Prevalence of Subclinical Cushing’s Syndrome in 70 Patients with Adrenal Incidentaloma: Clinical, Biochemical and Surgical Outcomes

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Abstract. Subclinical Cushing’s syndrome (SCS) is being detected with increased frequency in patients with adrenal incidentaloma. In the current study, we evaluated the prevalence of SCS in 70 patients with adrenal incidentaloma and compared the main findings on them with other patients with nonfunctional adrenal incidentaloma (NFA). Overnight 3 mg dexamethasone (DXM) suppression test to exclude cortisol hypersecretion, and high dose DXM suppression test to find out patients with SCS, were applied to all subjects. Afterwards, biochemical and clinical findings of patients with SCS were compared with the other patients with NFA. Four of the 70 patients with adrenal incidentaloma were found to have SCS, with a prevalence of 5.7%. Basal ACTH and DHEA-S levels were significantly lower (p<0.05 and p<0.01, respectively), and midnight cortisol and 24-hour urinary free cortisol levels were significantly higher in patients with SCS (p<0.001 and p<0.05, respectively). Biochemical and metabolic bone parameters were similar in patients with SCS and in patients with NFA. Hypertension, diabetes mellitus, and obesity were more common in patients with SCS. One of the patients with SCS developed adrenocortical insufficiency following unilateral adrenalectomy which lasted for about 6 months. Suppressed ACTH and DHEA-S levels, and high midnight cortisol levels may be some clues for SCS in patients with adrenal incidentaloma. Since patients with SCS frequently have risk factors for atherosclerosis such as hypertension, diabetes, and obesity, and the surgical management of SCS with adrenalectomy may offer an advantage. Patients undergoing adrenalectomy should be followed for the development of adrenal insufficiency.

Key words: Subclinical Cushing’s syndrome, Adrenal incidentaloma

CLASSIC Cushing’s syndrome is a rare disease with an estimated incidence of 1 case per 500,000 persons [1]. On the other hand, autonomous glucocorticoid production without specific signs and symptoms of Cushing’s syndrome which is termed as subclinical Cushing’s syndrome (SCS), is much more frequent, especially on the occasion of incidentally detected adrenal masses. With the routine use of imaging techniques such as ultrasound (USG) and computerized tomography (CT), adrenal masses are being detected with increased frequency [2–4]. Incidentally discovered adrenal masses are mostly benign, asymptomatic lesions [3, 5], often arbitrarily considered as nonfunctioning tumors. Recent studies have reported increasing evidence that subtle cortisol production and abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis are more frequent than previously thought. Depending on the diagnostic criteria and the study design used, SCS is found in 5% to 20% of patients with adrenal incidentaloma [6–17]. It is assumed that glucocorticoid production in these patients is insufficient to cause a clinically recognizable syndrome. The progression to overt Cushing’s syndrome is unclear and probably is very low [1, 18, 19]. But Barzon et al. found the estimated cumulative risk to develop overt Cushing’s syndrome of 12.5% after one year when considering only patients with subclinical autonomous...
glucocorticoid overproduction [20].

The lack of both standard endocrine tests and criteria for the definition of SCS limits the possibility of a correct evaluation and management of these patients. The use of 24-hour urinary free cortisol (UFC) excretion is not a sensitive indicator of this low grade hypercortisolemia [8], because increased values are late findings usually associated with emerging clinical signs of Cushing’s syndrome [8]. Beside this, absence of suppression on dexamethasone (DXM) testing has been reported in many studies [21–23].

The spectrum of biochemical findings in SCS includes the followings: Blunted diurnal variation of cortisol secretion, low dehydroepiandrosterone sulfate (DHEA-S), low or suppressed plasma ACTH and blunted response of ACTH to CRH stimulation, missing cortisol suppression after low dose overnight or classical DXM suppression test, missing cortisol suppression after high dose DXM suppression test and elevated urinary free cortisol [24]. The best means to uncover autonomous cortisol secretion is the overnight DXM suppression test, which rarely fails to detect SCS. To reduce false positive results, a higher DXM dose (3 mg instead of 1 or 2 mg) can be preferred [24]. When the short DXM suppression testing failed to suppress morning cortisol, further investigation is required, including a confirmatory high dose DXM suppression test (8 mg).

Long-term prospective studies assessing the outcome of patients with subclinical hypercortisolism are limited. The progression towards overt Cushing’s syndrome seems to occur only in few cases in the short-term [19, 24–29]. Although patients with Cushing’s syndrome have clearly established complications, the morbidity of patients with subclinical disease is less clear, and controversy exists as to the risk of progression from subclinical to overt hypercortisolism [18, 19, 25, 30, 31]. However, recent evidence suggests that SCS might also be associated with increased risk for hypertension, diabetes, obesity, or osteoporosis [8, 26, 32–35]. On the contrary, whether patients with SCS should undergo adrenalectomy is a matter of debate. There are not enough clinical trials on the subject. Some authors suggest surgical treatment for the young patients (<50 years), and the patients with a recent history of weight gain, substantial obesity, arterial hypertension, diabetes mellitus, and osteopenia [8]. These findings are all associated with the hypercortisolemic states already. Besides, in the study of Reincke et al., it was pointed out that patients with SCS had to be followed for the possible adrenocortical insufficiency after unilateral adrenalectomy. In that study, four of the seven patients with SCS who underwent unilateral adrenalectomy developed adrenocortical insufficiency [8].

In the current study, we aimed to evaluate the presence of SCS attributable to autonomous cortisol production in patients with incidentally discovered adrenal masses. Furthermore the clinical picture of the presentation in those patients and the surgical outcomes of the patients who underwent adrenalectomy were determined so as to illuminate the therapeutic approach to the patients with adrenal incidentaloma.

Subjects and Methods

Patients

In the current study, we prospectively evaluated 70 patients (50 women and 20 men) with incidentally detected adrenal masses who were referred to the Department of Endocrinology and Metabolic Diseases of the Ankara University, School of Medicine, Ankara, Turkey. The mean age of the patients was 54.45 ± 11.73 years (age range 29–78 years). The mean size of the adrenal masses was 2.94 ± 1.79 cm (range 0.5–10 cm). All the incidentalomas were discovered by abdominal USG, CT, or magnetic resonance imaging performed for the evaluation of unrelated diseases. Patients with known extraadrenal malignancies were excluded. None of the patients showed specific signs or symptoms of hormone production excess, nor were they on hormonal treatment. Patients with severe or paroxysmal hypertension, hypokalemia (<3.5 mEq/L) and clinical signs of hypercortisolism or hyper-androgenism were also excluded. Vanillylmandelic acid and metanephrine to exclude the presence of pheochromocytoma, plasma renin activity and aldosterone in the upright position to exclude an aldosterone-secreting adenoma, were performed. Although the patients were all free of signs or symptoms of hypercortisolism, in order to determine the frequency of SCS in these patients with adrenal incidentaloma, we also evaluated the baseline plasma ACTH and serum cortisol at 08.00 and 24.00 hour for the diurnal rhythm and 24-h UFC excretion. Moreover
dehydroepiandrosterone sulfate (DHEA-S) concentrations were measured because low DHEA-S level is accepted as a biochemical finding in SCS. Afterwards, all patients underwent 3 mg overnight DXM suppression testing to exclude cortisol hypersecretion. We preferred 3 mg instead of 1 or 2 mg for the overnight suppression to minimize the false positive results [24]. A serum cortisol level suppressed under 3 μg/dl, in this test, excludes significant autonomous cortisol secretion. In patients who failed to achieve serum cortisol suppression to below 3 μg/dl after the administration of 3 mg DXM, a high dose DXM test was performed (2 mg, four times a day for two days). Subclinical Cushing’s syndrome was diagnosed when high dose DXM failed to suppress serum cortisol and 24-h UFC levels below the 50% of the baseline values.

In patients with SCS, insulin dependent diabetes mellitus, osteoporosis, diffuse obesity, and hypertension are more common findings and we tried to compare our patients with SCS to the other patients with adrenal incidentaloma for those clinical findings. Lipid profiles (total and HDL, LDL-cholesterol, triglyceride), calcium, phosphorous, alkaline phosphatase, parathyroid hormone (PTH), vitamin D (25 OH D3) levels were also determined and bone mineral density (BMD) was evaluated by dual-energy x-ray absorptiometry (DEXA). BMD was measured on the lumbar spine and at the right femoral neck. Individual BMD values were expressed as Z-score, which represents the difference between individual value and mean of the reference age- and sex-matched population, expressed in standard deviations. T-score was also determined. The reference population adopted was the international pooled sample provided by the densitometer manufacturer [36].

All the patients were followed up at regular intervals at 6 and 12 months and then every year for a median period of 24 months for the possible increase in tumour size and adrenal hyperfunction. Patients who underwent adrenal surgery (10 patients) were also examined histopathologically and they were all followed after operation as well. We followed our patients who underwent adrenal surgery, especially the ones who had been accepted to have autonomous cortisol secretion, for the development of possible adrenal insufficiency. In addition, the improvement in the metabolic status, change in body weight, improvement in blood pressure for the hypertensive subjects, and improvement in glycemic profiles in the diabetic subjects were all recorded for the patients who underwent adrenalectomy in the follow-up period.

Endocrine assessment

All hormonal analyses were performed in the endocrinology laboratory of Ankara University, School of Medicine. Plasma ACTH was measured by sequential immunometric assay (IMMULITE 2000 ACTH, Diagnostic Products Corporation, Los Angeles, CA, USA; normal range 5–50 pg/ml). Serum cortisol and DHEA-S were measured by electrochemiluminescence immunoassay (Elecsys Systems 1010/2010/ Modular Analytics E170, Roche Diagnostics GmbH, Mannheim, Germany; normal range 6.2–19.4 μg/dl for the morning cortisol and 2.0–9.0 μg/dl for the midnight cortisol; and Elecsys Systems 1010/2010/ Modular Analytics E170, Roche Diagnostics GmbH, Mannheim, Germany; normal range 70–300 μg/dl in females and 100–300 μg/dl in males, respectively). Urinary free cortisol was measured by radioimmunoassay (DSL-2100 ACTIVE, Webster, TX, USA; normal range 9–156 μg/day). In all these methods intra- and interassay coefficients of variations were <10%.

Statistical analyses

The results are expressed as mean ± SD. Statistical analyses was performed by using nonparametric test (Mann-Whitney U test) for the comparison of patients with SCS and the other patients with adrenal incidentaloma. Chi square test was used to compare the prevalences of hypertension, diabetes mellitus, and obesity in patients with SCS and in patients with nonfunctional adrenal adenoma (NFA). Also Spearman’s R coefficient was used for the correlation analyses between parameters determined in the study. Significance was considered to be at \( p<0.05 \).

Results

In the current study, we evaluated 70 patients with incidentally detected adrenal masses without any signs or symptoms of hormonal excess. We also compared the clinical picture and metabolic status of the patients with SCS with the other patients with NFA. Mean
body mass index of those patients with adrenal incidentaloma was 29.04 ± 4.04 kg/m² (range 20–38 kg/m²). Table 1 shows the results of hormonal analyses investigating the HPA axis.

Mean ACTH, morning and midnight cortisol, 24-h UFC, and DHEA-S levels were found to be in normal ranges and the diurnal cortisol rhythm was intact in general. Mean cortisol level following 3 mg DXM administration at midnight was 2.77 ± 3.79 μg/dl, but there were patients who failed to suppress morning cortisol under 3 μg/dl (no = 12; 17.91%). Those patients underwent the high dose DXM suppression test for the confirmation of SCS. Four patients fulfilled the criteria for SCS after the high dose DXM test, and according to our results, the prevalence of SCS in adrenal incidentaloma was 5.7%. The hormonal values related to the HPA axis of our patients with SCS is seen in Table 2.

In patients with SCS basal ACTH values were all under 15 pg/ml. Furthermore DHEA-S levels were found to be low and midnight cortisol levels were high. All these results were compatible with Cushing’s syndrome. Only one patient with SCS had 24-h UFC over the normal range (patient 3).

In Table 3, the diurnal rhythm of patients whose plasma cortisol concentrations was not suppressed by 3 mg DXM, but suppressed by 8 mg DXM test is shown. Diurnal rhythm was found to be normal in those patients. Fig. 1 shows the comparison of the hormonal values of patients with SCS and patients with NFA. In addition, biochemical and metabolic bone parameters of the patients with SCS and the patients with NFA are also shown in Table 4.

The hormonal and biochemical comparison of the SCS cases and the other patients with adrenal incidentaloma displayed that basal ACTH and DHEA-S levels were significantly lower in patients with SCS than the patients with NFA (p<0.05 and p<0.01, respectively). Although morning cortisol levels did not differ significantly, midnight cortisol and 24-h UFC levels were significantly higher in patients with SCS (p<0.001 and p<0.05). However, none of the biochemical parameters (total cholesterol, LDL and HDL-cholesterol, triglyceride), and none of the metabolic bone parameters

### Table 1. Hormonal evaluation of adrenal incidentalomas (mean ± SD) (ranges are given in parenthesis)

<table>
<thead>
<tr>
<th>ACTH (pg/ml)</th>
<th>Morning cortisol (μg/dl)</th>
<th>Midnight cortisol (μg/dl)</th>
<th>24-h UFC (μg/day)</th>
<th>DHEA-S (μg/dl)</th>
<th>Cortisol after 3 mg DXM (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.62 ± 13.85 (5–67)</td>
<td>15.22 ± 5.97 (7.11–36.60)</td>
<td>3.47 ± 3.60 (1.0–17.60)</td>
<td>66.54 ± 44.46 (6–192)</td>
<td>115.00 ± 146.16 (16.3–840)</td>
<td>2.77 ± 3.79 (0.39–21)</td>
</tr>
</tbody>
</table>

DXM: Dexamethasone, DHEA-S: Dehydroepiandrosterone sulfate, UFC: Urinary free cortisol

### Table 2. Hypothalamic-pituitary-adrenal axis in patients with subclinical Cushing’s syndrome.

<table>
<thead>
<tr>
<th>Patient</th>
<th>ACTH (pg/ml)</th>
<th>Morning cortisol (μg/dl)</th>
<th>Midnight cortisol (μg/dl)</th>
<th>24-h UFC (μg/day)</th>
<th>DHEA-S (μg/dl)</th>
<th>Cortisol after 3 mg DXM (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>17.5</td>
<td>8.79</td>
<td>54</td>
<td>18</td>
<td>3.27</td>
</tr>
<tr>
<td>2</td>
<td>9.5</td>
<td>14.7</td>
<td>15.4</td>
<td>95.4</td>
<td>19.1</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>14</td>
<td>17.6</td>
<td>192</td>
<td>45</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>&lt;5</td>
<td>19.4</td>
<td>15.2</td>
<td>92</td>
<td>25.5</td>
<td>18.6</td>
</tr>
</tbody>
</table>

DXM: Dexamethasone, DHEA-S: Dehydroepiandrosterone sulfate, UFC: Urinary free cortisol

### Table 3. Diurnal cortisol rhythm of patients whose plasma cortisol concentrations are not suppressed by 3 mg DXM, but suppressed by 8 mg DXM test.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Morning cortisol (μg/dl)</th>
<th>Midnight cortisol (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>5.77</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>9.91</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>22.7</td>
<td>6.7</td>
</tr>
<tr>
<td>5</td>
<td>11.9</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>18.5</td>
<td>1.75</td>
</tr>
<tr>
<td>7</td>
<td>13.7</td>
<td>4.42</td>
</tr>
<tr>
<td>8</td>
<td>18.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

DXM: Dexamethasone
determined (calcium, phosphorous, alkaline phosphatase, parathyroid hormone, and vitamin D, lumbar or femoral BMD and Z or T scores) were found to be significantly different in patients with NFA than in patients with cortisol secreting adrenal adenoma. There were no significant age, sex, and BMI difference between hypercortisolemic patients and the others, either.

Of 10 patients who have been operated on because of the large tumor size or subclinical hypercortisolism, the histopathological results are as follows: four adrenocortical adenoma (including our three SCS cases), four myelipoma, one adrenocortical hyperplasia, and one adrenal cortical carcinoma.

Among those operated on patients with NFA, two were hypertensive and three were diabetic. No change in their metabolic status, physical findings, or biochemical parameters occurred in the follow-up period. Cortisol suppression has been recovered postoperatively in all three patients with SCS who underwent unilateral adrenalectomy. One of them developed adrenal insufficiency after unilateral adrenalectomy.

Table 4. Biochemical and metabolic bone parameters of patients with subclinical Cushing’s syndrome and patients with nonfunctional adrenal masses

<table>
<thead>
<tr>
<th></th>
<th>Nonfunctional adrenal incidentaloma cases (n = 66)</th>
<th>Subclinical Cushing’s syndrome cases (n = 4)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose</td>
<td>108.25 ± 31.17 (mg/dl)</td>
<td>113.00 ± 17.72 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>205.34 ± 43.12 (mg/dl)</td>
<td>225.75 ± 51.64 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>49.03 ± 12.20 (mg/dl)</td>
<td>44.50 ± 18.00 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>124.46 ± 34.89 (mg/dl)</td>
<td>148.25 ± 39.30 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>151.15 ± 74.36 (mg/dl)</td>
<td>165.00 ± 38.81 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.32 ± 0.59 (mg/dl)</td>
<td>9.57 ± 0.80 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.62 ± 0.71 (mg/dl)</td>
<td>2.80 ± 0.85 (mg/dl)</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>77.96 ± 32.49 (U/L)</td>
<td>99.00 ± 32.79 (U/L)</td>
<td>NS</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>35.45 ± 25.16 (pg/ml)</td>
<td>55.30 ± 42.83 (pg/ml)</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamine D</td>
<td>41.18 ± 19.95 (ng/ml)</td>
<td>19.95 ± 2.75 (ng/ml)</td>
<td>NS</td>
</tr>
<tr>
<td>Lumbar L2-L4 BMD</td>
<td>0.97 ± 0.17 (g/cm²)</td>
<td>0.95 ± 0.10 (g/cm²)</td>
<td>NS</td>
</tr>
<tr>
<td>Lumbar L2-L4, Z-score</td>
<td>0.032 ± 1.2</td>
<td>0.22 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Lumbar L2-L4, T-score</td>
<td>−0.97 ± 1.54</td>
<td>−1.16 ± 0.99</td>
<td>NS</td>
</tr>
<tr>
<td>Femoral neck BMD</td>
<td>0.81 ± 0.18 (g/cm²)</td>
<td>0.76 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Femoral neck, Z-score</td>
<td>0.67 ± 0.96</td>
<td>0.13 ± 1.39</td>
<td>NS</td>
</tr>
<tr>
<td>Femoral neck, T-score</td>
<td>−0.35 ± 1.23</td>
<td>−1.41 ± 2.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mann Whitney U test, NS: Not significant

Fig. 1. Comparison of hormonal levels in patients with subclinical Cushing’s syndrome and in patients with nonfunctional adrenal masses.
Mann Whitney U test, *p<0.001, **p<0.01, ***p<0.05
SCS: Subclinical Cushing’s syndrome, NFA: Nonfunctional adrenal adenoma
DHEA-S: Dehydroepiandrosterone sulfate, UFC: Urinary free cortisol, MC: Midnight cortisol, C: Morning cortisol
which lasted for six months (patient 4). She had to take glucocorticoid replacement in that period. Improvement in blood pressure occurred in two of them (patients 3 and 4), and we stopped anti-hypertensive drug in one of them (patient 3). The other patient taking anti-hypertensive medication has a better blood pressure and she is now normotensive with valsartan 160 mg/day. She had hypertension for about eight years before the adrenal surgery. Change in body weight was observed in all three patients with SCS who underwent adrenalectomy. Body mass index in patient 1 went down from 36.93 kg/m$^2$ to 34 kg/m$^2$ after a period of one year following adrenalectomy. In patient 3, BMI fell to 28 kg/m$^2$ from 29 kg/m$^2$, as well. Patient 4 lost weight after withdrawal of glucocorticoid supplementation. Her BMI was 30 kg/m$^2$ before operation. On the other hand she is still overweight with a BMI of 28.3 kg/m$^2$. Of the two diabetic SCS patients, only one could be operated (patient 1). She has now a better glycemic profile on treatment with just a suitable diabetic diet.

With the correlation analyses, we found that there were positive correlations between midnight cortisol and morning cortisol ($r = 0.463$, $p<0.001$), and 3 mg DXM suppression test ($r = 0.392$, $p<0.001$), and UFC ($r = 0.301$, $p<0.05$). So patients with higher midnight cortisol levels tended to have higher morning cortisol and UFC levels. In contrast, plasma cortisol levels following 3 mg DXM administration was inversely correlated with ACTH ($r = -0.382$, $p<0.001$) and DHEA-S ($r = -0.355$, $p<0.01$). This may reflect the low ACTH and DHEA-S levels in patients with SCS. We have also found a significant negative correlation between the results of DXM suppression test and the diurnal cortisol rhythm ($r = -0.784$, $p<0.001$, in the correlation between diurnal rhythm and 3 mg DXM test; $r = -0.847$, $p<0.001$, in the correlation between diurnal rhythm and 8 mg DXM test). So, patients who were not suppressed by 3 mg DXM, and by 8 mg DXM, including the patients with SCS, tended to have blunted diurnal cortisol rhythm.

**Discussion**

Incidentally detected adrenal masses constitute a clinical concern because of their increasing frequency. Most of them are benign and nonfunctional masses. However, careful diagnostic assessment of adrenal incidentalomas must be performed to exclude the presence of malignant and/or functioning lesions [37]. Recent studies have reported that some of these tumors have subtle autonomous cortisol secretion without signs and symptoms of Cushing’s syndrome which is called as subclinical Cushing’s syndrome. Unfortunately, there are conflicting data whether the patients with SCS have increased morbidity and mortality as seen in classic Cushing’s syndrome or not. Another difficult question is raised about the selection of the optimal management of these patients [2, 4, 24, 25, 38–40].

In the current study, we evaluated the clinical and biochemical picture of 70 patients with adrenal incidentaloma. We also determined the frequency of SCS in this patient population. Afterwards the clinical, biochemical and hormonal features of the patients with SCS were compared to those of other patients with adrenal incidentaloma.

Previous studies reported an occurrence of SCS that ranged 5–20% [6–17]. Hensen et al. found cortisol-producing adenomas in 6% of all incidentally detected adrenal tumors [6], whereas McLeod et al. reported a 5% prevalence cortisol-producing tumors in 122 patients with adrenal incidentaloma [7]. Autonomy of cortisol secretion shows significant diversity, and the frequency and the clinical significance of subclinical Cushing’s syndrome may be affected by its definition. This might explain the different frequencies in different studies, as there is yet no established criteria for the unquestionable diagnosis of SCS. However, we have evaluated the HPA axis of our 70 patients with adrenal incidentaloma to find out the real degree of prevalence of SCS among them.

In our study, prevalence of SCS was found to be 5.7%. This result is within the previously reported range. An important observation we have come up with is that in only 1/3 of nonsuppressors in the overnight 3 mg DXM test, autonomous cortisol hypersecretion was detected. Eight out of 12 nonsuppressed patients by 3 mg DXM showed adequate suppression after 8 mg DXM test. HPA axis of those patients was evaluated individually and it was noticeable that diurnal cortisol rhythm was intact in all of them, as shown in Table 3. So, they could not be easily accepted to have autonomous cortisol secretion. Overnight DXM suppression has been quoted as the best parameter for evaluating the presence of subclinical hypercortisolism [16]. In addition, our results confirm that blunted diur-
nal variation of cortisol secretion with high midnight cortisol levels, low or suppressed plasma ACTH, and low DHEA-S concentrations are also valuable findings for the diagnosis of SCS, since all four patients with SCS had those findings in our study. So, not only the overnight low dose DXM suppression test, but also some other findings like blunted diurnal cortisol rhythm, low plasma ACTH and DHEA-S levels, and high midnight cortisol levels must be considered in the establishment of the diagnosis of subclinical hypercortisolism. On the contrary, high 24-h UFC was found in only 1/4 of patients with SCS. This is not surprising, because increased UFC is generally a late finding in Cushing’s syndrome and it is less useful as a screening test [8, 24].

In our patient group, among the ones whose plasma cortisol levels were suppressed by 3 mg DXM, there was only one patient who did not show normal cortisol diurnal rhythm with a morning cortisol level of 9.87 μg/dl and a midnight cortisol level of 7.48 μg/dl. To the contrary, plasma cortisol level after 3 mg DXM suppression was found to be 1.5 μg/dl, for that patient. Besides, neither ACTH nor DHEA-S levels were lower than expected normal ranges in that patient. She had no signs or symptoms of hypercortisolism, either. So we did not accept her as a patient with a weak but autonomous cortisol secretion. Comparing the HPA axis of the patients with SCS and the patients who failed to suppress plasma cortisol under 3 μg/dl following 3 mg DXM but showed adequate suppression after 8 mg DXM, and the patients who suppressed plasma cortisol under desirable value following 3 mg DXM test, we realized that mean midnight cortisol levels and mean plasma cortisol levels after 3 mg DXM test were also significantly higher in patients with SCS than the patients suppressed by 3 mg DXM and the patients not suppressed by 3 mg DXM but suppressed by 8 mg DXM.

Similar to previous reports [37, 41], the mean plasma ACTH and DHEA-S levels are significantly lower and the mean midnight cortisol level is significantly higher in our cases with SCS as compared to the patients with NFA. One of our patients with SCS (patient 1) suppressed plasma cortisol under desirable level following 8 mg DXM. However, suppression in 24-h UFC was not observed in that patient, since we expected suppression in 24-h UFC level below 50% of the baseline. Failure in the suppression of 24-h UFC below the 90% of the baseline value in high dose 8 mg DXM testing, is in favour of autonomous hypercortisolism [42]. Although, 34 μg/day of 24-h UFC after 8 mg DXM seems to be low, it is not a suppressed value regarding the basal 24-h UFC level of that patient. Besides, the low ACTH level with the high midnight cortisol level, and the very low DHEA-S level all agreed with SCS in that patient. Moreover, the diurnal cortisol rhythm was absent in the same patient. Midnight cortisol is accepted as a very sensitive and predictive test for the evaluation of hypercortisolism. Midnight cortisol levels above 7.5 μg/dl have been taken as a specific criteria for the establishment of the diagnosis of Cushing’s syndrome, and they may distinguish patients with Cushing’s syndrome from pseudo-Cushing’s states [43, 44]. Patient 1 had a high midnight cortisol level of 8.79 μg/dl.

The comparison of the patients with NFA with those with SCS yielded no significant differences for age, sex, and BMI. Comparison of the biochemical and metabolic bone parameters of patients with SCS and patients with NFA revealed no significant difference, as well. Though hyperlipidemia, at least hypertriglycerideremia is expected in hypercortisolism and also in SCS [37], no difference in the lipid pattern has been observed in patients with SCS. Despite the abnormal biochemical markers of bone turnover in patients with SCS documented by previous studies [45], the BMD data, and Z-scores or T-scores are consistent with the lack of significant bone loss in patients with SCS. Only one patient with SCS has Z-score under −1 and T-score under −2.5 standard deviation at her femoral neck and that patient is a 69 year old postmenopausal women (patient 1). In the study of Torlontano et al., PTH levels were found to be higher in patients with SCS than in patients with NFA, but other bone turnover parameters like alkaline phosphatase, serum calcium and phosphate levels were not significantly different [45], and so resemble our results. However, due to the small number of observations, these results have to be considered with caution.

Arterial hypertension, obesity and diabetes mellitus are significantly more prevalent in patients with incidentally detected adrenal masses than in the general population. Reporting for the Adrenal Incidentaloma National Italian Study Group, Angeli et al. found that of 887 patients, 42% were hypertensive, 28% were obese, and 10% were diabetic [46]. In another study,
those respective prevalences were found to be 46%, 36%, and 21% for hypertension, obesity, and diabetes mellitus [8]. Patients with SCS were having similar or slightly higher prevalences of hypertension, obesity, and diabetes mellitus. The prevalences of hypertension, obesity, and diabetes mellitus are higher in our cases of SCS and adrenal incidentaloma in general. The respective prevalences of hypertension, obesity, and diabetes mellitus are 70% (49 of 70 patients), 35.7% (25 of 70 patients), and 34.3% (24 of 70 cases) in our adrenal incidentaloma cases, including our SCS cases. On the other hand, in a recent, cross-sectional, population-based survey, including 24788 subjects over 20 years of age, held in Turkey, the prevalences of hypertension, obesity, and diabetes were found to be 29%, 22%, and 7.2%, respectively [47]. Those prevalences are significantly lower than the prevalences of our patients with SCS and our patients with NFA. The respective prevalences of hypertension (100%), obesity (75%), and diabetes mellitus (50%) are much more higher among our SCS cases, and the differences reached statistical significance when compared with the prevalences in general population (for hypertension $p = 0.007$, for obesity $p = 0.036$, for diabetes $p = 0.028$). In addition, the differences of the prevalences of hypertension, obesity, and diabetes are also significantly higher in patients with NFA than in the general Turkish population (for hypertension 68.18% vs 29%, $p<0.001$; for obesity 33.33% vs 22%, $p<0.05$; for diabetes 33.33% vs 7.2%, $p<0.001$). On the contrary, those respective prevalences did not reach statistical significance between patients with SCS and patients with NFA, probably because of the small number of the patients in SCS group. If only the number of patients with SCS were higher, it could have been possible to find out significant differences for hypertension, obesity and diabetes prevalences among patients with SCS when compared with the patients with NFA, in our opinion. The high prevalences of hypertension, obesity and diabetes mellitus in patients with SCS might suggest that clinically silent hypercortisolism is probably not completely asymptomatic.

Correlation analyses did not show any significant correlation between the clinical presentation and the hormonal or biochemical parameters of patients with adrenal incidentaloma. Midnight cortisol levels were positively correlated with morning cortisol levels, UFC and low dose DXM suppression test. Moreover, mean plasma cortisol level following low dose overnight DXM test was inversely correlated with DHEA-S and ACTH levels. This may explain the higher midnight cortisol, and lower DHEA-S and ACTH levels in patients with SCS, because all these findings are also the expected hormonal findings in SCS.

None of the patients with NFA showed adrenal hyperfunction or tumor growth in the follow-up period. So none of them had to be operated on. Of 10 patients who have been operated on because of the large tumor size or subclinical hypercortisolism, only one of the patients with SCS developed adrenocortical insufficiency following surgery which lasted for about six months (patient 4). She needed glucocorticoid replacement in that period. She has not been on glucocorticoid replacement anymore and her HPA axis recovered now. Unfortunately, one of the patients with SCS could not be operated on due to her inappropriate cardiovascular status (patient 2).

Adrenalectomy should be recommended for masses greater than 4 cm in size because of the increased risk of malignancy [48, 49]. We also prefer adrenalectomy for the nonfunctional adrenal incidentalomas greater than 4 cm in diameter. Other nonfunctional incidentalomas less than 4 cm in size are followed clinically and radiographically. Of our seven patients who underwent adrenal surgery because of the large tumor size (range between 4 to 10 cm in diameter), only one was diagnosed as adrenocortical carcinoma and the tumour size was $6.5 \times 6.0 \times 3.0$ cm in that case.

Recovery of cortisol suppression was determined postoperatively in all three patients with SCS who underwent unilateral adrenalectomy. Besides body weight decreased in all three, blood pressure improved in two of the three, and glycemic regulation improved in the diabetic patient postoperatively, in patients with SCS who underwent adrenalectomy. All these results are in favour of the possible useful effects of adrenalectomy in patients with SCS.

In conclusion, the prevalence of SCS is 5.7% among our 70 cases of adrenal incidentaloma. Although patients with SCS do not have the classical clinical signs or symptoms of Cushing’s syndrome, some findings can be elicited with them that may stand for clues of cortisol hypersecretion such as suppressed ACTH levels, low DHEA-S levels, and high midnight cortisol levels. Though the low dose overnight DXM suppression test can be used as a screening test, it is
singly insufficient for a final diagnosis. Since patients frequently have risk factors for atherosclerosis such as hypertension, diabetes mellitus, and obesity, surgical management of SCS with adrenalectomy may offer an advantage. Patients undergoing adrenalectomy should be followed for the development of adrenal insufficiency.

References


