-suppressed plasma corticotrophin (normal value: 25 pg/ml) or (iii) UFC<110 µg/day. In control subjects, HPA axis tests were not routinely investigated, as none of them had an apparent adrenal lesion on ultrasonography or clinical findings of a hormone excess syndrome.

Cushing’s syndrome was based on a non-suppressed post DST cortisol (>1.8 µg/dl), elevated urinary free cortisol (>110 µg/dl/day) and/or disturbed diurnal rhythm of cortisol secretion (midnight cortisol level>7.5 µg/dl). Patients who had ACTH-dependent
Cushing’s syndrome were evaluated with bilateral petrosal sinus sampling. Subjects with undetectable ACTH levels were referred for adrenal imaging with CT.

Metabolic syndrome was defined according to the criteria established by the National Cholesterol Education Program Adult Treatment Panel III [17].

Distribution of continuous variables was assessed by the Kolmogorov–Smirnov normality test. According to the variable distribution, Independent samples t-test was used. Dichotomous variables were assessed with chi-square test. Logistic regression analysis was employed to assess independent variables that could be associated with development of NAFLD and metabolic syndrome.

Statistical analysis was performed using SPSS, version 11.0 for Windows. A p value less than 0.05 was accepted as statistically significant.

Results

In adenoma group, the mean adenoma diameter was 22.8±10.0 mm. There were 61 (47.0%) subjects with right, 61 (47.0%) with left and 8 (6.0%) with bilateral adrenal adenomas.

Hormonal evaluation of participants with adrenal adenomas and Cushing’s syndrome were as follows: morning cortisol (14.0±5.9 µg/dl vs. 24.6±7.7 µg/dl, p<0.001), midnight cortisol (5.7±3.6 µg/dl vs. 23.8±5.2 µg/dl, p<0.001), urinary free cortisol (41.3±26.4 µg/day vs. 149.8±87.1 µg/day, p<0.001) and post DST cortisol (1.6±1.1 µg/dl vs. 17.8±9.0 µg/dl, p<0.001), respectively.

Participants with Cushing’s syndrome were significantly younger than the remaining subjects. Gender and BMI were comparable due to the inclusion criteria. Prevalence of Type 2 DM, hypertension and hyperlipidemia were similar in adenoma and control groups, while Type 2 DM (55.0% vs. 23.8%, p=0.007) and hypertension (59.3% vs. 85.0%, p=0.044) were more common in Cushing’s syndrome group when compared to adenoma group (Table 1).

Non-alcoholic fatty liver prevalence was 30.7%, 65.0%, 39.4% in adenoma group, Cushing’s syndrome group and control group, respectively. Aminotransferase levels and GGT were significantly elevated in Cushing’s syndrome group when compared to adrenal adenoma group. Gamma-glutamyl transpeptidase and ALT levels were comparable between adrenal adenoma group and control

### Table 1. Anthropometric and metabolic characteristics and non-alcoholic fatty liver disease data of subjects with non-functioning adrenal adenomas, Cushing Syndrome and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Adrenal adenoma (n=130)</th>
<th>Cushing Syndrome (n=20)</th>
<th>Control Subjects (n=170)</th>
<th>p†</th>
<th>p‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56.4±10.6</td>
<td>45.1±11.3</td>
<td>57.2±9.8</td>
<td>&lt;0.001</td>
<td>0.517</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>105/25</td>
<td>16/4</td>
<td>141/34</td>
<td>0.965</td>
<td>0.965</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2±4.1</td>
<td>32.5±6.5</td>
<td>31.0±4.4</td>
<td>0.152</td>
<td>0.163</td>
</tr>
<tr>
<td>Type 2 DM (%)</td>
<td>23.8</td>
<td>55.0</td>
<td>23.5</td>
<td>0.007</td>
<td>0.989</td>
</tr>
<tr>
<td>Hypertension %</td>
<td>59.3</td>
<td>85.0</td>
<td>55.4</td>
<td>0.044</td>
<td>0.553</td>
</tr>
<tr>
<td>Hyperlipidemia %</td>
<td>57.2</td>
<td>75.0</td>
<td>66.4</td>
<td>0.149</td>
<td>0.152</td>
</tr>
<tr>
<td>NAFLD n, (%)</td>
<td>40, (30.7)</td>
<td>13, (65.0)</td>
<td>67, (39.4)</td>
<td>0.005</td>
<td>0.145</td>
</tr>
<tr>
<td>AST</td>
<td>19.4±8.1</td>
<td>27.7±15.9</td>
<td>21.4±7.2</td>
<td>0.002</td>
<td>0.042</td>
</tr>
<tr>
<td>ALT</td>
<td>22.9±22.3</td>
<td>33.0±15.6</td>
<td>22.1±12.7</td>
<td>&lt;0.001</td>
<td>0.691</td>
</tr>
<tr>
<td>GGT</td>
<td>27.0±19.0</td>
<td>76.4±60.3</td>
<td>27.8±23.7</td>
<td>&lt;0.001</td>
<td>0.795</td>
</tr>
</tbody>
</table>

Note that p† refers to comparisons between subjects with adrenal adenoma and Cushing Syndrome and p‡ refers to comparisons between subjects with adrenal adenoma and control subjects. Chi-square or Independent samples T Test was used for comparisons.

### Table 2 Predictors of non-alcoholic fatty liver disease and metabolic syndrome in non-functioning adrenal adenoma and Cushing Syndrome groups

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>OR</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAFLD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.16</td>
<td>0.984</td>
<td>0.352</td>
<td>0.951-1.018</td>
</tr>
<tr>
<td>Gender</td>
<td>0.307</td>
<td>1.359</td>
<td>0.319</td>
<td>0.535-3.541</td>
</tr>
<tr>
<td>Hypercortisolism</td>
<td>1.246</td>
<td>3.476</td>
<td>0.021</td>
<td>1.204-10.036</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAFLD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.522</td>
<td>1.686</td>
<td>0.069</td>
<td>0.926-3.003</td>
</tr>
<tr>
<td>Gender</td>
<td>0.269</td>
<td>1.309</td>
<td>0.604</td>
<td>0.473-3.621</td>
</tr>
<tr>
<td>Hypercortisolism</td>
<td>0.522</td>
<td>1.686</td>
<td>0.373</td>
<td>0.535-5.319</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>2.215</td>
<td>9.162</td>
<td>&lt;0.001</td>
<td>3.338-25.149</td>
</tr>
<tr>
<td>Model 3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Metabolic Syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.031</td>
<td>1.032</td>
<td>0.071</td>
<td>0.997-1.068</td>
</tr>
<tr>
<td>Gender</td>
<td>0.255</td>
<td>1.290</td>
<td>0.603</td>
<td>0.494-3.370</td>
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<tr>
<td>Hypercortisolism</td>
<td>2.358</td>
<td>10.571</td>
<td>0.004</td>
<td>2.162-51.688</td>
</tr>
</tbody>
</table>

NAFLD, non-alcoholic hepatosteatosis, OR, odds ratio

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resulting in increased glucose output, lipid synthesis and, finally, cortisone to cortisol and facilities the action of glucocorticoids in liver expression in the liver. This stimulates the conversion of inactive indole hydroxysteroid dehydrogenase type 1 ($\beta=2.358$, $p<0.004$, OR: 10.571).

discussed that, the presence of a silent adrenal adenoma was not associated with additional risk of developing fatty liver beyond the consequences of metabolic syndrome and insulin resistant state. Despite the clinical awareness of the close interrelation between Cushing’s syndrome and fatty liver, true incidence remains unknown. In a previous report, Rockall et al [19] demonstrated that 20% of patients had hepatic steatosis. However, this rate was not significantly different when compared to prevalence estimates reported to be 17%-33% in epidemiological studies [20]. In our study, we showed that individuals with Cushing’s syndrome had nearly a 2-fold increase in NAFLD prevalence when compared to the remaining participants. Additionally, steatosis observed in hypercortisolemic subjects was more severe. Logistic regression analysis revealed that, hypercortisolism strongly predicted the development of metabolic syndrome (OR: 10.571, $p=0.004$). However, when age, gender, hypercortisolism and metabolic syndrome were assessed in a regression model for their possible effects on NAFLD development, metabolic syndrome remained as the sole independent predictor for the development of fatty liver (OR: 9.162, $p<0.001$). These results suggest that cortisol excess is related with the development of fatty liver, mainly through its unfavorable metabolic effects. These include their contrary effects on insulin action in several tissues. They increase the turnover between stored energy (glycogen, triglyceride) and freely available fuel (glucose, free fatty acids) for mitochondrial oxidation. Glucocorticoids also raise blood pressure and glucose and increase visceral adipose tissue that is strongly associated with insulin resistant state [21,22].

Two issues in our study may cause concern. The first one is the detection of steatosis by ultrasonography instead of CT or magnetic resonance imaging. Despite its limitations such as being operator dependent and the lack of providing quantitative information, it was demonstrated that sensitivity, specificity and positive predictive value of ultrasonography to detect steatosis was as high as 80 to 100% [23]. Second issue was the lack of HPA axis parameters in control group. We did not perform any hormone measurements in control group because they did not have adrenal gland tumors or clinical findings related to any hormone excess syndromes.

In conclusion, we did not demonstrate an increased NAFLD prevalence in subjects with non-functioning adrenal adenomas while apparent hypercortisolism was associated with both increased prevalence and increased severity of NAFLD via its unfavorable metabolic effects. The causative relation between clinically non-functioning adrenal adenomas and unfavorable metabolic status has been a debate so far. Despite several data regarding this condition, there are still unequivocal issues that need to be elucidated with further studies.
References