Evaluation of Diagnostic Tests for ACTH-Dependent Cushing’s Syndrome

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Abstract. We evaluated the usefulness and accuracy of diagnostic tests for adrenocorticotropic hormone (ACTH)-dependent Cushing’s syndrome, based on our experience of 88 cases, including 73 cases with Cushing’s disease, and 15 cases with ectopic ACTH syndrome (EAS). In our study, 0.5 mg of dexamethasone failed to suppress the morning cortisol secretion in 100% of cases with Cushing’s disease and EAS. Plasma ACTH levels were significantly increased by desmopressin (DDAVP) in 86% of cases with Cushing’s disease, especially in microadenomas (90%), while these levels were not affected in normal subjects. In EAS, 44% responded to DDAVP. Plasma ACTH levels were increased in response to the human corticotropin-releasing hormone (CRH) test in 100% of microadenomas and 73% of macroadenomas with Cushing’s disease, but only in 27% of cases with EAS. A high dose (8 mg) of dexamethasone suppressed the morning cortisol secretion in 89% of microadenomas with Cushing’s disease, and in 82% of all cases with Cushing’s disease, while it did in only 20% of cases with EAS. Taken together, the 0.5 mg dexamethasone suppression test (DST) and DDAVP test are considerably useful for the screening of ACTH-dependent Cushing’s syndrome. The CRH test and 8 mg DST would be effective for the diagnosis of Cushing’s disease, because our study shows a sensitivity of 81% in cases with Cushing’s disease when these tests are considered together. These data were submitted to prepare the diagnostic criteria for Cushing’s disease, suggested by the working group of the Ministry of Health, Labour, and Welfare of Japan.

Key words: ACTH, Cortisol, Cushing’s disease, Pituitary, Adrenal gland

ADRENOCORTICOTROPIC hormone (ACTH)-dependent Cushing’s syndrome results from chronic glucocorticoid excess with clinically characteristic signs and symptoms, caused by an ACTH-producing tumor [1]. Cushing’s disease is primarily caused by a pituitary ACTH-secreting tumor, while ectopic ACTH syndrome (EAS) is considered to be a result of extrapituitary ACTH-secreting tumors, such as bronchial carcinoid, thymic carcinoid, pheochromocytoma, and so on [2]. The diagnosis and differential diagnosis of ACTH-dependent Cushing’s syndrome are challenging problems in clinical endocrinology.

Although Cushing’s disease is usually recognized because of the Cushingoid appearance, with the characteristic features of central obesity, moon face, skin atrophy, purple striae and buffalo hump, and the clinical criteria are clear, a debate exists in the literature over the biochemical diagnosis of this disease [3]. Recently, the diagnostic criteria for Cushing’s disease have been reported by the Ministry of Health, Labour, and Welfare of Japan [4]. In the guideline, the following endocrinological findings were considered as diagnostic criteria: 1) The presence of a Cushingoid appearance; 2) Evidence of autonomic or abnormal secretion of ACTH, such as (a) normal-high ACTH and cortisol levels, and (b) high levels of urinary excretion of free cortisol; 3) Screening tests show (a) incomplete suppression of cortisol (> 5 µg/dl) by a low-dose (0.5 mg) overnight dexamethasone suppression test (DST), (b) high cortisol levels (> 5 µg/dl) during night time sleeping, and (c) response of plasma ACTH levels to the desmopressin (DDAVP) test; 4) The dif-
Differential diagnosis of the Cushing’s disease from EAS shows (a) normal or exaggerated response of plasma ACTH levels to the human corticotropin-releasing hormone (hCRH) test, (b) suppression of cortisol (less than half, compared with the basal level) by high-dose (8 mg) overnight DST, (c) the presence of a pituitary adenoma by magnetic resonance imaging (MRI), and (d) positive results by a selective venous sampling test. DST and CRH tests are well known for the differential diagnosis of Cushing’s disease [3, 5]. ACTH release would be caused by an abnormal increase in vasopressin type 1b receptors in response to a vasopressin type 2 receptor agonist, DDAVP, on pituitary corticotroph adenoma cells [6], and has been used for the differential diagnosis of ACTH-dependent Cushing’s syndrome [7]. Nevertheless, the data in Cushing’s disease has been known to overlap with some cases of EAS [2]. In discussing the present diagnostic criteria, we refer to our extensive data.

In the present study, we evaluated the usefulness and accuracy of the DST, the hCRH test, and the DDAVP test for the screening and differential diagnosis of 88 cases with ACTH-dependent Cushing’s syndrome, including 73 cases with Cushing’s disease, and 15 cases with EAS.

Subjects and Methods

Subjects

From 1978 to 2008, we encountered 88 cases of ACTH-dependent Cushing’s syndrome at Tokyo Women’s Medical University Hospital and Hirosaki University School of Medicine & Hospital. The DST, hCRH test, and DDAVP test were examined to evaluate ACTH secretory dynamics, and 73 cases were diagnosed as Cushing’s disease (15 males and 58 females; ages 35.3 ± 1.5) due to pituitary adenomas and 15 cases as EAS (8 males and 7 females; ages 40.2 ± 4.1), and were confirmed after the operations. Informed consent was obtained before commencing the study, and all tests were performed according to clinical guidelines.

Diagnostic tests in ACTH-dependent Cushing’s syndrome

Dexamethasone suppression test (DST)

Dexamethasone (0.5, 1, or 8 mg) was administered p.o. at 23:00. Blood samples were taken at 08:00 the next morning, and then plasma cortisol levels were determined. More than 5 µg/dl of the cortisol level was considered no suppression (a positive result) by low-dose DST. Less than half the levels of cortisol, compared with the basal level, were found to be suppressed by high-dose (8 mg) DST.

Desmopressin (DDAVP) test

A single dose (4 µg) of DDAVP (Kyowa Hakko Kirin Co., Tokyo, Japan) was intravenously injected to each subject under fasting conditions. Blood samples were taken before and 30, 60, 90, and 120 min after injecting DDAVP, and then plasma ACTH levels were determined. More than a 1.5-fold increase in the ACTH level compared with the basal level was considered a significant response.

Corticotropin-releasing hormone (CRH) test

A single dose (100 µg) of hCRH (Mitsubishi Tanabe Pharma, Osaka, Japan) was intravenously injected to each subject under fasting conditions in the morning. Blood samples were taken before and 30, 60, 90, and 120 min after injecting hCRH, and then plasma ACTH levels were determined. A greater than 1.5-fold increase in the ACTH level compared with the basal level was considered a significant response.

3. Assays

Plasma cortisol levels were measured by radioimmunoassay (Amersham Pharmacia Biotech, Buckinghamshire, UK, or TFB Co., Tokyo, Japan). Plasma ACTH levels were measured by immunoradiometric assay (IRMA) using an assay kit (ACTH IRMA kit, Mitsubishi Kagaku Iatron, Tokyo, Japan).

Results

Low-dose dexamethasone suppression test (DST)

All cases with Cushing’s disease and EAS showed an incomplete suppression of plasma cortisol levels (> 5 µg/dl) after 0.5 mg of DST, but plasma cortisol levels were suppressed to < 5 µg/dl by 1 mg of dexamethasone in 3 (4%) out of 73 cases with Cushing’s disease (Fig. 1A and Table 1). In all cases with EAS, plasma
DIAGNOSTIC TESTS FOR CUSHING’S DISEASE

EAS, plasma ACTH levels were significantly increased in response to DDAVP injection in 1 case (33%) out of 3 bronchial carcinoids, and in 3 cases (50%) of an additional 6 lung cancers (Fig. 2B and Table 1).

**Corticotropin-releasing hormone (CRH) test**

Plasma ACTH levels were significantly increased following hCRH injection in all 62 cases of pituitary microadenomas (n=62) and macroadenomas (n=11) are shown as closed triangles and closed circles, respectively. (B) Ectopic ACTH syndrome. The results of the hormonal data in bronchial carcinoids (n=6) and other lung cancers (n=9) are shown as closed squares and closed circles, respectively.

Table 1. Summary of the diagnostic tests in ACTH-dependent Cushing’s syndrome.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>DST</th>
<th>DDAVP test</th>
<th>CRH test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Suppression/Total (%)</td>
<td>Suppression/Total (%)</td>
<td>Responder/Total (%)</td>
</tr>
<tr>
<td>CD</td>
<td>62/62 (100)</td>
<td>59/62 (95)</td>
<td>55/62 (89)</td>
</tr>
<tr>
<td>Macro</td>
<td>1/11 (100)</td>
<td>1/11 (100)</td>
<td>5/11 (45)</td>
</tr>
<tr>
<td>Total</td>
<td>73/73 (100)</td>
<td>70/73 (96)</td>
<td>60/73 (82)</td>
</tr>
<tr>
<td>EAS</td>
<td>6/6 (100)</td>
<td>6/6 (100)</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>Br ca</td>
<td>9/9 (100)</td>
<td>9/9 (100)</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>Others</td>
<td>9/9 (100)</td>
<td>9/9 (100)</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>15/15 (100)</td>
<td>15/15 (100)</td>
<td>3/15 (20)</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>96.0</td>
<td>82.0</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>0</td>
<td>0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

More than 5 μg/dl of the plasma cortisol level was considered no suppression (a positive result) by low-dose DST. Compared with the basal level, plasma cortisol level less than half was considered a suppressive effect induced by a high-dose (8 mg) DST, dexamethasone suppression test; DDAVP, desmopressin; CRH, corticotropin-releasing hormone; CD, Cushing’s disease; EAS, ectopic ACTH syndrome; Micro, pituitary microadenomas; Macro, pituitary macroadenomas; Br ca, bronchial carcinoids; Others, other lung cancers.

Sensitivity and specificity show the results for the diagnosis of CS from ACTH-dependent Cushing’s syndrome.

cortisol levels were not suppressed by 0.5 or 1 mg of DST (Fig. 1B, and Table 1).

**Desmopressin (DDAVP) test**

Plasma ACTH levels were significantly increased following the DDAVP injection in 19 (90%) out of 21 cases of pituitary microadenomas, but not in 1 case of macroadenomas (Fig. 2A and Table 1). In cases of EAS, plasma ACTH levels were significantly increased in response to DDAVP injection in 1 case (33%) out of 3 bronchial carcinoids, and in 3 cases (50%) of an additional 6 lung cancers (Fig. 2B and Table 1).

**Fig. 1.** Dexamethasone suppression test (DST) in ACTH-dependent Cushing’s syndrome. Changes in the plasma cortisol levels by dexamethasone are shown. (A) Cushing’s disease. The results of the hormonal data in pituitary macroadenomas (n=11) and microadenomas (n=62) are shown as closed triangles and closed circles, respectively. (B) Ectopic ACTH syndrome. The results of the hormonal data in bronchial carcinoids (n=6) and other lung cancers (n=9) are shown as closed squares and closed circles, respectively.
Desmopressin (DDAVP) test in ACTH-dependent Cushing’s syndrome. Changes in the plasma ACTH level in response to DDAVP are shown. (A) Cushing’s disease (CD). The results of the hormonal data in a pituitary macroadenoma (n=1) and microadenomas (n=21) are shown as closed triangles and closed circles, respectively. (B) Ectopic ACTH syndrome (EAS). The results of the hormonal data in bronchial carcinoids (n=3) and other lung cancers (n=6) are shown as closed squares and closed circles, respectively.

**Screening Test**
1. Incomplete suppression of plasma cortisol level (> 5 µg/dl) by low-dose (0.5 mg) overnight DST
2. High plasma cortisol level (> 5 µg/dl) during night time sleep
3. Response of plasma ACTH levels to desmopressin test

**Diagnostic Test**
1. Normal or exaggerated response of plasma ACTH levels to CRH test
2. Suppression of plasma cortisol level (less than half, compared with the basal level) by high-dose (8 mg) overnight DST
3. Presence of pituitary adenoma as determined by MRI
4. Selective venous sampling test

Screening test: (1) is essential and either (2) or (3) is requisite to positive response.
Diagnostic test: If (3) is negative, (4) is requisite to diagnosis of Cushing’s disease.
Fig. 3. CRH test in ACTH-dependent Cushing’s syndrome. Changes in the plasma ACTH level in response to CRH are shown. (A) Cushing’s disease. The results of the hormonal data in pituitary macroadenomas (n=11) and microadenomas (n=62) are shown as closed triangles and closed circles, respectively. (B) Ectopic ACTH syndrome (EAS). The results of the hormonal data in bronchial carcinoids (n=5) and other lung cancers (n=10) are shown as closed squares and closed circles, respectively.
86% (19/22) of cases with Cushing’s disease, especially in cases of microadenomas (90%). On the other hand, in cases with EAS, 44% (4/9) responded to DDAVP. A prior study also shows that 30-60% of cases with EAS responded to DDAVP [8, 9]. These results suggest that the DDAVP test is a sensitive screening test for ACTH-dependent Cushing’s syndrome, but does not discriminate it effectively from EAS. The DDAVP test or 0.5 mg DST gives priority to a high sensitivity, and is considerably useful for the screening of ACTH-dependent Cushing’s syndrome.

In a prior study, the CRH test has shown that increments of 35% and 20% above baseline ACTH and cortisol levels, respectively, produce a good result as a positive response in Cushing’s disease [10]. Kaye and Crapo suggested that the diagnostic criteria consistent with Cushing’s disease consist of an increase of 20% from the basal level in peak plasma cortisol, or an increase in peak plasma ACTH of 50% following the administration of ovine CRH [11]. When these criteria are used for the plasma ACTH responses in the differential diagnosis of Cushing’s syndrome, the test has a sensitivity of 86% and a specificity of 95%, while plasma cortisol responses give an improved sensitivity of 91% and a similar specificity of 95% [11]. In our results, plasma ACTH levels increased more than 1.5-fold in response to hCRH in almost all cases of Cushing’s disease, including 100% (62/62) and 73% (8/11) of cases with microadenomas and macroadenomas, respectively. On the other hand, even in cases with EAS, 27% (4/15) responded to hCRH. Reimondo et al. reported that an ovine CRH-induced ACTH percentage increment of 50% produced sensitivity of 86%, and a specificity of 90% [12]. They conclude that the ovine CRH test is likely to be the most reliable noninvasive diagnostic procedure for the differential diagnosis of ACTH-dependent Cushing’s syndrome [12]. Taken together, these results suggest that the hCRH test would be effective in distinguishing Cushing’s diseases from EAS, although it is not possible to discriminate completely between Cushing’s disease and EAS using the CRH test alone.

After high-dose (8 mg) DST, the morning plasma cortisol levels were suppressed in 89% (55/62) of microadenomas with Cushing’s disease, and 82% (60/73) of all Cushing’s disease cases. Our results are consistent with those of previous studies showing that the efficiency in Cushing’s disease is almost 80% [13]. In cases with EAS, a high-dose of dexamethasone suppressed 50% (3/6) of bronchial carcinoids and 0% (0/9) of other lung cancers. Salgano et al reported that a high dose of dexamethasone caused a high false-positive rate in lung carcinoid tumors [2]. These results suggest that a high-dose dexamethasone test is also useful for the diagnosis of Cushing’s disease, although we have to pay attention to the results produced in macroadenomas with Cushing’s disease and in cases of bronchial carcinoids with EAS [14].

When both the CRH test and 8 mg DST were considered together, our study showed a sensitivity of 81%, and a specificity of 60% in Cushing’s disease cases. Therefore, the CRH test and 8 mg DST would be effective for distinguishing Cushing’s diseases from EAS, although it is not possible to discriminate completely between Cushing’s disease and EAS by the CRH test or 8 mg DST alone. Based on our recent unpublished data, both the CRH test and 8 mg DST would achieve a higher specific measure for the diagnosis of Cushing’s disease when combined with MRI [15, 16], because the test results, along with the presence of a pituitary adenoma offer > 90% specificity in Cushing’s disease. In fact, MRI shows a high specificity to diagnose Cushing’s disease [17]. The ability of MRI to detect pituitary ACTH-secreting adenomas in patients with Cushing’s disease is, however, limited, because the calculated accuracy for detecting a pituitary source of ACTH is reported to be almost 60% for MRI [18]. In some cases of EAS, (18) F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) would be helpful to detect the localization [19]. Cavernous or inferior petrosal sinus sampling is also often used in the differential diagnosis of Cushing’s syndrome [20]. In conclusion, the diagnosis of Cushing’s disease is still one of the challenging problems in clinical endocrinology. We have to consider the usefulness and accuracy of each diagnostic test for ACTH-dependent Cushing’s syndrome, because the data in Cushing’s disease partially overlap with some cases of EAS.

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References