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Limited Diagnostic Utility of Plasma Adrenocorticotropic Hormone for Differentiation between Adrenal Cushing Syndrome and Cushing Disease

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Background: Measurement of the plasma adrenocorticotropic hormone (ACTH) level has been recommended as the first diagnostic test for differentiating between ACTH-independent Cushing syndrome (CS) and ACTH-dependent CS. When plasma ACTH values are inconclusive, a differential diagnosis of CS can be made based upon measurement of the serum dehydroepiandrosterone sulfate (DHEA-S) level and results of the high-dose dexamethasone suppression test (HDST). The aim of this study was to assess the utility of plasma ACTH to differentiate adrenal CS from Cushing' disease (CD) and compare it with that of the HDST results and serum DHEA-S level.

Methods: We performed a retrospective, multicenter study from January 2000 to May 2012 involving 92 patients with endogenous CS. The levels of plasma ACTH, serum cortisol, 24-hour urine free cortisol (UFC) after the HDST, and serum DHEA-S were measured.

Results: Fifty-seven patients had adrenal CS and 35 patients had CD. The area under the curve of plasma ACTH, serum DHEA-S, percentage suppression of serum cortisol, and UFC after HDST were 0.954, 0.841, 0.950, and 0.997, respectively (all $P<0.001$). The cut-off values for plasma ACTH, percentage suppression of serum cortisol, and UFC after HDST were 5.3 pmol/L, 33.3%, and 61.6%, respectively. The sensitivity and specificity of plasma ACTH measurement were 84.2% and 94.3%, those of serum cortisol were 95.8% and 90.6%, and those of UFC after the HDST were 97.9% and 96.7%, respectively.

Conclusion: Significant overlap in plasma ACTH levels was seen between patients with adrenal CS and those with CD. The HDST may be useful in differentiating between these forms of the disease, especially when the plasma ACTH level alone is not conclusive.

Keywords: Adrenal Cushing syndrome; Cushing disease; Adrenocorticotropic hormone; High-dose dexamethasone suppression test; Dehydroepiandrosterone sulfate

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INTRODUCTION

Cushing syndrome (CS) is a rare disorder characterized by excessive cortisol secretion from the adrenal glands [1]. This condition occurs in two distinct forms: adrenocorticotropic hormone (ACTH)-dependent CS and ACTH-independent CS, which are traditionally identified using a plasma ACTH assay. Low levels of plasma ACTH are indicative of ACTH-independent CS because excess autonomously released cortisol suppresses pituitary ACTH secretion [2,3]. In many cases, however, the distinction between disease subtypes remains unclear. Previous studies have reported high interassay variability and low reproducibility of plasma ACTH assays [4,5]. In one study, only 85% of all measurements were interpreted correctly, and this rate dropped to 60% in patients with suspected ACTH-independent CS [6]. Plasma ACTH values must be interpreted with caution because the wide variation in patient results often leads to difficulty in differentiating between ACTH-dependent and ACTH-independent CS.

The high-dose dexamethasone suppression test (HDST) has been a mainstay of biochemical testing for distinguishing Cushing' disease (CD) from ACTH-producing ectopic CS [7]. However, this test is rarely performed to distinguish ACTH-dependent CS from adrenal CS. Moreover, the sensitivity and specificity of the HDST for diagnosing CD are 65% to 100% and 60% to 100%, respectively [8-11].

Serum dehydroepiandrosterone sulfate (DHEA-S) is the most abundant adrenal androgen in blood. Serum DHEA-S levels generally remain within the high-normal range in patients with CD, but are low in patients with adrenal CS because of low plasma ACTH [12-14]. However, the normal range of serum DHEA-S is wide, making serum DHEA-S a poor choice as a first-line test for the differential diagnosis of CS.

Here, we investigated the diagnostic utility of plasma ACTH levels for distinguishing between adrenal CS and CD. We also compared the discriminatory role of the plasma ACTH level with that of the HDST results and serum DHEA-S level.

METHODS

Study subjects

We retrospectively collected data from 100 patients diagnosed with CD or adrenal CS from January 2000 to May 2012 in Seoul National University Hospital, Seoul National University Bundang Hospital, and Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul, Korea [15]. Only 92 patients with CD and adrenal CS were included. Eight patients were excluded due to the presence of ectopic, recurrent, cyclic, or subclinical CS, including two patients with ectopic CS and four with recurrent CS. This study was approved by the institutional review board of each participating hospital.

The diagnosis of CD was made by inferior petrosal sinus sampling or brain magnetic resonance imaging and confirmed by postsurgical pathologic examination or the presence of postsurgical adrenal insufficiency. Thirty-two patients were treated surgically, along with an additional three patients treated by Gamma knife surgery. The diagnosis of adrenal CS was made by abdominal computed tomography and confirmed by postsurgical pathologic examination. Furthermore, all patients with adrenal CS became hypoadrenal after removal of a solitary adrenal adenoma.

Plasma ACTH levels were measured in all patients with CS. The serum cortisol level or urine free cortisol (UFC) level after the HDST was measured in 77 patients, and the serum DHEA-S level was measured in 41 patients.

Laboratory measurements

Serum cortisol and UFC levels were measured using a radioimmunoassay kit (IMMUNOTEC, Prague, Czech Republic). The reference range for 24-hour UFC was 53.6 to 318.0 nmol/day. We calculated the percentage suppression of the serum cortisol concentration at 8:00 AM and UFC after HDST by daily administration of 8 mg of dexamethasone for 2 days. The serum DHEA-S level was measured using a competitive binding radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA, USA). The reference range of DHEA-S was 950 to 11,670 nmol/L. Blood samples were collected in a prechilled tube containing ethylenediaminetetraacetic acid (EDTA), immediately separated at 4°C, and stored at −80°C until needed. The morning plasma ACTH level was measured using an immunoradiometric assay (CIS-Bio International, Saclay, France). The limit of detection by the immunoradiometric assay was 0.4 pmol/L, and the interassay and intra-assay coefficients of variability were 9% to 45% and 1% to 17%, respectively [6]. The reference range of ACTH at 8:00 to 9:00 AM was 2.2 to 19.8 pmol/L. The mean plasma ACTH level was used when multiple tests were performed.

Statistical analysis

To evaluate the association between diagnosis and baseline characteristics or biochemical findings, Fisher exact test was used for categorical variables and the Mann-Whitney test was

used for quantitative variables. Receiver operating characteristic (ROC) curve analyses were used to determine the discrimination values for response to HDST, plasma ACTH, and serum DHEA-S. The optimal cut-off points for adrenal CS were chosen using the Youden index (sensitivity+specificity-1) [16]. We calculated the sensitivity, specificity, positive predictive value, and negative predictive value for each biochemical test based on the optimal cut-off points. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and MedCalc version 12.5.0 (MedCalc Software, Mariakerke, Belgium). *P* value of <0.05 was considered statistically significant.

RESULTS

Adrenal CS was present in 57 patients (62.0%) and CD in 35 patients (38.0%). No significant differences in age, sex, serum cortisol level, or 24-hour UFC level were observed between the

Table 1. Baseline Characteristics and Laboratory Results of Patients with Adrenal Cushing Syndrome and Cushing Disease

Characteristic	Adrenal Cushing syndrome (*n*=57)	Cushing disease (*n*=35)	*P* value
Age at diagnosis, yr	40 (31–54)	40 (28–54)	0.834
Female sex	51 (89.5)	28 (80.0)	0.230
Body mass index, kg/m^2	24.1 (21.6–27.0)	23.6 (22.2–26.4)	0.816
24-Hour UFC, nmol/day	1,535 (1,004–3,022)	1,199 (611–1,961)	0.136
Serum cortisol, nmol/L	499 (417–624)	593 (397–712)	<0.001
ACTH, pmol/L	3.1 (0.2–4.7)	22.2 (12.1–29.9)	<0.001
DHEA-S, nmol/L	677 (453–1,982)	6,321 (3,610–8,850)	0.001
Serum cortisol after LDST, nmol/L	535 (430–601)	290 (212–444)	0.001
UFC after LDST, nmol/day	1,311 (806–2,324)	312 (146–624)	0.001
Percentage suppression of serum cortisol after HDST, %	–0.7 (–13.3 to 17.5)	88.5 (62.6–92.8)	<0.001
Percentage suppression of UFC after HDST, %	–3.1 (–78.2 to 24.0)	94.4 (88.9–97.4)	<0.001
Patients with repeated ACTH measurement	29 (50.9)	18 (51.4)	0.959
Mass size (adrenal or pituitary), mm	18.5 (10.0–30.0)	2.2 (0.1–5.0)	
Patients with adrenal carcinoma	1 (1.8)	-	
Patients with pituitary adenoma of >10 mm	-	3 (8.6)	

Values are expressed as median (interquartile range) or number (%).
UFC, urinary free cortisol; ACTH, adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone sulfate; LDST, low-dose dexamethasone suppression test; HDST, high-dose dexamethasone suppression test.

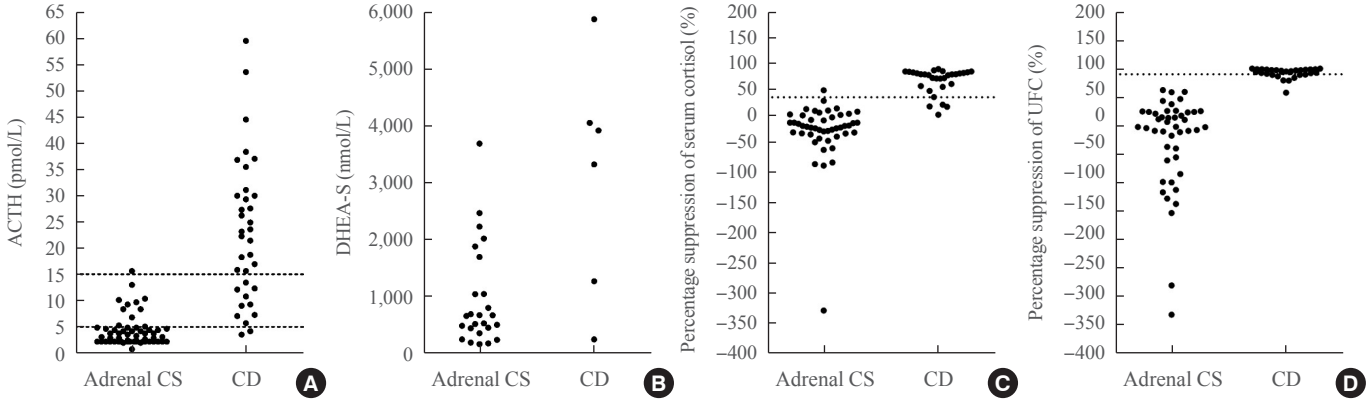

Fig. 1. (A) Plasma adrenocorticotropic hormone (ACTH) and (B) serum dehydroepiandrosterone sulfate (DHEA-S) levels. Percentage suppression of (C) serum cortisol and (D) urine free cortisol (UFC) after high-dose dexamethasone suppression test in patients with Cushing disease (CD) and adrenal Cushing syndrome (CS).

two groups (Table 1). Compared with patients with adrenal CS, those with CD exhibited higher plasma ACTH (median, 22.2 pmol/L vs. 3.1 pmol/L; $P<0.001$) (Fig. 1A) and serum DHEA-S levels (median, 6,321 nmol/L vs. 677 nmol/L; $P=0.001$) (Fig. 1B); however, some overlap in the plasma ACTH levels was detected between patients with adrenal CS and CD in the range of 3.5 to 15.6 pmol/L (Fig. 1A). The median percentage suppression of serum cortisol after the HDST was 88% in patients with CD. Serum cortisol suppression of >50% after the HDST occurred in 26 (87%) of 30 patients with CD. The median percentage suppression of UFC after the HDST was 94% in patients with CD. UFC was suppressed by >90% in 22 patients (73%) with CD (Fig. 1C, D). Of the 45 patients with adrenal CS, serum cortisol was suppressed by >50% in only one patient, while UFC was not suppressed by >90% in any patients with adrenal CS (Fig. 1C, D). The percentage suppression of serum cortisol and UFC after the HDST was greater in patients with CD than in those with adrenal CS ($P<0.001$).

No significant differences in the basal serum cortisol or 24-hour UFC level were detected between patients with a suppressed ACTH level of <2.2 nmol/L and those without plasma ACTH suppression (Table 2). The serum cortisol and UFC levels after the HDST and the percentage suppression after the HDST did not differ between the two groups.

ROC curve analyses were used to assess the diagnostic power of the plasma ACTH level, serum DHEA-S level, and percentage suppression of serum cortisol as well as the UFC level after the HDST in differentiating adrenal CS from CD (Table 3). The area under the curve was highest for UFC after HDST (Fig. 2). The sensitivity for plasma ACTH levels of <2.2 pmol/L was only 33.3%, although the specificity was 100.0%. For the HDST, the sensitivity and specificity of the percentage suppres-

Table 2. Comparison of Clinical and Biochemical Characteristics in Patients with Adrenal Cushing Syndrome Based upon Suppression of Plasma ACTH

Characteristic	Suppressed ACTH ($n=21$)	Normal/high ACTH ($n=36$)	P value
Age at diagnosis, yr	39 (32–54)	42 (30–56)	0.785
Female sex	20 (95.2)	31 (86.1)	0.397
Body mass index, kg/m^2	22.8 (20.7–27.1)	24.1 (22.2–27.1)	0.457
Adrenal mass size, mm	30.0 (25.0–33.5)	30.0 (25.0–34.8)	0.784
24-Hour UFC, nmol/day	1,120 (639–1,997)	1,348 (593–1,886)	0.832
Serum cortisol, nmol/L	505 (436–668)	499 (414–624)	0.841
ACTH, pmol/L	1.98 (2.11–2.18)	4.44 (3.37–6.45)	<0.001
DHEA-S, nmol/L	478 (236–570)	1,048 (627–2,589)	0.017
Serum cortisol after LDST, nmol/L	535 (430–706)	532 (372–582)	0.571
UFC after LDST, nmol/day	1,018 (847–2,672)	1,098 (599–2,139)	0.415
Percentage suppression of serum cortisol after HDST, %	4.6 (–17.9 to 18.8)	–4.2 (–13.2 to 14.9)	0.580
Percentage suppression of UFC after HDST, %	–2.2 (–66.1 to 16.4)	–7.3 (–91.1 to 24.7)	0.878

Values are expressed as median (interquartile range) or number (%).
ACTH, adrenocorticotropic hormone; UFC, urinary free cortisol; DHEA-S, dehydroepiandrosterone sulfate; LDST, low-dose dexamethasone suppression test; HDST, high-dose dexamethasone suppression test.

Table 3. Efficacy of Biochemical Parameters Used for Diagnosing Adrenal Cushing Syndrome

Parameter	Cut-off value	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %
ACTH, pmol/L	5.3	0.954	0.889–0.987	84	94	96	79
DHEA-S, nmol/L	2,464	0.810	0.693–0.936	86	85	92	73
Suppression of serum cortisol, %	–33.3	0.950	0.877–0.986	96	91	94	94
Suppression of UFC, %	–61.6	0.997	0.949–1.000	98	94	96	97

AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; ACTH, adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone sulfate; UFC, urine free cortisol.

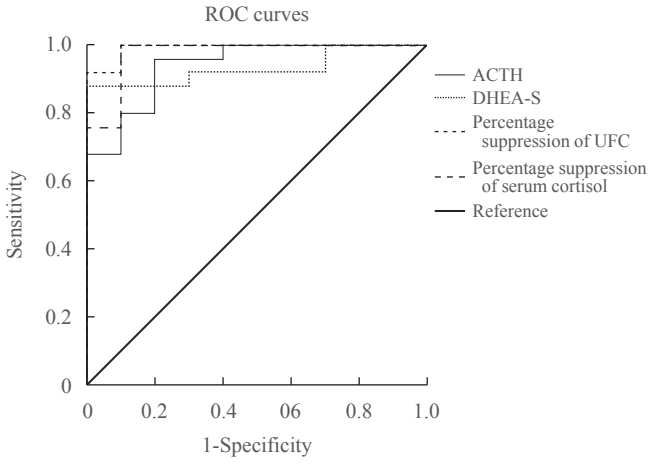

Fig. 2. Receiver operating characteristic (ROC) curves for plasma adrenocorticotropic hormone (ACTH), serum dehydroepiandrosterone sulfate (DHEA-S), percentage suppression of serum cortisol, and urine free cortisol (UFC) after the high-dose dexamethasone suppression test. The black line represents the results equivalent to chance.

sion of serum cortisol at 50% was 97.6% and 86.2%, respectively, and those of suppression of UFC at 90% were 100.0% and 73.4%, respectively (data not shown). According to the Youden index, the optimal cut-off values of plasma ACTH and the percentage suppression of serum cortisol and UFC after the HDST were 5.3 pmol/L, 33.3%, and 61.6%, respectively. Using these cut-off points, the sensitivity and specificity of the HDST were higher than those of plasma ACTH, and the sensitivity and specificity of DHEA-S were lower than those of plasma ACTH. Suppression of UFC after the HDST was the most powerful diagnostic tool for differentiating between adrenal CS and CD among the tests examined.

Of the three patients with CD plus adrenocortical adenoma, one patient exhibited no plasma ACTH suppression, while the other two patients showed plasma ACTH suppression to <2.2 pmol/L. However, the percentage suppression of serum cortisol and UFC were >50% and 90%, respectively, for all patients (Table 4). Of the 57 patients with adrenal CS, nine had pituitary

Table 4. Characteristics of Patients with Cushing Disease with Adrenocortical Adenoma

Patient no.	Sex	Age, yr	ACTH, pmol/L	HDST, %		Pituitary mass, mm[a]	Adrenal mass, mm[a]
				Serum cortisol	UFC		
1	F	69	38.3	99	99	10	10
2	M	66	3.5	89	97	5	10
3	F	73	22.2	90	-	5	10

In the HDST, % indicates percentage suppression of serum cortisol and UFC after HDST.
ACTH, adrenocorticotropic hormone; HDST, high-dose dexamethasone suppression test; UFC, urine free cortisol.
[a]Longest diameter of mass.

Table 5. Characteristics of Patients with Adrenal Cushing Syndrome with Pituitary Adenoma

Patient no.	Sex	Age, yr	ACTH, pmol/L	HDST, %		DHEA-S, nmol/L	Adrenal mass, mm[a]	Pituitary mass, mm[a]
				Serum cortisol	UFC			
1	F	24	8.4	29.0	14	665	37	4
2	F	33	3.5	19.0	−152	510	27	7
3	F	21	8.4	−29.0	10	8,717	10	4
4	F	27	2.0	−64.0	−467	185	27	3
5	F	51	4.8	−111.0	11	-	32	4
6	M	40	5.4	−1.3	−10	1,878	30	3
7	F	40	4.4	-	-	-	10	2
8	M	53	1.8	-	−17	2,464	10	3
9	M	57	3.7	6.7	−36	-	20	5

In the HDST, % indicates percentage suppression of serum cortisol and UFC after HDST.
ACTH, adrenocorticotropic hormone; HDST, high-dose dexamethasone suppression test; UFC, urine free cortisol; DHEA-S, dehydroepiandrosterone sulfate.
[a]Longest diameter of mass.

tumors; of these, seven exhibited plasma ACTH levels of >2.2 pmol/L. No serum cortisol or UFC suppression after the HDST was observed in any of these patients (Table 5).

We also analyzed the diagnostic value of the HDST in patients with CD with pituitary macroadenoma. In this study, only three of the 35 patients with CD also had pituitary macroadenomas. The HDST was performed in only two of these patients; both serum cortisol and UFC were suppressed in all patients. Although the percentage of patients with pituitary macroadenoma was <10%, the HDST was not helpful in patients with pituitary macroadenoma.

DISCUSSION

We investigated the utility of plasma ACTH levels in the differential diagnosis of CS compared with that of the HDST results and serum DHEA-S levels. Using suppressed plasma ACTH level (<2.2 pmol/L) as the discriminatory parameter, our data show that a substantial number of patients with adrenal CS may remain unidentified. The HDST may therefore be a useful additional for the differential diagnosis of CS in patients with plasma ACTH levels of 3.5 to 15.6 pmol/L.

The plasma ACTH assay is the mainstay in the differential diagnosis of CS [15,17]. Its convenience and low cost make it a viable choice for both hospitals and outpatient clinics. Plasma ACTH concentrations are detectable in 60% of patients with adrenal CS, and within the normal range in the remaining patients [18]. Similarly, 33.3% of patients with adrenal CS in our study had plasma ACTH concentrations of <2.2 pmol/L. Accordingly, plasma ACTH was of limited value for the differential diagnosis of CS.

Low interassay reproducibility and high variability of plasma ACTH levels may contribute to the poor diagnostic performance of this assay. Although plasma ACTH levels had a lower diagnostic accuracy than did the HDST in detecting adrenal CS, the sensitivity of plasma ACTH was 100% in patients with plasma ACTH levels of <2.2 pmol/L. Thus, further confirmation of adrenal CS may be unnecessary in patients with plasma ACTH levels below the limit of detection. However, in patients with plasma ACTH levels of 3.5 to 15.6 pmol/L, it is often difficult to definitely diagnose adrenal CS using this assay alone. Among patients with plasma ACTH levels within this range, HDST was able to successfully identify all patients with adrenal CS.

Previous studies have reported poor diagnostic accuracy of the HDST in discriminating adrenal CS. However, the positive and negative predictive values of the serum cortisol and UFC levels after the HDST in our patients with adrenal CS were approximately 95%, significantly higher than that of the plasma ACTH level. Neither the serum cortisol nor UFC level was found to be suppressed after the HDST in any patient with adrenal CS. Moreover, when the plasma ACTH level was within the range of 3.5 to 15.6 pmol/L, the specificity of the percentage suppression of serum cortisol and UFC was 100%. These results suggest that the utility of the HDST as a diagnostic tool may have been underestimated. Furthermore, the HDST was helpful in identifying the source of hormone secretion when both pituitary and adrenal nodules were detected by radiologic studies. In our study, 14% of patients with adrenal CS had pituitary adenomas, and 31% of patients with CD had adrenocortical adenomas. These observations demonstrate a clear ability of the HDST to distinguish between adrenal CS and CD in these situations, whereas the plasma ACTH level alone had little discrimination value.

Unlike the HDST, measurement of the serum DHEA-S level did not improve the ability to diagnose adrenal CS when added to plasma ACTH assays. Because serum DHEA-S is directly regulated by plasma ACTH, it was expected to be lower in patients with adrenal CS than in those with CD. However, in this study, the serum DHEA-S levels demonstrated a wide range of overlap between patients with adrenal CS and those with CD. The poor diagnostic value of the serum DHEA-S level may reflect the complex mechanisms of adrenal androgen secretion [12].

Our study included only 35 of 92 patients (38%) with CD compared with 57 patients (62%) with adrenal CS. Such a distribution is unusual given the normal distribution of cases, with CD typically more common than adrenal CS [19]. However, this finding is not without precedent; similar results have also been reported in a Korean population [20]. In that study, the prevalence of adrenal CS was slightly higher than that of CD, and ectopic CS was very rare. This distribution is thought to have been due to the low detection rate of pituitary tumors and ectopic CS in Korea, although racial differences may also be involved. The unusual distribution of patients our study population may have influenced the outcomes of some of our statistical tests; different cutoff values may be possible in a larger or more normally distributed cohort. Similarly, it is conceivable that the high prevalence of adrenal causes could inflate the positive and negative predictive values of the HDST.

As with all studies, the analyses presented here are not without limitations. First, the biochemical data were retrospectively retrieved from patients' medical records, meaning that the num-

ber of biochemical tests for each patient was not equal. Second, plasma ACTH levels may deteriorate in response to handling error after blood collection. Under ideal conditions, blood samples should be collected in a prechilled tube containing EDTA, immediately separated at 4°C, and then stored at −80°C until needed. Even with modern ACTH assays, ACTH levels may deteriorate quickly in whole blood unless cooled and separated and the plasma quickly frozen until needed. It is therefore possible that ACTH levels in patients with CD may have fallen after the blood samples were collected despite the fact that the study was performed with the same protocol and assay method at each institution. Third, simultaneous pituitary and adrenocortical tumors were identified in some patients, as above. These findings include both CD plus an adrenal incidentaloma as well as adrenal CS plus a pituitary incidentaloma. Last, the corticotropin-releasing hormone (CRH) stimulation test was not performed in our study because of limited reagent availability in Korea. It would be of interest to examine whether stimulated ACTH levels in response to CRH better discriminate between adrenal CS and CD.

In conclusion, our study has demonstrated that the plasma ACTH assay has limited value in the differential diagnosis of CS. The diagnostic accuracy of the HDST is higher than that of the plasma ACTH level, whereas the serum DHEA-S level is less useful than the plasma ACTH level. The HDST is particularly beneficial in identifying patients with adrenal CS when the plasma ACTH level is not suppressed to <2.2 pmol/L. We therefore recommend using the HDST for the differential diagnosis of CS when plasma ACTH levels alone are inconclusive.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Pecori Giraldi F. Recent challenges in the diagnosis of Cushing's syndrome. Horm Res 2009;71 Suppl 1:123-7.
2. Kuhn JM, Proeschel MF, Seurin DJ, Bertagna XY, Luton JP, Girard FL. Comparative assessment of ACTH and lipotropin plasma levels in the diagnosis and follow-up of patients with Cushing's syndrome: a study of 210 cases. Am J Med 1989;86(6 Pt 1):678-84.
3. Nieman LK, Chrousos GP, Oldfield EH, Avgerinos PC, Cutler GB Jr, Loriaux DL. The ovine corticotropin-releasing hormone stimulation test and the dexamethasone suppression test in the differential diagnosis of Cushing's syndrome. Ann Intern Med 1986;105:862-7.
4. Vogeser M, Engelhardt D, Jacob K. Comparison of two automated adrenocorticotropic hormone assays. Clin Chem 2000;46:1998-2000.
5. Howe LA, Smeaton T. Anomalous results with the CIS-Sorin adrenocorticotrophic hormone radioimmunoassay kit. Clin Chem 1979;25:816-7.
6. Pecori Giraldi F, Saccani A, Cavagnini F; Study Group on the Hypothalamo-Pituitary-Adrenal Axis of the Italian Society of Endocrinology. Assessment of ACTH assay variability: a multicenter study. Eur J Endocrinol 2011;164:505-12.
7. Newell-Price J, Perry L, Medbak S, Monson J, Savage M, Besser M, et al. A combined test using desmopressin and corticotropin-releasing hormone in the differential diagnosis of Cushing's syndrome. J Clin Endocrinol Metab 1997;82:176-81.
8. al-Saadi N, Diederich S, Oelkers W. A very high dose dexamethasone suppression test for differential diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf) 1998;48:45-51.
9. Kaye TB, Crapo L. The Cushing syndrome: an update on diagnostic tests. Ann Intern Med 1990;112:434-44.
10. Aron DC, Raff H, Findling JW. Effectiveness versus efficacy: the limited value in clinical practice of high dose dexamethasone suppression testing in the differential diagnosis of adrenocorticotropin-dependent Cushing's syndrome. J Clin Endocrinol Metab 1997;82:1780-5.
11. Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, et al. Diagnosis and complications of Cushing's syndrome: a consensus statement. J Clin Endocrinol Metab 2003;88:5593-602.
12. Barbetta L, Dall'Asta C, Re T, Colombo P, Travaglini P, Ambrosi B. Androgen secretion in ectopic ACTH syndrome and in Cushing's disease: modifications before and after surgery. Horm Metab Res 2001;33:596-601.
13. Yamaji T, Ishibashi M, Sekihara H, Itabashi A, Yanaihara T. Serum dehydroepiandrosterone sulfate in Cushing's syndrome. J Clin Endocrinol Metab 1984;59:1164-8.
14. Kleiber H, Rey F, Temler E, Gomez F. Dissociated recovery of cortisol and dehydroepiandrosterone sulphate after treatment for Cushing's syndrome. J Endocrinol Invest 1991;14:489-92.
15. Gilbert R, Lim EM. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. Clin Biochem Rev 2008;29:103-6.

16. Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 2005;16:73-81.
17. Klose M, Kofoed-Enevoldsen A, Ostergaard Kristensen L. Single determination of plasma ACTH using an immunoradiometric assay with high detectability differentiates between ACTH-dependent and -independent Cushing's syndrome. Scand J Clin Lab Invest 2002;62:33-7.
18. Invitti C, Pecori Giraldi F, de Martin M, Cavagnini F. Diagnosis and management of Cushing's syndrome: results of an Italian multicentre study. Study Group of the Italian Society of Endocrinology on the Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis. J Clin Endocrinol Metab 1999;84:440-8.
19. Oldfield EH, Doppman JL, Nieman LK, Chrousos GP, Miller DL, Katz DA, et al. Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing's syndrome. N Engl J Med 1991;325:897-905.
20. The Korean Society of Endocrinology; The Survey Committee for Endocrine Disease in Korea. The incidence and clinical characteristics of Cushing's syndrome in Korea. J Korean Soc Endocrinol 2000;15:31-45.