Turkish Journal of Endocrinology and Metabolism, (2003) 1 : 1-10 **ORGINAL ARTICLE**

Desmopressin Stimulation Test: Worth to Use in Clinical Practise?

Rıfat Emral Vedia Tonyukuk Hasan Sav Demet Çorapçıoğlu Nuri Kamel Gürbüz Erdoğan

Department of Endocrinology and Metabolic Diseases, Ankara University, School of Medicine, Ankara, Turkey

Differential diagnosis of Cushing's disease (CD) is still problematic. Desmopressin testing may be an alternative tool to differentiate patients with CD. We assessed the effects of desmopressin (DDAVP) on the release of ACTH and cortisol (F) when given to patients with Cushing's syndrome (CS) of varied aetiologies and normal individuals (NI).

Fourteen patients with CD formed group I, nine patients with adrenal CS and three patients with ectopic ACTH secretion formed group II and fourteen NI formed group III. Net increments (Δ) and percent increments (Δ%) in F and ACTH were calculated following 10 μg DDAVP. ΔF% over 20% and in ΔACTH% over 50% were accepted as positive response to the test.

ΔF and ΔF% were significantly higher in group I when compared to group II and III ($p<0.01$ and $p<0.001$, respectively). ΔACTH and ΔACTH% were also significantly higher in group I comparing with group II and III ($p<0.001$ and $p<0.001$, respectively). Positive response was observed in 10/14 (71.42%) of patients in group I, in 3/12 (25%) of patients in group II and in 3/14 (21.42%) of healthy subjects in group III. Interestingly 2/3 of patients with ectopic ACTH secretion showed positive response. The sensitivity of the test was 71.42% and the specificity was 76.92%. The diagnostic accuracy was found to be 75%.

These data provide some evidence that DDAVP testing is not an enough diagnostic tool for differential diagnosis of CD, since hyperresponsive patients to the peptide are not so uncommon in ectopic ACTH dependent CS.

Keywords: Desmopressin stimulation test, Cushing's disease, ectopic ACTH secretion

Introduction

The diagnosis of Cushing's syndrome (CS) remains one of the most challenging tasks in clinical neuroendocrinology (1). Cushing's disease (CS) is the most common form of the hyper-cortisolemic states. The best diagnostic approach to patients with suspected CD continues to evolve. There is, however, no such thing as the single, simple, perfect, non-invasive test that will, in every case, allow the differential diagnosis of CD. The highest degree of accuracy is most likely to be obtained by using a variety of tests that assess the spectrum of different physiological responses to a variety of agents (1). For those patients in whom the diagnosis of CS remains doubtful, the dexamethasone-CRH test (2) or stimulation with desmopressin (3) may be resolutive.

In the investigation of patients with CS, multiple reports have demonstrated that CRH is a useful diagnostic test in both diagnosis and differential diagnosis of the disorder (4). CRH causes a significant increase in serum cortisol in the great majority of patients with CD, whilst in patients with adrenal Cushing's syndrome (AC) or the ectopic ACTH syndrome (EC) such a rise is seen only extremely rarely (4). Similar results have been described also in studies employing arginine vasopressin (AVP) and its synthetic analogue lysine

Correspondence address:

Rıfat Emral
İbn-i Sina Hospital
D-block, 10.floor
06100 Samanpazarı-Ankara/Turkey
Phone : +90 312 3094717
Fax : +90 312 3094505
E-mail : rifatemral@yahoo.com

vasopressin (LVP) (5-8). These investigations had clearly established that AVP was an ACTH secretagogue as well (9). The pituitary effects of AVP are secondary to specific binding of the peptide to the V3 receptor subtype, whose presence has been detected on human corticotrophs (10,11). But studies reported marked side effects with AVP and LVP, including nausea, abdominal pain and flushing. Another synthetic and long-acting AVP analogue, desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP), is characterized by a more selective activity on the renal V2 receptors (12) and is generally free of unpleasant side effects (13). Although its potential activity on pituitary V3 receptors remains uncertain, bolus administration of this compound produces significant ACTH and cortisol responses in the vast majority of patients with CD (14-16). However, contrasting results have been reported: some studies demonstrated no significant DDAVP-induced stimulation of ACTH and cortisol levels (14,17,18) whereas others demonstrated a significant cortisol increase in some normal subjects (19,20). In general it is accepted that normal subjects do not respond to DDAVP (18,21). So another possible application of this test could be to differentiate patients with CD from normal individuals (NI) or pseudo-Cushing states.

The present study was designed to assess the effects of desmopressin on the release of ACTH and cortisol when given as an intravenous bolus to patients with CS of varied aetiologies and to healthy subjects. We therefore evaluated the clinical value of the DDAVP test in differential diagnosis of CD by comparing ACTH and cortisol responses of patients with CD, AC, EC and NI.

Table 1. Clinical and laboratory characteristics of the patients with Cushing's syndrome

Patient no	Age/Sex (yr)	BMI (kg/m^2)	Diagnosis[1]	Treatment[2]	UFC[3] (μg/24 h)	Baseline ACTH[4] (pg/ml)	Serum cortisol[5] (μg/dl)		
							Baseline	Low-dose DST	High-dose DST
1	34/F	26.6	CD/Mi[a]	TN/TS	592	60.3	56.9	102.0	36.0
2	33/F	37.8	CD/Mi	TN/TS	1456	61.5	42.0	27.5	17.7
3	28/F	22.2	CD/Mi	TN/TS	260	75.4	22.4	9.5	0.5
4	17/M	30.0	CD/CH	TN/TS	150	26.0	100.0	6.3	2.8
5	33/F	36.0	CD/Mi[a]	TN/TS	78	53.7	20.8	7.3	0.5
6	33/F	32.0	CD/Mi[a]	TN/TS	320	73.8	28.0	26.0	1.0
7	37/F	32.8	*CD/Mi	TN/TS	132	67.0	20.45	10.9	6.9
8	30/F	26.0	CD/Mi	TN/TS	420	126.0	55.6	44.7	4.0
9	30/F	30.0	CD/Mi	TN/TS	138	56.0	24.0	13.1	4.2
10	53/M	28.0	*CD/Mi	TN/TS	162	128.0	27.5	5.7	3.1
11	33/M	27.1	CD/Ma	TN/TS	545	65.0	25.0	23.7	12.9
12	25/F	32.0	CD/Mi[a]	TN/TS	138	81.4	17.0	12.6	7.5
13	39/F	38.0	CD/Mi	TN/TS	106	46.7	19.2	20.0	2.0
14	35/F	34.9	*CD/Mi[a]	TN/TS	200	84.7	32.1	10.4	36.0
15	30/F	26.0	AC/AA	UA[r]	335	8.9	47.5	35.0	31.0
16	29/F	42.0	AC/AA	UA[r]	290	10.7	27.0	28.2	33.2
17	47/M	33.0	AC/NH	BA	406	7.2	17.7	21.2	32.8
18	50/F	38.4	AC/AA	UA[l]	412	7.4	14.5	34.1	19.8
19	31/F	27.5	AC/AA	UA[l]	267	13.6	13.0	11.0	10.7
20	44/M	29.0	AC/AA	UA[l]	342	15.0	16.6	15.9	15.0
21	39/M	49.5	AC/AA	UA[l]	336	5.0	21.4	23.0	16.6
22	61/F	25.0	AC/AA	UA[r]	170	9.7	31.8	39.2	34.5
23	21/F	21.3	AC/C	UA[r]	951	6.7	38.7	40.1	39.0
24	55/M	25.0	EC/LC[a]	T,Died	928	122.0	32	25.7	23
25	69/F	31.0	*EC/CC	Died	1814	180.0	80	100	130
26	35/M	24.6	*EC/BC	UA[r]+T	295	29.9	29.19	36.39	35.48

[1] CD: Cushing's disease (Mi: microadenoma, Mi[a]: microadenoma with positive ACTH immunostaining; Ma: macroadenoma; CH: corticotroph hyperplasia); AC: Adrenal Cushing's syndrome (AA: adrenal adenoma; NH: bilateral nodular hyperplasia; C: adrenal carcinoma); EC: Ectopic ACTH dependent Cushing's syndrome (LC[a]: small cell lung carcinoma with positive ACTH immunostaining; CC: colon carcinoma; BC: bronchial carsinoid);

[2] TN/TS: Transnasal transsphenoidal hypophysectomy; UA: Unilateral adrenalectomy (r: right; l: left); BA: Bilateral adrenalectomy; T: Thoracotomy.

[3] UFC: Urinary Free Cortisol (normal range: 9-156 μg/24 h)

[4] Plasma ACTH normal range: 5-50 pg/ml

[5] Serum cortisol normal range: 6.2-19.4 μg/dl

* Patients who underwent inferior petrosal sinus sampling

Materials and Methods

Subjects

Twenty-six patients with CS (14 with CD, nine with AC, three with EC) and 14 healthy volunteers, totally 40 subjects were enrolled into the study. Local ethical committee approval was obtained and all subjects gave fully informed consent. Their sexes, ages, body mass indexes (BMI) were recorded. Fourteen patients with CD (11 women, three men; mean age 32.85±7.58 years, age range 17-51 years, BMI 30.97±4.71 kg/m^2, BMI range 22.65-38.05 kg/m^2) formed the first group (group I) while 12 patients with CS of varied aetiologies rather than the pituitary dependent hypercortisolism (seven women, five men; mean age 42.58±14.13 years, age range 21-69 years, BMI 31.03±8.36 kg/m^2, BMI range 21.33-49.50 kg/m^2) formed the second group (group II) and 14 healthy individuals (10 women, four men; mean age 38.85±14.50 years, age range 18-75 years, BMI 31.12±8.92 kg/m^2, BMI range 18.37-50.17 kg/m^2) without any signs of hypercortisolism consisted of the third group (group III). The third group served as a control for desmopressin testing. Those three groups were sex, age and BMI matched. Details of patients with CS are provided in Table 1.

The initial diagnosis of CS was based on the standart criteria: clinical features, high urinary cortisol, loss of circadian rhythm of plasma cortisol, and absent suppression after low-dose dexamethasone tests (1 mg orally overnight and 2 mg/day orally for 48 h). The differential diagnosis included high dose dexamethasone test (8 mg/day orally for 48 h) and measurement of plasma ACTH concentrations (22). Morphological assessment was performed by pituitary and adrenal magnetic resonance imaging (MRI) and/or computerized tomography (CT). When indicated, bilateral petrosal sinus sampling with or without CRH was also employed (23). Inferior petrosal sinus sampling (IPSS) was required to confirm the origin of ACTH hypersecretion in three patients with CD (nos 7, 10, 14) and in two patients with EC (nos 25, 26). A pituitary adenoma was suggested by MRI in 11 cases with CD and 10 were microadenoma (nos 1-10) while only one was macroadenoma (no 11). Diagnosis of pituitary dependent CS in the rest three patients with CD became decisive if the patient cured after transnasal-transsphenoidal (TN/TS) hypohysectomy (nos 12, 13), or if positive ACTH immunostaining was seen in the removal pituitary tissue and if central to peripheral gradients of ACTH and cortisol were over 3 in the inferior petrosal sinus sampling (no 14). Patients were judged in remission of disease six months after surgery if they had hypocortisolism requiring glucocorticoid substitution therapy or, in case normal serum and urinary cortisol levels, if they had clinical remission of symptoms and a normal suppressibility of serum cortisol level after low-dose dexamethasone testing (24). Twelve patients with CD (85.71%) met the criteria for surgical remission. Nine patients with AC underwent adrenalectomy and except one, all had unilateral adrenalectomy. That patient whom underwent bilateral adrenalectomy had bilateral adrenal macronodular hyperplasia. Eight patients with AC (88.88%) cured after adrenalectomy, but the patient with adrenocortical carcinoma (no 23) did not cure though her clinical picture improved. Two out of three patients with EC died. One of them had small cell lung carcinoma (no 24) and histological proof obtained by immunoreactivity to ACTH of surgically removal tumoural tissue. The other patient with EC had colon carcinoma (no 25) and she died because of acute abdomen caused by colon perforation. The ectopic source of ACTH in this patient was proved by the absence of central to peripheral gradient of ACTH and cortisol in the inferior petrosal sinus sampling. The third patient with EC had bronchial carsinoid tumour (no 26), 1x0.5 cm in diameter in thorax CT and his clinical picture was conflicting. He was referred to our department with the classical signs and symptoms of CS and hormonal determinations proved hypercortisolism. Though his presentation was suitable with CD (hypermelanosis, normal or high serum ACTH levels, normal suppressibility of serum cortisol level after high-dose dexamethasone suppression test), no pituitary adenoma was detected in the MRI of pituitary. Adrenal CT showed bilateral adrenal hyperplasia and an adenoma in the right adrenal gland. Inferior petrosal sinus sampling with CRH showed no lateralization between right and left part of pituitary gland and central/peripheral ratios of ACTH and cortisol were about 1/1. These results were taken in favour of AC, so the patient underwent unilateral adrenalectomy. But hypercortisolism persisted afterall and the patient was accepted as EC and throcal approach was done afterwards. Desmopressin stimulation test was applied to this patient after unilateral adrenalectomy.

Fourteen NI, served as control group, consisted of healthy subjects and they were all applied 1-mg overnight dexamethasone suppression test for the exclusion of hypercortisolism.

Desmopressin stimulation testing

The desmopressin stimulation tests were performed between 0800 and 0830h, with the patients fasting and resting in the bed. A cannula was placed in an anterior forearm vein 30 minutes before the begining of the test. Two baseline blood samples were drawn 30 minutes apart. After the collection of baseline blood samples, 10 μg DDAVP (Minirin/DDAVP, Ferring) was injected as an i.v. bolus and blood samples for plasma ACTH and serum cortisol (F) determinations were taken at 30, 60, 90 and 120 minutes after DDAVP administration. The samples for ACTH measurement were collected into iced EDTA tubes and the tubes were immersed in an ice bath following collection. The plasma was centrifugated in a refrigerated centrifuge for its separation from the cells and then it was freezed immediately in plastic tubes. The specimens for cortisol measurement were placed in standart sampling tubes. Plasma treated with EDTA and freezed at once at –20^{o}C and kept in the refrigerater untill assay.

Blood pressure and pulse were monitored at 15 minutes intervals thoughout the test. Patients were instructed to report any side effects after the i.v. injection of DDAVP and were advised to avoid excessive fluid intake for the remainder of that day.

Hormonal assays

Plasma ACTH level was measured by a comercially available chemiluminescence immunoassay (IMMULITE 2000 ACTH assay, Diagnostic Products Corporation, Los Angeles, CA, USA). The assay sensitivity is 5 pg/ml and the intraassay coefficient of variations are 8.7%, 6.8% and 9.5% at ACTH concentrations of 23, 40 and 421 pg/ml, respectively, while interassay coefficient of variations are 10.0%, 8.7% and 6.1% at ACTH conentrations of 24, 89 and 496 pg/ml, respectively. Undetectable ACTH levels were assigned a value of 5 pg/ml for statistical analysis.

Serum cortisol was measured by a commercially available electrochemiluminescence immunoassay (ECLIA, Roche Elecsys® Systems 1010/2010, Geel, Belgium). Intraassay coefficient of variations are 1.6%, 1.5% and 1.6% at cortisol concentrations of 7.35, 20.3 and 46.0 μg/dl. Interassay coefficient of variations are 20% at the cortisol concentration of <0.29 μg/dl. Lowest cortisol concentration was <0.036 μg/dl with this assay and this value represented the assay sensitivity as well.

Response criteria

In each patient with CS and in NI, ACTH and F rises (Δ%) greater than 50% and 20% from baseline levels (mean value of samples taken at –30 and 0 minutes), respectively, were considered as positive responses to DDAVP stimulation test, as previously defined (22,25,26).

Statistical analysis

Variables among groups are expressed as the mean ± standart deviation. Hormonal secretory responses are expressed as absolute peak values, absolute net increments (Δ) and percent increments (Δ%) over baseline values were determined. Intragroup statistical evaluation was carried out by Wilcoxon test, whereas intergroup differences were evaluated by oneway ANOVA. The prevalence of responses to DDAVP in the group of patients with CD was compared to that in the other groups by Chi-squared analysis. For the comparison of absolute peak and Δ values of ACTH and cortisol after DDAVP, between groups, Kruskal-Wallis test was used. Sensitivity was defined as the ratio of true positive to the sum of true positive and false negative results. Specificity was defined as the ratio of true negative to the sum of true negative and false positive results. Accuracy was defined as the ratio of the sum of true positive and true negatives to the sum of true and false positive and true and false negative results. Receiver-operator-characteristic (ROC) curves were used to establish the usefulness of percent increments of ACTH and cortisol after the DDAVP stimulation test for the differentiation of CD from the other groups and areas under the curves (AUC) were determined, as well. *P* values less than 0.05 were considered to be significant.

Results

Plasma cortisol responses

In patients with CD 10 μg desmopressin significantly ($p<0.01$) increased cortisol (baseline 24.60 ± 12.15; peak 48.16 ± 30.47 μg/dl) levels. Cortisol net increment (ΔF) was found to be 23.55 ± 27.46 μg/dl, while ΔF% was 104.21 ± 117.93% in

patients with CD. Cortisol responses greater than 20% from baseline were observed in 10/14 (71.42%) of patients with CD.

In patients with AC or EC, desmopressin caused a significant increase ($p<0.05$) in cortisol levels as well (baseline 31.04 ± 20.27; peak 38.14 ± 36.40 μg/dl), but this increament was not as much as the one seen in patients with CD. The individual analysis of cortisol responses to DDAVP showed that in two patients with EC the ACTH/cortisol rises fulfilled the criteria of a positive response (nos 25 and 26), so in the second group, the highest net increments after DDAVP, for cortisol, belonged to these two patients. In this group, ΔF was found to be 7.10 ± 16.92 μg/dl, whereas ΔF% was 13.69 ± 21.08%. Positive cortisol response was observed in 3/12 (25%) of patients in the group (two of them were the patients with EC).

When we determined the responses of the control subjects to DDAVP, we realized that there was no significant rise in cortisol levels compared to the baseline (baseline 15.35 ± 5.40; peak 16.05 ± 5.04 μg/dl). Both ΔF and ΔF% were found to be lower than the other groups (ΔF 0.27 ± 3.50 μg/dl and ΔF% 4.23 ± 33.17%). Cortisol responses greater than 20% from baseline were observed in 3/14 (21.42%) of normal individuals.

When we compared cortisol responses in three groups, it was obvious that patients with CD (group I) gave the highest responses to DDAVP in general. Peak cortisol levels following DDAVP administration were significantly higher in patients with CD and other patients with CS (patients with AC or EC) when compared to NI (mean rank of peak cortisol; 27.61, 24.00 and 10.39; $p>0.05$, $p<0.001$ and $p<0.001$, respectively). Mean ΔF was significantly higher in patients in group I when compared to the patients in group II and normal individuals in group III ($p<0.05$ and $p<0.001$, respectively) while no such significant difference was found between group II and group III. It was the same for ΔF%; patients in group I had significantly higher ΔF% when compared to patients in group II and normal individuals in group III ($p<0.01$ and $p<0.001$, respectively). However, ΔF% did not differ between group II and III.

ROC curve for the percent increment of cortisol after DDAVP is shown in Figure1.

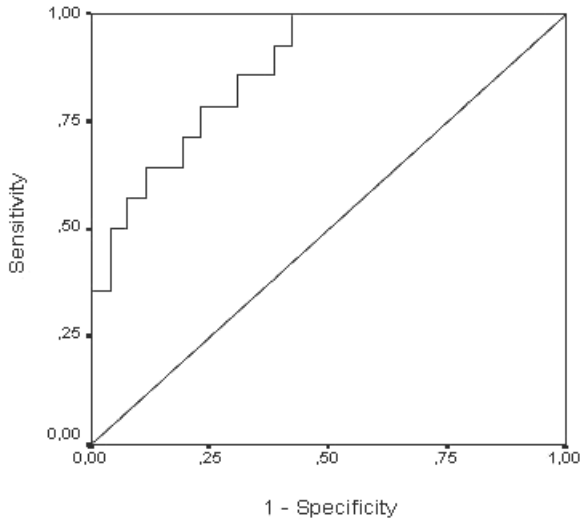

Figure 1. ROC curve for the percent increment of cortisol after DDAVP

From the analysis of ROC curve, sensitivity of desmopressin stimulation test according to positive cortisol response, for differential diagnosis of CD, was 78.6% and specificity of the test was 73.1% . AUC was 0.837 ± 0.072 and it was statisticaly significant ($p<0.01$).

Plasma ACTH responses

Following DDAVP administration patients with CD showed significant increase in ACTH levels (baseline 77.51 ± 66.22; peak 222.36 ± 275.85 pg/ml; $p<0.001$). ACTH net increment (ΔACTH) was found to be 145.41 ± 215.08 pg/ml, while ΔACTH% was 170.30 ± 142.85%.

ACTH responses greater than 50% from baseline were observed in 10/14 (71.42%) of patients with CD. This percent was the same with cortisol response in this group.

Plasma ACTH levels increased in the second group after DDAVP significantly (baseline 35.63 ± 57.40; peak 64.39 ± 133.72 pg/ml, $p<0.01$). When the patients with EC were excluded, increase in plasma ACTH was not significantly different from baseline, following DDAVP. This might reflect that patients with AC were unresponsive to DDAVP administration in general. In this group, ΔACTH was found to be 28.75 ± 79.44 pg/ml and ΔACTH% was 51.93 ± 64.78%. According to the positive ACTH response criteria 3/12 (25%) patients in this group were responsive to the peptide.

Normal individuals responded to DDAVP with a significant increase in ACTH level as well (baseline 17.71 ± 8.15; peak 23.11 ± 9.77 pg/ml, $p<0.05$), and ΔACTH was 2.50 ± 9.28 pg/ml, while ΔACTH% was 10.02 ± 77.43%. ACTH responses greater than 50% from baseline were observed in 3/14 (21.42%) of normal individuals.

Comparing the three groups, we found that there were differences for peak ACTH, ΔACTH, and ΔACTH% between group I and the others and all the parameters were significantly higher in group I when compared to group II and III ($p<0.001$ and $p<0.001$, respectively). No differences was found between group II and III for those parameters.

ROC curve for the percent increment of ACTH after DDAVP is shown in Figure 2.

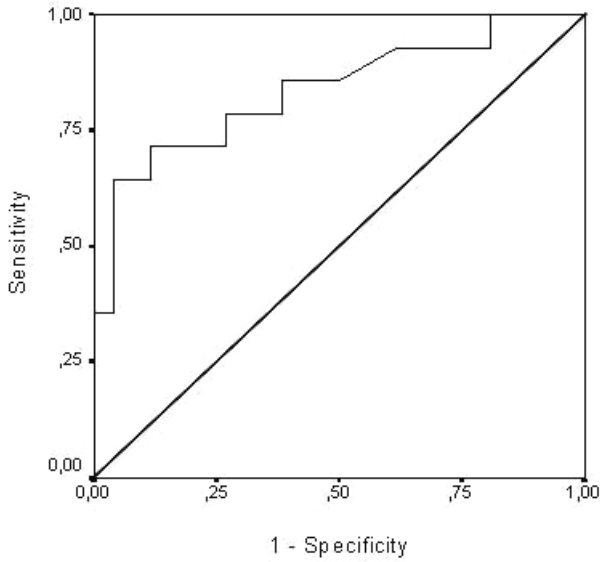

Figure 2. ROC curve for the percent increment of ACTH after DDAVP

From the analysis of ROC curve, according to positive ACTH response, for differential diagnosis of CD, sensitivity of desmopressin stimulation test was found to be 78.6%, and the specificity was 76.9%. AUC was 0.871 ± 0.055 and it was statisticaly significant ($p<0.01$).

Table 2, 3 and 4 shows the baseline, Δ and Δ% values of group I (patients with CD), group II (patients with AC and EC) and group III (control subjects), respectively.

Figure 3 and 4 gives the individual data showing the percentage change from baseline of cortisol (ΔF%) and ACTH (ΔACTH%) following desmopressin administration in group I (patients with CD), group II (patients with AC and EC) and group III (NI). The lines crossing the y-axes at 20% in figure 1 and at 50% in figure 2, representing the cut point of the positive response criteria in our study.

According to both cortisol and ACTH responses, the sensitivity of desmopressin stimulation test in differential diagnosis of Cushing's disease was found to be 71.42% and the specificity was found to be 76.92%, in our study. The diagnostic accuracy of the test was 75%.

Table 2. Levels of plasma ACTH (pg/ml) and serum cortisol (μg/dl) after 10 μg desmopressin test in patients with Cushing's disease

Patient no	Serum cortisol[1] (μg/dl)			Plasma ACTH[2] (pg/ml)		
	Basal	Δ	Δ%	Basal	Δ	Δ%
1	21.6	89.9	416.2	62.6	107.3	171.3
2	39.8	50.2	126.1	95.0	109.0	114.7
3	13.0	11.2	86.1	97.7	34.3	35.1
4	15.7	21.0	133.7	55.0	99.0	180.0
5	17.4	13.4	77.1	34.0	67.0	197.0
6	29.2	26.8	91.7	57.4	151.6	264.1
7	23.2	-3.2	-13.7	47.0	22.0	46.8
8	53.0	0	0	299.0	841.0	281.2
9	19.8	6.2	31.3	49.8	145.2	291.5
10	37.6	7.3	19.4	72.0	19.0	26.3
11	28.7	37.2	129.6	58.0	315.0	543.1
12	15.8	1.4	8.8	41.7	13.1	31.4
13	7.4	5.0	67.7	63.4	37.7	59.4
14	22.2	63.2	284.6	52.5	74.5	141.9

[1] Normal range: 6.2-19.4 μg/dl
[2] Normal range: 5-50 pg/ml

Table 3. Levels of plasma ACTH (pg/ml) and serum cortisol (μg/dl) after 10 μg desmopressin test in patients with adrenal Cushing's syndrome and ectopic ACTH-dependent Cushing's syndrome

Patient no	Serum cortisol[1] (μg/dl)			Plasma ACTH[2] (pg/ml)		
	Basal	Δ	Δ%	Basal	Δ	Δ%
1	28.0	0	0	8.5	1.0	11.7
2	27.5	0.8	2.9	12.0	2.0	16.6
3	20.1	3.3	16.4	7.0	12.9	184.2
4	29.1	0	0	8.0	1.0	12.5
5	15.9	4.7	29.5	13.6	2.5	18.3
6	13.0	-0.7	-5.3	14.0	1.7	12.1
7	19.5	-0.3	-1.5	4.8	1.9	39.5
8	24.6	0.8	3.2	8.0	1.7	21.2
9	40.9	-0.7	-2.9	12	1.0	8.33
10	29.0	9.0	31.0	95.5	10.5	10.9
11	90.5	59.7	65.9	200.0	280.0	140.0
12	34.3	8.6	25.0	38.0	55.9	147.1

[1] Normal range: 6.2-19.4 μg/dl
[2] Normal range: 5-50 pg/ml

Table 4. Levels of plasma ACTH (pg/ml) and serum cortisol (μg/dl) after 10 μg desmopressin test in normal individuals

Patient no	Serum cortisol[1] (μg/dl)			Plasma ACTH[2] (pg/ml)		
	Basal	Δ	Δ%	Basal	Δ	Δ%
1	16.4	-2.6	-15.85	12.0	4.0	33.3
2	6.92	6.4	93.6	13	3.0	23.0
3	16.5	-2.7	-16.3	12.0	2.0	16.6
4	18.2	3.0	16.4	19.0	15.0	78.9
5	21.1	0.3	1.4	27.0	-1.0	-3.7
6	16.7	6.0	35.9	22.0	7.0	31.8
7	23.0	-3.6	-15.8	32.0	-11.0	-34.3
8	8.1	-2.9	-35.5	8.5	-19.5	-229.4
9	10.2	1.5	15.5	16.0	16.0	100.0
10	14.8	4.0	27.0	14.0	4.0	28.5
11	7.3	-0.8	-10.9	33.5	14.5	43.2
12	16.8	-4.3	-33.0	13.0	2.0	15.3
13	15.8	-0.5	-3.1	16.0	-3.0	-18.7
14	23	0	0	18.0	10.0	55.5

[1] Normal range: 6.2-19.4 μg/dl
[2] Normal range: 5-50 pg/ml

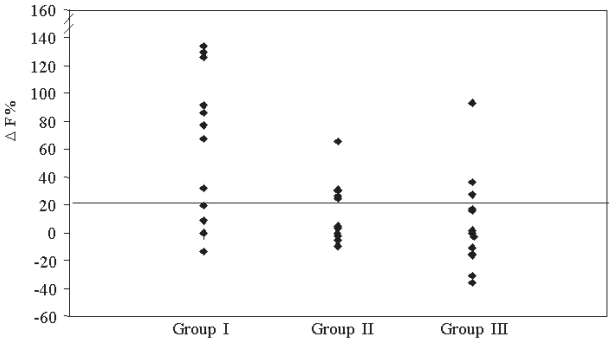

Figure 3. Individual data showing ΔF% following desmopressin administration in three groups
Group I: Patients with Cushing's disease
Group II: Patients with adrenal Cushing's syndrome and ectopic ACTH syndrome
Group III: Normal individuals

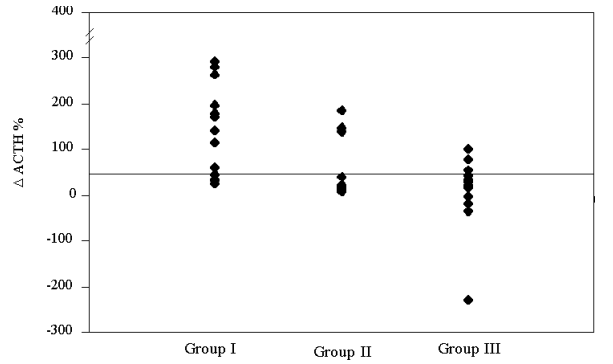

Figure 4. Individual data showing ΔACTH% following desmopressin administration in three groups
Group I: Patients with Cushing's disease
Group II: Patients with adrenal Cushing's syndrome and ectopic ACTH syndrome
Group III: Normal individuals

Side effects

After desmopressin injection, no significant side effects which caused interruption of the test were noticed in any patients. Only one patient complained about flushing during the test and it was transient.

Discussion

Cushing's syndrome is a constellation of clinical signs and symptoms resulting from chronic glucocorticoid excess. There is no diagnostic laboratory test to confirm the existence and then to determine the cause of Cushing's syndrome with a 100% sensitivity and specificity. Many authors have compared the value of different tests for this purpose but the results have not been enough to find the best diagnostic test. So new approaches are going on in this area.

The desmopressin test has recently been introduced in clinical practise as an adjunctive tool in the differential diagnosis of ACTH-dependent Cusing's syndrome. In this study, we decided to determine the diagnostic accuracy of desmopressin stimulation test by comparing the cortisol and ACTH responses to DDAVP injection of patients with CD, AC, EC and healthy individuals.

The present study confirms the ability of desopressin to stimulate ACTH and cortisol secretion in most patients with CD, as previously reported by the other studies (14-16,26-28). Both absolute net increment and peak levels of cortisol and ACTH were found to be significantly higher from baseline levels in patients with CD.

Malerbi *et al.* measured the cortisol response to 5 or 10 μg of intravenous desmopressin in patients with CS and in normal subjects (14). Fifteen of 16 (94%) patients with CD and two patients with adrenal nodular hyperplasia exhibited a response, though none of the eight patients with adrenal tumour and one patient with EC responded to the test. Furthermore, responses occurred in two out of 15 normal individuals. Tsagarakis *et al.* found that 21/25 of patients (84%) with CD showed cortisol increase greater than 20% from baseline, but only 3/20 (15%) of obese patients had the similar response. ACTH responses greater than 50% from baseline were observed in 23/25 (95%) of patients with CD and 3/20 (15%) of obese patients in the same study (26). These results are more or less the same with our findings. But we found less responders in patients with CD (71.42%) and more

responders in healthy controls (21.42%) according to cortisol and ACTH response criteria. Moreover, an other important aspect of our study is that, 2/3 (66.66%) of patients with ectopic ACTH syndrome (nos 25, 26) had positive responses to DDAVP with an extend increase in both cortisol and ACTH levels. In fact, cortisol and/or ACTH rises were reported in occasional patients with ectopic ACTH syndrome (15,29-31). This might question the value of the desmopressin test in the differential diagnosis of ACTH-dependent Cushing's syndrome. Table 5 shows the results of DDAVP test in patients with EC in different studies.

Table 5. Desmopressin testing results of patients with ectopic ACTH secretion in different studies.

	Number of subjects	DDAVP dose	Response criteria (according to ΔF% and ΔACTH%)	Number of responsive subjects
Malerbi et al*	1	10	-	0
Sakai et al	3	5	120%**	0
Newel-Price et al	5	10	20%-35%	1 and 3
Arlt et al	1	10	20%-35%	1
Colombo et al	1	10	20%-50%	0
Terzolo et al	5	10	20%-50%	2
Tsagarakis et al	5	10	20%-50%	3
Emral et al	3	10	20%-50%	2
Total	24			9 and 11

* Response criteria in this study was taken as an increase above baseline higher than four times the intra-assay coefficient of variation at baseline concentration

** According to ΔACTH%

*** Number of responsive patients according to ΔF% and number of responsive patients according to ΔACTH% , respectively

Two additional detailed studies found that ACTH-secreting bronchial carcinoid tumors overexpressed the V2 and V3 receptor subtypes (32,33). One of the patients with EC had bronchial carcinoid in our study and he responded to DDAVP as well. Consistent with previous findings, nearly one of four patients with ectopic ACTH responded to desmopressin. Because few patients with occult ectopic ACTH have been studied with desmopressin in vivo, the documentation of even one case with a false positive test raises serious questions about its usefulness.

Our findings shows that DDAVP test is not suitable for differentiation between ACTH-dependent Cushing syndrome. Fortunately, positive cortisol or ACTH responses were only observed 1/9 (11.11%) of patients with AC. On the other hand, as it is well known, in general, it is not difficult to differentiate adrenal and pituitary origin of hypercortisolism. Determination of plasma ACTH level is usually enough in that situation. Individual analysis of the responders in patients with AC showed that one of these patients had bilateral macronodular hyperplasia (no 17). In the study of Malerbi et al, it was reported that two patients with adrenal nodular hyperplasia responded to DDAVP, as well (14). These results may show that patients presenting with bilateral nodular hyperplasia can respond to DDAVP but it is obvious that more studies are needed on the subject.

In different studies, sensitivity and specificity of DDAVP test were found dissimilar in different occasions. In the study of Malerbi et al. (3) the sensitivity of the test was found to be 100% while the specificity was much more lower with 64%, when the test was used in differentiating Cushing's disease from depression. Comparing the patients with Cushing's disease and patients with obesity, Tsagarakis et al. found the sensitivity of DDAVP test 92% and specificity 85%, according to ACTH responses (26). By using ROC analysis, Terzolo et al. found the sensitivity of the test 87% and the specificity 89%, and the test was not found reliable in determining the aetiology of Cushing's syndrome when compared with CRH test (30). In the current study, the sensitivity and the specificity of the DDAVP test were found to be lower when compared to those studies and the same results were found whether 20% increase in cortisol or 50% increase in ACTH was taken as positive response criteria; the sensitivity was 71.42% and the specificity was 76.92%. Diagnostic accuracy was found to be 75%. When ROC curves are used to calculate the sensitivity and specificity of the test; the sensitivity was 78.6% whereas the specificity was 73.1% for cortisol increment, and the sensitivity was again 78.6% while the specificity was 76.9% for ACTH increment. AUCs for both cortisol and ACTH increase were statisticaly significant but this is not enough to approve the usefulness of desmopressin stimulation test. So it is hard to say that the test has superiority when compared with the classical diagnostic tests, like high-dose dexamethasone suppression test, in differential diagnosis of Cushing's disease.

In conclusion, these data provide some evidence that the desmopressin stimulation test is not an enough diagnostic tool for differential diagnosis of ACTH-dependent Cushing's syndrome, because hyperresponsive patients to the peptide are not so uncommon in ectopic ACTH-dependent Cushing's syndrome. On the other hand, the test has high

false negative and false positive results. Occasionally, DDAVP can be used in certain indications, may be instead of CRH, since it is cheap and safe, and easy to find. But we think that the test is not worthy in routine use to differentiate patients with Cushing's syndrome from healthy subjects or to differentiate patients with Cushing's disease from adrenal Cushing's syndrome or ectopic ACTH-dependent Cushing's syndrome, since better discriminating diagnostic tests with higher sensitivity and specificity are in clinical practical use.

References

1. Newell-Price J, Grossman AB. The differentail diagnosis of Cushing's syndrome. *Ann Endocrinol* **62:** 173-179, 2001.
2. Yanovski JA, Cutler JR. GB, Chrousos GP, Nieman LK. Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. *JAMA* **269:** 2232-2238, 1993.
3. Malerbi DA, Fraboso MCBV, Vieira Filho AHG, Brenlha EML, Mendonça BB. Cortisol and adrenocortico-tropin response to desmopressin in women with Cushing's disease compared with depressive illness. *J Clin Endocrinol Metab* **81:** 2233-2237, 1996.
4. Orth DN. Corticotropin-releasing hormone in humans. *Endocr Rev* **13:** 164-191, 1992.
5. Coslovsky R, Wajchenberg BL, Nogueira O. Hyperresponsiveness to lysine vasopressin in Cushing's disease. *Acta Endocrinol* **75:** 125-132, 1974.
6. Catania A, Cantalamessa L, Orsatti A, et al. Plasma ACTH response to corticotropin releasing factor in patients with Cushing's disease. Comparison with the lysine-vasopressin test. *Metabolism* **33:** 478-481, 1984.
7. Krieger DT, Luria T. Plasma ACTH and cortisol responses to TRF, vasopressin or hypoglycemia in Cushing's disease and Nelson's syndrome. *J Clin Endocrinol Metab* **44:** 361-368, 1977.
8. Tabarin A, San Galli F, Dezou S, et al. The corticotropin-releasing factor test in the differential diagnosis of Cushing's syndrome: a comparison with the lysine-vasopressin test. *Acta Endocrinol* **123:** 331-338, 1990.
9. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotrophin and β-endorphin. *Science* **213:** 1394-1397, 1981.
10. Antoni F. Novel ligand specificity of pituitary vasopressin receptors in the rat. *Neuroendocrinology* **39:** 186-188, 1984.
11. Sugimoto Y, Sato M, Mochizuki S, Watanabe Y, Hashimoto S, Kawashima H. Molecular cloning and functional characterization of the cDNA encoding the human V1b vasopressin receptor. *J Biol Chem* **269:** 27088-27092, 1994.
12. Sawyer WH, Acosta M, Manning M. Structural changes in the arginine vasopressin molecule that prolong its antidiuretic action. *Endocrinology* **95:** 140-149, 1974.
13. Richardson DW, Robinson AG. Desmopressin. *Ann Intern Med* **103:** 228-239, 1985.
14. Malerbi DA, Mendonca BB, Liberman B, et al. The desmopressin stimulation test in the differential diagnosis of Cushing's syndrome. *Clin Endocrinol* **38:** 463-472, 1993.
15. Newell-Price J, Perry L, Medbak S, et al. A combined test using desmopressin and corticotrophinreleasing hormone in the differential diagnosis of Cushing's syndrome. *J Clin Endocrinol Metab* **82:** 176-181, 1997.
16. Colombo P, Passini E, Re T, Faglia G, Ambrosi B. Effect of desmopressin on ACTH and cortisol secretion in states of ACTH excess. *Clin Endocrinol* **46:** 661-668, 1997.
17. Andersson KE, Arner B, Hedner P, Mulder JL. Effects of 8-lysine-vasopressin and synthetic analogues on release of ACTH. *Acta Endocrinol* **69:** 640-648, 1972.
18. Gaillard RC, Riondel AM, Ling N, Muller AF. Corticotropin releasing factor activity of CRF 41 in normal man is potentiated by angiotensin II and vasopressin but not by desmopressin. *Life Sci* **43:** 1935-1944, 1988.
19. Rado JP, Juhos E. Effect of 1-deamino-8-D-arginine vasopressin (DDAVP) on plasma cortisol (hydrocortisone). *J Clin Pharmacol* **16:** 333-337, 1976.
20. Scott LV, Medback S, Dinan TG. ACTH and cortisol release following intravenous desmopressin: a dose-response study. *Clin Endocrinol* **51:** 653-658, 1999.
21. Ceresini G, Freddi M, Paccotti P, Valenti G, Merchenthaler I. Effects of ovine corticotropin-releasing hormone injection and desmopressin coadministration on galanin and adrenocorticotropin plasma levels in normal women. *J Clin Endocrinol Metab* **82:** 607-668, 1997.
22. Kaye T, Crapo L. The Cushing's syndrome: an update on diagnostic tests. *Ann Intern Med* **122:** 434-444, 1990.
23. Boscaro M, Rampazzo A, Paoletta A, et al. Selective venous sampling in differential diagnosis of ACTH-dependent Cushing's syndrome. *Neuroendocrinology* **55:** 264-268, 1992.
24. Losa M, Mortini P, Dylgjeri S, et al. Desmopressin stimulation test before and after pituitary surgery in patients with Cushing's disease. *Clin Endocrinol* **55:** 61-68, 2001.
25. Ambrosi B, Colombo P, Bochicchio D, Bassetti M, Masini B, Faglia G. The silent corticotrophinoma: is clinical diagnosis possible? *J Endocrinol Invest* **15:** 443-452, 1992.
26. Tsagarakis S, Vasiliou V, Kokkoris P, Stavropoulos G, Thalassinos N. (1999) Assessment of cortisol and ACTH responses to the desmopressin test in patients with Cushing's syndrome and simple obesity. *Clin Endocrinol* **51:** 473-4777.
27. Sakai Y, Horiba N, Tozawa F, et al. Desmopressin stimulation test for diagnosis of ACTH-dependent Cushing's syndrome. *Endocr J* **44:** 687-695, 1997.

28. Moro M, Putignano P, Losa M, Invitti C, Maraschini C, Cavagnini F. The desmopressin test in the differential diagnosis between Cushing's disease and pseudo-Cushing states. *J Clin Endocrinol Metab* **85:** 3569-3574, 2000.

29. Arlt W, Dahia PLM, Callies F, et al. Ectopic ACTH production by a bronchial carcinoid tumour responsive to desmopressin in vivo and in vitro. *Clin Endocrinol* **47:** 623-627, 2001.

30. Terzolo M, Reimondo G, Ali A, et al. The limited value of the desmopressin test in the diagnostic approach to Cushing's syndrome. *Clin Endocrinol* **54:** 609-616, 2001.

31. Tsagarakis S, Tsigos C, Vasiliou V, et al. The desmopressin and combined CRH-desmopressin tests in the differential diagnosis of ACTH-dependent Cushing's syndrome : Constraints imposed by the expression of V2 vasopressin receptors in tumors with ectopic ACTH secretion. *J Clin Endocrinol Metab* **87:** 1646-1653, 2002.

32. Dahia PLM, Ahmed-Shuaib A, Jacobs RA, et al**.** Vasopressin receptor expression and mutation analysis in corticotropin-secreting tumors. *J Clin Endocrinol Metab* **81:** 1768-1771, 1996.

33. De Keyzer Y, Lenne F, Auzan C. The pituitary V3 vasopressin receptor and the corticotroph phenotype in ectopic ACTH syndrome. *J Clin Invest* **97:** 1311-1318, 1996.