Clinical significance of screening for subclinical Cushing’s disease in patients with pituitary tumors

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Abstract. Cushing’s syndrome (CS) is a clinical state caused by chronic excess of glucocorticoid, and results in hypertension, impaired glucose tolerance, and dyslipidemia. Recently, a mild state of pituitary CS without typical Cushingoid appearance (subclinical Cushing’s disease; SCD) has been identified. However, the true prevalence of SCD and its effect on metabolic disorders remain obscure. The aim of this prospective study was to determine the prevalence of SCD according to the guideline proposed by the working group of the Japanese Ministry of Health, Welfare and Labor, and to assess the outcome of surgery on metabolic disorders. The prevalence of SCD was investigated in 105 consecutive patients diagnosed with pituitary adenomas by MRI. ACTH-dependent hypercortisolism was diagnosed based on the results of the 0.5 mg dexamethasone suppression test (serum cortisol >3.0 μg/dL) plus one positive finding of the following two tests: midnight serum cortisol level >5.0 μg/dL or ACTH increase >50% after 1-deamino-5-D-arginine vasopressin (DDAVP) challenge. The final diagnosis of SCD was established by positive staining for ACTH in surgically-excised pituitary adenoma. Three patients (4.8%) were diagnosed with SCD among 62 patients with pituitary adenoma. Transsphenoidal adenomectomy partially resulted in improvement of blood pressure and glucose metabolism in SCD patients. Our results emphasize the importance of SCD screening in patients with pituitary tumors, especially in those patients with metabolic disorders.

Key words: Subclinical Cushing’s disease, ACTH-dependent hypercortisolism, Prevalence, Metabolic disorders

CUSHING’S SYNDROME (CS), a clinical state caused by chronic excess of glucocorticoid (GC) [1], is divided into adrenocorticotropic hormone (ACTH)-dependent CS, such as ACTH-producing pituitary adenoma [Cushing’s disease (CD)] and ACTH-independent CS, such as cortisol-producing adrenal tumors [2, 3]. The clinical features of CS include central obesity, moon face, hirsutism, and plethora [4, 5]. GC excess in CS results in hypertension, impaired glucose tolerance, dyslipidemia, osteoporosis, depression and cognitive impairment [5, 6].

In addition to the classical overt CS, mild state of adrenal CS (subclinical Cushing’s syndrome; SCS) [7] and that of CD (SCD) [8] have been identified in recent years. These subclinical types are defined as conditions lacking typical physical features of CS, while harboring autonomy in cortisol and/or ACTH secretion. Among the subclinical types, SCS was reported to be associated with high prevalence of risk factors for cardiovascular diseases, such as hypertension, hyperglycemia, hyperinsulinemia, dyslipidemia and atherosclerotic plaques [9]. Previous case reports described partial or total reversal of metabolism disorders following surgical treatment of SCS [10, 11]. These studies warrant screening for hypercortisolism in patients with adrenal tumor but without typical physical features of CS.

With regard to SCD, the working group of the Japanese Ministry of Health, Welfare and Labor, proposed in 2006 a guideline on the diagnosis of SCD (Revised in 2010) (Table 1) [12]. Currently, the diagnosis of SCD in patients with pituitary tumor is established according to this guideline. However, the prev-
Table 1 Criteria for the diagnosis of subclinical Cushing’s disease according to the Ministry of Health, Labour, and Welfare, Japan (2010).

<table>
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<th>Step</th>
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| 1. Suspicion of SCD | (1) Suspicion or presence of pituitary lesion on MRI.  
(2) Normal–high plasma ACTH with normal morning cortisol level.  
(3) Absence of typical Cushingoid appearance (moon face, central obesity or dorsocervical fat pad (buffalo hump): purple striae, thin skin and easy bruising, proximal myopathy. In children, decrease in weight gain rate with obesity).  
Perform screening tests when (1), (2) and (3) are positive. |
| 2. Screening tests | (1) Incomplete suppression of plasma cortisol level (>3 μg/dL) in low-dose (0.5 mg) overnight dexamethasone suppression test (DST).  
(2) High plasma cortisol level (>5 μg/dL) during nocturnal sleep.  
(3) Response of plasma ACTH level to desmopressin (DDAVP) test (>50% increase in plasma ACTH level after test).  
(4) High salivary cortisol level (>1.5, compared with mean level for the hospital) during nocturnal sleep.  
Perform differential diagnosis when (1) and one other test are positive. |
| 3. Differential diagnosis of Cushing’s disease from ectopic ACTH syndrome | (1) Suppression of plasma cortisol level (<50%, compared with basal level) following high-dose (8 mg) overnight DST.  
(2) Normal or exaggerated plasma ACTH response to human CRH test (>50% increase in plasma ACTH level after the test).  
(3) Presence of pituitary adenoma on MRI.  
(4) Positive results in a selective sinus sampling test. |

Diagnostic Criteria  
Reliable cases: Meeting 1, 2 and 3(1), (2), (3) and (4).  
Almost reliable cases: Meeting 1, 2 and 3(1), (2) and (3).  
Suspected cases: Meeting 1 and 2.

Subjects
We recruited patients with solitary pituitary tumors scheduled for surgery and admitted to our department between January 2012 and December 2014. Each patient was contacted and informed about the purpose of the SCD screening test. Patients diagnosed preoperatively with lesions other than pituitary adenomas by magnetic resonance imaging (MRI) were excluded from the screening test, such as patients with Rathke’s cleft cyst, craniopharyngioma, meningioma and hemangioma. The screening test was conducted according to the guideline by the other 105 consecutive patients (males, 53; females, 52) with MRI-based diagnosis of pituitary adenomas. These 105 patients included patients with functional pituitary adenoma, such as overt CS, growth hormone-secreting pituitary adenoma and prolactin-secreting pituitary adenoma. These patients were of a median age of 50 years (range, 21-87), body mass index (BMI) of 22.7 kg/m² (16.4-48.8) and pituitary tumor size of 19 mm (range, 2-44).

The study protocol was approved by the Human Ethics Committee of Osaka University (no. 15025) and conformed to the Declaration of Helsinki.

Diagnosis of SCD
The guideline used for the diagnosis of SCD is shown in Table 1. Based on this guideline, the diagnosis of SCD was established through three steps. The diagnostic protocol did not restrict screening the subjects for non-functional pituitary adenoma. Therefore, the screening test for SCD was performed in those patients with suspected pituitary adenomas who showed evidence of hormonal activity (e.g., growth hormone-secreting pituitary adenoma and prolactin-secreting pituitary adenoma). The prevalence of SCD was evaluated in all patients with pituitary tumors, and in patients who were excluded for apparently functional adenoma and tumors other than adenoma.

Step 1. Patients with suspected diagnosis of SCD
The presence of pituitary adenoma was detected on MRI in all patients prior to hospitalization. Plasma ACTH and serum cortisol were measured in the early morning after overnight fast. Typical Cushingoid features, such as moon face, central obesity or dorsocervi-
Screening for subclinical CD

Step 2. Screening test

The low-dose dexamethasone suppression test (DST) was performed using the following protocol: 0.5 mg dexamethasone was administered at 2300 h, and blood sample was collected next morning for measurement of serum cortisol level. The normal response to 0.5 mg DST was considered suppression of serum cortisol to less than 3.0 μg/dL. The midnight cortisol sample was collected at 2300 h, and the normal serum cortisol level at midnight was considered less than 5.0 μg/dL.

The desmopressin (DDAVP) test was performed using the following protocol: DDAVP (4 μg) was injected intravenously and blood samples were taken before and 30, 60, 90, 120 minutes after injection. Any increase in ACTH after DDAVP administration of >50% was considered paradoxical response. Salivary cortisol level was not evaluated in our hospital. Patients with abnormal results of low-dose DST and one or more other tests were suspected to have either SCD or ectopic ACTH syndrome (EAS), and underwent further testing to establish the correct diagnosis (Step 3).

Step 3. Differential diagnosis of Cushing’s disease from EAS

SCD was differentiated from EAS by three tests; the corticotropin-releasing hormone (CRH) test, high-dose (8 mg) dexamethasone suppression test and selective sinus sampling test. However, pituitary adenoma was detected on MRI in all our patients, and all decided to receive transsphenoidal adenomectomy at admission. For these reason, SCD was diagnosed in this study by histopathological examination of the surgically excised lesions and confirmation by immunohistochemical staining for ACTH.

Hormonal assays

Plasma ACTH was measured by electrochemiluminescence Immunoassay (ECLusys ACTH kit, Roche Diagnostics, Tokyo, Japan). Serum cortisol was measured by chemiluminescent enzyme immunoassay (Access cortisol kit, Beckman Coulter, Tokyo, Japan). This kit has a sensitivity of 0.4 μg/dL, with an intra-assay coefficient of variation (CV) of <4.3% and an inter-assay CV of <5.9%.

Results

Step 1. The case who was suspected the presence of SCD

Out of the 105 patients, low plasma ACTH level was detected in 1 patient and low serum cortisol in 8 patients. Furthermore, typical Cushingoid features were identified in 2 patients, and were subsequently diagnosed with overt Cushing’s disease (Fig. 1). The remaining 94 patients underwent the screening tests.

Step 2. Screening tests

Of the 94 patients, 24 (25.5 %) showed incomplete suppression of plasma cortisol level after overnight 0.5 mg DST, and accordingly underwent further tests (midnight serum cortisol level and DDAVP test). Based on the results of these tests, 10 patients were diagnosed to have ACTH-dependent hypercortisolism.

Step 3. Differential diagnosis of Cushing’s disease from EAS

The above 10 patients underwent transsphenoidal adenomectomy. The surgically-excised adenomatous lesions were examined histopathologically and immunohistochemically, and were confirmed to be ACTH-positive in 3 patients (2 females and 1 male) and ACTH-negative in 6 patients (3 females and 3 males). The last patient was diagnosed Rathke’s cleft cyst but not pituitary adenoma.

Prevalence of SCD in pituitary adenomas

By our screening strategy, we detected 3 SCD patients (2.9%) among 105 patients with pituitary tumors (Table 2). These patients did not have the comorbidity of the other functional adenomas. After the exclusion of patients with functional adenomas (2 overt CS, 17 growth hormone-secreting pituitary adenomas and 15 prolactin-secreting pituitary adenomas), 8 patients with Rathke’s cleft cysts and 1 patient with chordoma, the estimated prevalence of SCD was 4.8% (3/62) among patients with other pituitary adenoma.

Surgical outcome of the three patients with SCD

The three SCD patients were treated with a standard regimen of hydrocortisone replacement within two weeks after surgery. None developed symptoms of adrenal insufficiency after withdrawal of hydrocortisone treatment. To confirm the remission of endogenous hypercortisolism, we repeated the 0.5 mg DST after treatment with hydrocortisone. All three patients
showed normal cortisol response (serum cortisol: Patient 1: 1.0 μg/dL, Patient 2: 2.8 μg/dL, Patient 3: 1.4 μg/dL).

We also evaluated the change in metabolic disorders after surgery (Table 3). The metabolic parameters were measured before and at 2 months after surgery. Body mass index (BMI) did not change in all patients. Blood pressure after surgery improved in Patients 1 and 2, but no change was noted in Patient 3. Hemoglobin A1c (HbA1c) improved after surgery in the two diabetic patients (Patients 1 and 2), whereas no change after surgery was noted in the normoglycemic patient (Patient 3). With respect to dyslipidemia, LDL cholesterol improved in two patients (Patients 1 and 3) whereas Patient 2 had already been treated with statin after surgery. No consistent changes were noted in triglyceride and HDL cholesterol.

![Diagram](image_url)

**Table 2** Prevalence of SCD in patients with pituitary tumors and pituitary adenomas excluding patients with apparently functional adenomas.

<table>
<thead>
<tr>
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<th>All patients with pituitary tumors (n=105)</th>
<th>Exclusion of patients with apparently functional adenomas (n=62)</th>
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<tbody>
<tr>
<td>SCD, n=3</td>
<td>2.9 %</td>
<td>4.8 %</td>
</tr>
<tr>
<td>NFA, n=59</td>
<td>56.2 %</td>
<td>95.2 %</td>
</tr>
<tr>
<td>CD, n=2</td>
<td>1.9 %</td>
<td>–</td>
</tr>
<tr>
<td>GHoma, n=17</td>
<td>16.2 %</td>
<td>–</td>
</tr>
<tr>
<td>PRLoma, n=15</td>
<td>14.3 %</td>
<td>–</td>
</tr>
<tr>
<td>Rathke’s cleft cyst, n=8</td>
<td>7.6 %</td>
<td>–</td>
</tr>
<tr>
<td>Chordoma, n=1</td>
<td>1.0 %</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 2** *SCD, Subclinical Cushing disease; NFA, Non-functioning pituitary adenoma; CD, Cushing disease; GHoma, Growth hormone-secreting pituitary adenoma; PRLoma, Prolactin-secreting pituitary adenoma.*
Discussion

In this prospective study, we investigated the presence of SCD in a group of consecutive and non-selected cohort of patients with pituitary tumors, and found the estimated prevalence of SCD to be 2.9% (or 4.8% after excluding apparently functional adenoma and tumors other than adenoma). Furthermore, we evaluated the changes in metabolic disorders in SCD patient after surgery, and demonstrated that treatment of SCD improved blood pressure and glucose metabolism.

Toini et al. [13] reported recently that the prevalence of subclinical-mild ACTH-dependent hypercortisolism (AH) in patients with pituitary incidentaloma was about 5% (histologically confirmed AH, 4.4% and biochemically diagnosed AH, 7.3%). They defined subclinical-mild AH by criteria similar to those of the Japanese guideline [12]. The prevalence of SCD in our cohort is similar to those estimated in the above study. Several studies have examined previously the prevalence of SCS in patients with adrenal incidentaloma (AI) [14-17]. The estimated prevalence of SCS was usually less than 10% (range, 6–23%) in patients with AI. At first glance, the prevalence of SCD in pituitary adenomas seems to be lower than that of SCS in AI. However, the diagnosis of SCD in our study was confirmed by endocrinological and histopathological examinations, whereas the diagnosis of SCS in previous studies was confirmed by endocrinological methods only. Thus, the difference in the prevalence of SCD and SCS might be due to differences in the methods of diagnosis. In the present study, the prevalence of SCD was evaluated in patients who were confirmed the presence of pituitary tumor on MRI and scheduled for surgery. Thus, 84% of the subjects (88 of 115 patients) and 97% of the subjects excluding those with functioning tumors (60 of 62 patients) had macroadenomas. In other words, our results reflect the prevalence of SCD in macroadenomas, whereas the prevalence of whole SCD remains unclear including patients with microadenomas or suspicion of pituitary lesion on MRI.

In the screening tests (Step 2) 10 patients were diagnosed to have ACTH-dependent hypercortisolism. However 7 of 10 patients were excluded from the diagnosis of SCD. The reason is that one patient was suffering from depression causing the pseudo-Cushing’s syndrome [12] and the rest 6 patients have taken the medicines that were reported to affect dexamethasone metabolism (e.g., atorvastatin, clonazepam or terbinafine) [18]. The condition described above might con-
In this study, we evaluated the outcome of surgery for SCD on metabolism disorders. Transphenoidal adenomectomy improved blood pressure and glucose metabolism in two of the three patients. Taniguchi et al. [19] screened 77 hospitalized patients with diabetes mellitus for subclinical hypercortisolism and detected SCD in 2 of these patients. The metabolic clearance rate of glucose was evaluated in these patients by the glucose clamp test, an index of insulin sensitivity, which showed significant improvement after surgery. In addition, the blood pressure of the two patients decreased after surgery. Considered together with our results, we assume that surgical excision of the pituitary adenoma in SCD patients with hypertension or/and impaired glucose tolerance can result in improvement of metabolic disorders. However, further prospective studies of larger number of patients are needed to confirm our findings in the small number of SCD patients.

In conclusion, the present study demonstrated a prevalence of SCD of 4.8% in patients with pituitary adenomas, and that transphenoidal adenomectomy partially resulted in improvement of blood pressure and glucose metabolism. Our results advocate the importance of SCD screening in patients with pituitary tumors, especially those with metabolic disorders.

**Disclosure**

The authors declare no conflict of interest.

### References