Development and Validation of a 0.5 mg Dexamethasone Suppression Test as an Initial Screening Test for the Diagnosis of ACTH-dependent Cushing’s Syndrome

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Abstract. For the diagnosis of Cushing’s syndrome (CS), the overnight 1 mg dexamethasone suppression test (DST) has been widely used as a standard low-dose DST. However, it is evident that 1 mg DST may not be sensitive enough to detect CS when the cortisol cut-off concentration is 5 µg/dL. Therefore, we developed and validated 0.5 mg DST as a new screening method for diagnosis of ACTH-dependent CS. To compare 0.5 mg DST with 1 mg DST, 110 patients with ACTH-dependent CS were enrolled, including 88 with Cushing’s disease (CD), 8 with subclinical CD and 14 with ectopic ACTH syndrome, as well as 134 control subjects. Subjects were given either 0.5 mg or 1 mg dexamethasone orally at 23:00 on different days, with blood samples collected the following morning between 8:00 and 9:00 to determine plasma cortisol concentration. The area under the receiver operator characteristics curve observing the 0.5 mg DST was higher than that of the 1 mg DST. The most sensitive and specific cut-off value of plasma cortisol concentration using 0.5 mg DST was found to be 3.05 µg/dL with 99.1% sensitivity and 98.4% specificity, identical to the 3 µg/dL cut-off currently used in the Japanese guideline for diagnosis of subclinical CD. In conclusion, 0.5 mg DST is a sensitive and specific screening test for diagnosis of ACTH-dependent CS. We recommend 0.5 mg DST with a cortisol cut-off concentration of 3 µg/dL to be used as the initial step in diagnosing ACTH-dependent CS.

Key words: ACTH-dependent Cushing’s syndrome, Cushing’s disease, Dexamethasone suppression test, Screening

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from East and Southeast Asia are leaner than those in Western countries, thus it has been suggested that the 1 mg LDDST may be too strong in suppressing plasma cortisol concentration in Japanese patients. In recent years, patients with subclinical CS have been diagnosed as both ACTH-independent [8-11] and -dependent [12-14]. In these patients, plasma cortisol concentration is easily suppressed by DST as their levels of excess cortisol are only mild. Therefore, we attempted to develop and validate a 0.5 mg LDDST to provide a more reliable and sensitive screening test for patients potentially suffering from ACTH-dependent CS.

Subjects and Methods

Patients and controls

We evaluated 110 patients diagnosed with ACTH-dependent CS including overt CD, subclinical CD and EAS, as well as 134 controls including healthy volunteers, patients with type 2 diabetes mellitus (DM), essential hypertension and simple obesity in four independent University Hospitals (Table 1). All patients with overt CD and subclinical CD had transsphenoidal surgery (TSS) performed and their ACTH production determined by immunohistochemistry. All patients with EAS underwent surgery for original tumors and their ACTH production determined by immunohistochemistry. For the patients with type 2 DM, the LDDST was performed after hospitalization with their plasma glucose under therapeutic control. In patients with simple obesity, pituitary and adrenal disorders were denied using clinical tests including the magnetic resonance imaging and the computed tomography. None of controls had clinical signs of Cushing’s syndrome. None of participants received any medication affecting glucocorticoid metabolism, e.g. rifampicin or mitotane. The clinical profiles of patients with ACTH-dependent CS and controls are summarized in Table 2.

Study design

All patients and controls provided written consent. As a primary screening step for diagnosis of CS, 0.5 mg and 1 mg dexamethasone were administered orally at 23:00 hours on different days, and blood samples were collected after 30-min bed rest the following morning between 8:00 and 9:00 hours for determination of plasma cortisol concentration.

Hormone assays

Plasma ACTH was measured using immunoradiometric assay (ACTH IRMA kit, Mitsubishi Kagaku Iatron, Tokyo; minimum detection limit 5 pg/mL), and

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<th>Table 1. Clinical profile of patients and control subjects</th>
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<tr>
<td><strong>ACTH dependent Cushing’s syndrome</strong></td>
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<tr>
<td>Cushing’s disease</td>
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<tr>
<td>subclinical Cushing’s disease</td>
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<td>ectopic ACTH syndrome</td>
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<td><strong>controls</strong></td>
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<td>healthy volunteer</td>
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<td>essential hypertension</td>
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<td>type 2 diabetes mellitus</td>
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<td>simple obesity</td>
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<th>Table 2. Clinical profiles of controls and patients with ACTH-dependent Cushing’s syndrome</th>
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<tr>
<td><strong>controls</strong> n=134</td>
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<tr>
<td>age (y) 45.2 ± 15.7</td>
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<tr>
<td>Female/Male 47/87</td>
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<tr>
<td>body weight (kg) 75.6 ± 23.2</td>
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<td>Body mass index 28.1 ± 9.6</td>
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<td>micro/macro pituitary adenoma</td>
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<td>Basal plasma ACTH (pg/mL) 47.3 ± 28.6</td>
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<td>Basal plasma cortisol (µg/dL) 14.2 ± 5.6</td>
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<tr>
<td><strong>ACTH-dependent Cushing’s syndrome</strong> n=110</td>
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<tr>
<td>age (y) 48.8 ± 16.1</td>
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<tr>
<td>Female/Male 77/33</td>
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<tr>
<td>body weight (kg) 58.7 ± 8.7</td>
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<tr>
<td>Body mass index 23.4 ± 3.2</td>
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<td>micro/macro pituitary adenoma</td>
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<td>Basal plasma ACTH (pg/mL) 108.2 ± 82.8</td>
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<tr>
<td>Basal plasma cortisol (µg/dL) 31.2 ± 18.4</td>
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* by chi-square test, ** by unpaired t test
plasma cortisol was determined by radioimmunoassay (cortisol kit, TFB, Tokyo, Japan; minimum detection limit 1 µg/dL) at each hospital.

Statistical analysis

Values are expressed as mean ± SD. For continuous variables, difference between groups was analyzed using a two-tailed unpaired Student’s t test. For categorical variables, differences were analyzed using a chi-square test. A probability value of \( p < 0.05 \) was considered significant. Receiver operator characteristic (ROC) plots were used to determine the threshold for each treatment. All calculations were performed using Prism v. 4.0 (Graphpad software Inc., USA) software.

Results

Characteristics of patients and controls

The mean age of patients with ACTH-dependent CS and controls were 48.8 ± 16.1 and 45.2 ± 15.7 years old (\( p > 0.05 \) by unpaired t test), respectively. The body weight of controls was greater than that of patients with ACTH-dependent CS (75.6 ± 23.2 vs. 58.7 ± 8.7 kg, \( p < 0.0001 \) by unpaired t test; Table 2), because the control group included patients with simple obesity. By the same reason, the body mass index was also higher in controls than that of patients with ACTH-dependent CS (Table 2).

Plasma cortisol concentrations in patients with ACTH-dependent CS and controls at basal

Basal plasma cortisol concentrations in patients with ACTH-dependent CS were 31.2 ± 18.4 µg/dL, significantly higher than control subjects (14.2 ± 5.6 µg/dL, \( p < 0.0001 \); Table 1).

Plasma cortisol levels after 0.5 mg or 1 mg dexamethasone suppression test in controls and patients with ACTH-dependent CS.

Plasma cortisol concentrations after 0.5 and 1 mg DSTs in patients with ACTH-dependent CS were greater than control subjects (27.8 ± 19.1 vs. 1.3 ± 0.7 µg/dL, \( p < 0.0001 \), and 25.3 ± 19.2 vs. 1.1 ± 0.3 µg/dL, \( p < 0.0001 \), respectively) (Figures 1 and 2). Observing the control group, plasma cortisol concentrations after 1 mg DST were significantly lower than those after 0.5 mg DST (1.1 ± 0.3 vs. 1.3 ± 0.7 µg/dL, \( p < 0.05 \)).

Fig. 1. Plasma cortisol concentrations following the 0.5 mg DST in controls (filled circles) and patients with ACTH-dependent CS (open circles). Lines represent mean plasma cortisol concentrations. Plasma cortisol concentrations after the 0.5 mg DST in patients with ACTH-dependent CS were significantly higher than controls (\( p < 0.0001 \)).

Fig. 2. Plasma cortisol concentrations after the 1 mg DST in controls (filled circles) and patients with ACTH-dependent CS (open circles). Lines represent mean plasma cortisol concentrations. Plasma cortisol concentrations after the 1 mg DST in patients with ACTH-dependent CS were significantly higher than controls (\( p < 0.0001 \)).
under the ROC curve of 0.5 mg DST was greater than that of 1 mg DST. The most sensitive and specific cut-off value of plasma cortisol concentration for 0.5 mg and 1 mg DSTs were 3.05 and 1.70 µg/dL, respectively (Table 3). Following the 1 mg DST, the plasma cortisol concentration cut-off value was less than 1.70 µg/dL, very close to the lower assay limit of most cortisol assay kits, providing high sensitivity but low specificity. However, following the 0.5 mg DST, the plasma cortisol concentration cut-off value was 3.05 µg/dL, maintaining both high sensitivity and specificity.

**Discussion**

The LDDST as well as measuring night-time plasma cortisol concentrations are used as primary screening tests in diagnosing CS. Originally, Liddle devel-
posed a 2 mg 2-day DST using urinary excretion of 17-
hydroxycorticosteroid [15], while Nugent et al. built
on this to develop a single dose DST [16]. This test
provided the first diagnostic test for CS. Because the
radioimmunoassay for cortisol in plasma has become
readily available, the DST has now been developed
into an overnight LDDSt using 1 mg dexametha-
sone. The 1 mg DST is currently used as the standard
screening test [5], with a ‘normal’ response follow-
ing 1 mg DST to be less than 5 µg/dL plasma cortisol
concentration [3, 17]. Blenthen et al. reported that the
overnight 1 mg DST suppressed plasma cortisol con-
tent to less than 5 µg/dL in some patients with CS,
while the majority of healthy subjects had plasma
cortisol concentrations of less than 2 µg/dL [18].
Findling et al. reevaluated LDDST in patients with CS
and revealed that 18% of CD were missed when the
plasma cortisol cut-off value was 5 µg/dL after 1 mg
DST [19], a finding supported by Gorges et al. [20].
To increase the tests sensitivity, the plasma cortisol
collection cut-off value of 5 µg/dL after 1 mg DST
was reduced to 1.8 µg/dL [21]. This increased the sen-
sitivity but decreased the specificity (to less than 80%) as
was expected [1, 4, 19]. In 2008, the Endocrine
Society published a new set of clinical guidelines for
the diagnosis of CS, including both ACTH-dependent
and -independent types [7]. The guidelines recom-
end 24-h urinary free cortisol excretion, overnight
1 mg DST, or measurement of late night salivary cor-
tisol as initial tests. To achieve greater sensitive ef-
cacy, the guidelines further propose that the plasma
cortisol collection cut-off value following 1 mg DST
should be reduced to 1.8 µg/dL, as discussed above.

When the 1 mg DST was developed, some suggest-
ed that 1 mg dexamethasone might be too strong to
suppress plasma cortisol concentration in Japanese
patients with CS as people in Eastern and Southeastern
Asian countries are generally leaner than those in
Western countries. In addition, subclinical CS has
been found to be both adrenal [8-11] and pituitary [12,
13] types. Subclinical CS frequently presents glucose
intolerance [10, 22], hyperlipidemia [10], osteoporosis
[23], or hypertension [8] due to mild elevation of
cortisol secretion, without usual signs of CS. In
patients with subclinical CS, cortisol levels are usually
within the normal, healthy range in the early morning,
but display resistance to suppression by LDDST [24].
Therefore, we developed and evaluated the 0.5 mg
DST as a new primary screening test for diagnosis of
ACTH-dependent CS.

Previously, the majority of investigators used 5 µg/
dL of plasma cortisol concentration as the cut-off val-
ue to distinguish CS from non-CS individuals. Our re-
results using ROC analysis revealed that the cut-off val-
ue of plasma cortisol after 1 mg DST to be 1.70 µg/dL.
The sensitivity and specificity of our test was 96.2 and
98.1%, respectively. And the cut-off value was very
close to that suggested in the clinical guidelines pub-
lished by the Endocrine Society [7]. Therefore, our re-
results support the notion that the cut-off value of plasma
cortisol concentration should be lowered. However,
in lowering the cut-off value the plasma cortisol immu-
noassays become the limiting factor as the lowest
limit range of current immunoassays is about 1.0 µg/
dL. Furthermore, concentrations detected within the
lower range of competitive immunoassays are less re-
liable than concentrations within the ‘normal’ range.
Because of these limiting factors, it becomes apparent
that using the 0.5 mg DST with a cut-off of 3.05 µg/dL
may be more useful and reliable than using a cut-off of
1.70 µg/dL following the 1 mg DST. When using a
cut-off value of less than 1.5 µg/dL, the specificity be-
came remarkably low (88.7%). This revealed the cor-
tisol cut-off line with the greatest power was within a
very narrow range when using 1 mg DST. In contrast,
the specificity of the 0.5 mg DST was 96.1%, despite
using a cut-off value of 2.75 µg/dL, which achieved
100% sensitivity. These results suggest that the 0.5 mg
DST is useful and reliable.

As we had anticipated, plasma cortisol concentra-
tions after the 0.5 mg DST were significantly correlated
with those after the 1 mg DST. However, great prog-
nostic value may still be of importance in patients with
plasma cortisol concentrations of less than 5 µg/dL af-
after the 1 mg DST. Indeed, 4 CD patients and 5 subclin-
ical CD patients were misdiagnosed when utilizing the
plasma cortisol cut-off value of 5 µg/dL following the 1
mg DST. Furthermore, 2 CD patients and 2 subclinical
CD patients were missed when the cortisol cut-off was
at 1.70 µg/dL. Therefore, performing the 0.5 mg DST
may identify at-risk patients with greater accuracy, and
is less likely to miss patients requiring treatment.

The desmopressin test is performed to differentiate
pseudo-CS and healthy subjects from ACTH-dependent
CS patients [25, 26]. The CRH test and inferior pet-
rosal or cavernous sampling of plasma ACTH are per-
formed to distinguish CD from ectopic ACTH syn-
drome [27-32]. However, the specificities of these tests
are not 100% accurate. Therefore, to achieve an accurate and true diagnosis, the primary screening test must be not only sensitive but also specific. The current study suggests the 0.5 mg DST to be superior to the 1 mg DST. The present Japanese clinical guidelines for the diagnosis of CD outline the cut-off value of plasma cortisol concentration following the 0.5 mg DST to be 3 µg/dL. Using this cut-off the current study calculated the sensitivity and specificity to be identical to that of the guidelines. Therefore, our results support the use of 3 µg/dL as the cortisol cut-off value for the 0.5 mg DST. The determination of salivary cortisol obtained late at night has been recommended as one of the initial diagnostic tests due to its convenience. However, this test can detect overt CS but not subclinical CS [33], further supporting the necessity of the 0.5 mg DST to provide a sensitive method of diagnosing subclinical CS.

It has been suggested that plasma cortisol values are varied among cortisol assay kits [34]. In this study, we used the same assay method for plasma cortisol. The results obtained with cortisol assay kit used in this study is very similar to those obtained with liquid chromatography-tandem mass spectrometry, which is thought to be the most reliable method for the determination of cortisol. Therefore, it will be necessary to titrate the cortisol cut-off value after standardization of various cortisol assay kits. In addition, in this study, we studied using patients with type 2 DM, essential hypertension and simple obesity as controls but it may be also necessary to analyze using patients with non-functioning pituitary adenoma in the future.

The Japanese clinical guidelines for the diagnosis of subclinical ACTH-independent CS recommend using a plasma cortisol concentration of 3 µg/dL as the cut-off value following the overnight 1 mg DST. It is yet to be determined whether our current results can apply to cases of ACTH-independent CS, warranting further investigation.

In conclusion, the 0.5 mg DST is a sensitive and specific screening test for the diagnosis of ACTH-dependent CS. We recommend the 0.5 mg DST to be used with a cortisol cut-off value of 3 µg/dL as the initial step in diagnosing ACTH-dependent CS.

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