

The desmopressin test in the diagnosis of Cushing's disease

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Abstract: Introduction. A precise and early diagnosis of the underlying cause of Cushing's syndrome is necessary for successful treatment. The treatment of choice in ACTH-dependent Cushing's syndrome of pituitary origin is transsphenoidal pituitary adenoma resection. In patients, without visible pituitary adenoma on magnetic resonance imaging (MRI), surgical treatment should only be considered if the results of dynamic tests are unequivocal. **Objectives.** To assess usefulness of the desmopressin test in comparison with high-dose dexamethasone suppression test and CRH test in establishing the diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin. **Patients and methods.** 15 patients with ACTH-dependent Cushing's syndrome were studied. Pituitary microadenoma was surgically and histologically confirmed in all patients (only in 10 of them did pituitary MRI show a microadenoma). A control group consisted of 15 subjects, in whom Cushing's syndrome and other endocrine abnormalities were excluded. The following procedures were performed in all studied patients: daily rhythm of cortisol secretion, high-dose dexamethasone suppression test, CRH test and desmopressin test. **Results.** False negative responses were observed: in 1 patient to dexamethasone, in 1 patient to CRH and in 2 patients to desmopressin. In the other patients results of all 3 tests were positive. In 4 out of 5 patients without microadenoma seen on pituitary MRI, the desmopressin test yielded positive results. In the control group the results of dexamethasone and CRH test were positive in all patients, whereas the desmopressin test gave negative results (neither ACTH nor cortisol secretion increased after desmopressin administration in any patient). **Conclusions.** 1) The desmopressin test performed together with high-dose dexamethasone suppression test and CRH test may, in some cases, enhance the precision of final diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin. 2) The desmopressin test allows a very precise identification of healthy subjects.

Key words: Cushing's disease, desmopressin test

INTRODUCTION

The main diagnostic dilemma in ACTH-dependent Cushing's syndrome is to identify and locate (pituitary or especially extrapituitary location) an ACTH-producing tumor [1-3]. The most common cause of ACTH-dependent Cushing's syndrome is a pituitary microadenoma. However, pituitary microadenoma may not always be apparent on magnetic resonance imaging (MRI), whereas as many as 10% of population is known to have incidentaloma – a non-secreting pituitary adenoma [4]. Therefore, underlying cause of ACTH-dependent Cushing's syndrome can not be diagnosed simply on the basis of MRI scans with visible pituitary adenoma.

The dynamic tests are crucial in differential diagnosis. A number of tests is needed to confirm diagnosis in any case of

suspected ACTH-dependent Cushing's syndrome, as a single test is of limited diagnostic value. The high-dose dexamethasone suppression test and CRH (*corticotropin-releasing hormone*) test belong to the most common dynamic tests [2,5,6], while the desmopressin test is used less frequently [7-9]. The dynamic tests could replace an inferior petrosal sinuses sampling study with concomitant measurement of ACTH gradient, which according to some authors is a "gold standard" procedure in a diagnostic process of ACTH-dependent Cushing's syndrome of unknown origin, even though it is technically challenging and may lead to serious complications [2,10].

A precise and early diagnosis of the underlying cause of Cushing's syndrome is crucial for successful treatment. In ACTH-dependent Cushing's syndrome of pituitary origin a transsphenoidal pituitary adenoma resection is the treatment of choice. In patients without visible pituitary adenoma on MRI, qualification to surgical treatment should be supported by unequivocal results of dynamic tests.

The study aimed primarily to assess usefulness of the desmopressin test in comparison with high-dose dexamethasone suppression test and CRH test in establishing the diagnosis

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Table 1. Characteristics of patients with ACTH-dependent Cushing's syndrome of pituitary origin

Nr	Gender	Age	Cortisol				ACTH (pg/ml)	Pituitary microadenoma on MRI
			8.00 a.m.	6.00 p.m.	in dexamethasone suppression test			
			µg/dl	µg/dl	µg/dl*	%**		
1	M	38	26.60	11.90	1.76	93.4	57.30	+
2	F	33	26.20	17.20	3.07	88.3	13.26	–
3	M	24	14.90	18.30	1.57	89.5	18.66	+
4	F	37	32.55	17.01	1.10	96.6	37.20	+
5	F	50	37.60	27.60	2.34	93.8	20.70	+
6	F	48	24.70	28.03	14.30	42.1	11.00	+
7	F	45	40.00	36.40	12.10	69.7	34.00	+
8	M	22	21.70	27.80	1.19	94.5	15.00	+
9	F	38	29.00	17.45	7.86	72.9	15.04	+
10	F	44	30.98	21.60	6.50	79.0	31.30	+
11	M	45	27.43	32.63	4.73	82.7	20.30	–
12	M	51	24.90	25.00	4.85	80.5	52.00	–
13	M	41	30.40	31.50	12.40	59.2	60.33	–
14	M	41	30.00	36.00	11.80	60.6	34.58	–
15	F	34	31.00	31.20	3.30	89.3	19.00	+
X	8F	39.4±	28.53±	25.30±	5.92±	79.47±	29.31±	10 „+”
SEM	7M	2.13	1.51	1.91	1.15	3.94	4.08	5 „–”

Data presented as mean ± SEM

* Serum cortisol level after oral administration of 8 mg of dexamethasone at 11.00 p.m.

** Percentage suppression of serum cortisol

of ACTH-dependent Cushing's syndrome of pituitary origin. The second objective was to answer the question whether the desmopressin test is unequivocal enough to establish the final diagnosis and qualify for a transsphenoidal pituitary adenoma resection in patients without visible pituitary microadenoma on MRI scans.

PATIENTS AND METHODS

15 consecutive patients (8 women and 7 men) with ACTH-dependent Cushing's syndrome were included into the study. The pituitary origin was confirmed in all patients during the surgery using histological and immunohistochemical methods (all patients underwent transsphenoidal pituitary microadenoma resection). Tests were also carried out in 15 subjects (11 women and 4 men), in whom Cushing's syndrome and other endocrine abnormalities were excluded. Characteristics of both groups are shown in tables 1 and 2.

The diagnosis of Cushing's syndrome was established pre-operatively in all subjects based on: typical clinical symptoms, inappropriately high urinary free cortisol and high serum cor-

tisol levels found on at least 2 occasions before the admission to the inpatient department. Cortisol levels were checked again during hospitalization, and daily rhythm of cortisol secretion (serum cortisol values at 8.00 a.m. and 6.00 p.m.) proved to be abnormal in all patients. The normal ranges for serum cortisol levels were defined as follows: 5–15 µg/dl for morning concentrations and 2.5–7.5 µg/dl for evening values. Daily cortisol rhythm was considered pathological if the evening serum cortisol level was higher, comparable or did not decrease adequately compared to the morning values.

In all patients the morning (8.00 a.m.) serum ACTH was also measured. Afterwards, the patients underwent high-dose dexamethasone suppression test, CRH test and desmopressin test. The high-dose dexamethasone suppression test was performed as follows: 8 mg of dexamethasone were administered orally at 11.00 p.m. and blood samples for serum cortisol measurement were obtained twice, in the morning (8.00 a.m.) before and after dexamethasone administration. CRH test consisted of intravenous administration of 100 µg of CRH (Ferring) and blood sampling (from basilic vein) for serum cortisol and ACTH measurement at -30, 0, 20, 30, 60, 90, 120, 180, and 240 minutes. Desmopressin test involved intra-

Table 2. Results of CRH test and desmopressin test in patients with ACTH-dependent Cushing's syndrome of pituitary origin

Nr	CRH test						Desmopressin test					
	cortisol			ACTH			cortisol			ACTH		
	Δ $\mu\text{g/dl}$	$\Delta\%$	test	Δ pg/ml	$\Delta\%$	test	Δ $\mu\text{g/dl}$	$\Delta\%$	test	Δ pg/ml	$\Delta\%$	test
1	9.06	72.2	+	53.17	66.1	+	10.19	56.6	+	24.78	73.2	+
2	12.07	92.6	+	53.65	248.0	+	0	0	–	7.88	59.4	+
3	10.89	126.4	+	41.85	216.7	+	0	0	–	0.22	1.17	–
4	14.91	159.3	+	46.22	273.1	+	8.23	79.8	+	10.87	48.8	+
5	22.62	102.5	+	77.70	197.7	+	30.62	124.0	+	107.5	459.4	+
6	6.2	31.6	+	2.25	11.05	–	9.0	47.1	+	21.36	133.2	+
7	5.2	50.0	+	30.21	61.36	+	4.5	43.7	+	18.87	40.0	+
8	3.8	17.3	–	12.09	22.5	–	3.2	20.1	+	11.68	71.4	+
9	13.64	88.4	+	74.19	424.6	+	13.35	131.2	+	45.41	301.9	+
10	44.21	248.2	+	33.01	189.9	+	19.96	102.3	+	16.44	85.7	+
11	14.14	53.8	+	45.06	82.5	+	2.79	9.2	–	20.35	63.4	+
12	8.69	33.0	+	77.70	98.2	+	4.1	10.4	–	9.99	13.2	–
13	10.85	39.7	+	83.96	165.6	+	13.8	42.3	+	11.32	18.7	–
14	21.4	68.1	+	24.21	70.0	+	22.5	73.5	+	–	–	
15	16.71	105.9	+	–	–	–	9.0	39.1	+	–	–	

Δ – an increase (relatively to baseline value) in serum cortisol or ACTH level after CRH or desmopressin administration

$\Delta\%$ – an increase (relatively to baseline value) in serum cortisol or ACTH level after CRH or desmopressin administration given as the percentage of basal level

venous administration of 10 μg of desmopressin (Minirin Ferring) and blood sampling (from basilic vein) for plasma cortisol and ACTH measurement at -30, 0, 15, 30, 45, 60, 90, and 120 minutes. Serum cortisol concentration was measured using a RIA method (normal range for morning samples: 5–15 $\mu\text{g/dl}$, for evening samples 2.5–7 $\mu\text{g/dl}$). For serum ACTH measurement an IRMA method was used (normal range 10–60 pg/ml). The following interpretation of test results was adopted: high-dose dexamethasone test was positive if serum cortisol level obtained after dexamethasone administration decreased by at least 50%, compared with the basal value [11]. The Limit of detection for method used for cortisol concentration the measurement was 0.2 $\mu\text{g/dl}$. CRH test was positive if serum ACTH level increased by at least 35% (in terms of percentage of basal value - $\Delta\%$) over the basal value (calculated as a mean from -30' and 0' samples values), and cortisol level increased ($\Delta\%$) by at least 20% [12]. In agreement with other authors' recommendations [13], the same interpretation criteria were used for desmopressin test.

All patients with Cushing's syndrome underwent pituitary MRI and CT (*computed tomography*) of adrenal glands. The same imaging studies were performed in some subjects from the control group.

Statistical analysis of results was carried out. Arithmetical mean values and standard error of the mean (SEM) were calculated. Homogeneity of both groups in terms of the tested

parameters was tested with the Student's t test (if data had normal distribution) or Mann-Whitney U test (if data lacked normal distribution). Statistical significance was defined as $p < 0.05$.

RESULTS

Results in patients with ACTH-dependent Cushing's syndrome of pituitary origin

Serum ACTH values fell within normal range in all studied subjects (table 1).

High-dose dexamethasone suppression test was positive in 14 out of 15 patients. In 1 case the test yielded false-negative results. CRH test was positive for both ACTH and cortisol values in 12 patients and negative in one subject. In one woman positive response was observed only regarding the cortisol level (ACTH failed to increase significantly after CRH stimulation) and another woman had a positive test result with only serum cortisol response measured. The desmopressin test was positive for both ACTH and cortisol values in 8 patients but negative in 2 subjects (tables 4 and 5). In 2 other patients, after desmopressin administration the ACTH response was significant but cortisol failed to increase adequately. In one

Table 3. Characteristics of the control group

Nr	Gender	Age	Cortisol				ACTH pg/ml
			8.00 a.m.	6.00 p.m.	in dexamethasone suppression test		
			µg/dl	µg/dl	µg/dl*	%**	
1	F	27	12.80	2.6	1.82	85.8	13.89
2	M	27	11.93	3.4	1.51	87.3	18.91
3	M	20	12.11	5.2	1.42	88.2	18.66
4	F	56	8.19	3.1	1.65	79.8	15.68
5	F	40	10.16	2.8	2.03	80.0	24.34
6	F	39	12.40	6.1	1.50	87.9	121.37
7	F	32	11.25	3.6	1.32	98.2	15.63
8	F	27	10.80	7.0	1.89	82.5	16.04
9	M	21	9.61	3.1	0.61	93.6	21.40
10	F	38	8.71	2.8	1.20	86.2	18.42
11	F	58	12.30	5.1	2.57	79.1	17.90
12	M	23	7.30	2.7	1.15	84.2	24.77
13	F	43	13.00	5.3	2.90	77.7	21.47
14	F	29	14.10	6.3	2.00	85.2	17.23
15	F	66	11.21	4.8	1.44	87.2	24.51
X	11F	36.4±	11.05±	4.26±	1.66±	85.52±	18.74±
SEM	4M	3.53	0.48	0.37	0.14	5.30	0.96

* Serum cortisol level after oral administration of 8 mg of dexamethasone at 11.00 p.m.

** Percentage suppression of serum cortisol

man cortisol level rose significantly but ACTH response was not meaningful. In 2 other patients the test was positive with only serum cortisol measured. In 11 patients the results of all 3 tests were positive; in 1 man the dexamethasone test was negative with positive results of the remaining two tests; in another patient the CRH test was negative but the remaining two were positive and in last 2 patients the desmopressin test was negative but the remaining 2 tests were positive (table 2). In conclusion: the dexamethasone suppression test and CRH test were positive in 93% of patients, whereas the desmopressin test yielded positive results in 86% of subjects.

Pituitary microadenoma was recognizable on MRI scans in 10 out of 15 patients (67% of the studied group). In all patients without visible microadenoma on pituitary MRI, the dexamethasone suppression test and CRH test produced positive results, while the desmopressin test was positive in 4 out of 5 patients in this subgroup. Among patients with pituitary microadenoma recognizable on MRI, all 3 test yielded positive results in 7 cases. In the remaining 3 patients with visible microadenoma, 2 out of 3 tests performed were positive (in 1 case the dexamethasone suppression test and the CRH test, in 1 case the CRH test and the desmopressin test and in 1 case the dexamethasone suppression test and the desmopressin test).

Results in patients from the control group

In all studied subjects serum ACTH values were within normal range. The dexamethasone suppression test (table 3) as well as the CRH test were positive, and the desmopressin test was negative in all patients (table 4).

Pituitary MRI or CT of adrenal glands, if done, did not reveal any pathological structures.

DISCUSSION

The correct diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin may be difficult if pituitary or adrenal incidentaloma is present. Therefore, dynamic tests performed after initial screening examinations, are of crucial significance in the process of differential diagnosis. Nevertheless, result of any single test is insufficient for hundred per cent final diagnosis, and several tests should always be performed [2,3,7,12]. Diverse combinations of tests can be chosen [2,3,7,12,14]. The desmopressin test is used only in some centers. Desmopressin is a long-acting analog of vasopressin, with known antidiuretic and weak vasoconstrictive activity. Apart from that, desmo-

Table 4. Results of CRH test and desmopressin test in the control group

NR	CRH test						Desmopressin test					
	Cortisol			ACTH			Cortisol			ACTH		
	Δ $\mu\text{g/dl}$	$\Delta\%$	test	Δ pg/ml	$\Delta\%$	test	Δ $\mu\text{g/dl}$	$\Delta\%$	test	Δ pg/ml	$\Delta\%$	test
1	9.30	70.4	+	30.52	186.5	+	0.60	4.91	–	0.16	1.15	–
2	11.42	129.7	+	9.45	149.0	+	0.10	0.84	–	0.07	0.37	–
3	10.52	70.2	+	16.66	74.0	+	0	0	–	0	0	–
4	13.93	106.9	+	41.40	381.5	+	1.75	15.11	–	0.75	4.78	–
5	8.30	60.1	+	19.76	97.8	+	1.50	16.12	–	0	0	–
6	12.31	103.7	+	12.00	78.9	+	1.53	13.05	–	1.59	12.85	–
7	13.53	97.5	+	20.79	133.0	+	1.79	13.07	–	0	0	–
8	7.96	60.7	+	12.74	60.2	+	0.92	7.41	–	0	0	–
9	13.10	105.8	+	17.50	168.2	+	1.82	12.72	–	1.1	5.14	–
10	11.98	97.5	+	30.47	202.7	+	1.91	14.82	–	0	0	–
11	12.02	144.8	+	18.20	144.4	+	0	0	–	0	0	–
12	9.00	121.6	+	17.33	69.1	+	0	0	–	0	0	–
13	21.95	257.3	+	22.68	208.0	+	0.91	7.93	–	0	0	–
14	23.94	168.5	+	12.86	164.0	+	1.64	12.81	–	0	0	–
15	15.30	107.8	+	12.35	78.1	+	1.1	8.20	–	0	0	–

Δ – an increase (relatively to baseline value) in serum cortisol or ACTH level after CRH or desmopressin administration

$\Delta\%$ – an increase (relatively to baseline value) in serum cortisol or ACTH level after CRH or desmopressin administration given as the percentage of basal level

Table 5. Results of CRH test and desmopressin test in patients with ACTH-dependent Cushing's syndrome of pituitary origin (A) and in patients from the control group (B)

Group	Nr	CRH test				Desmopressin test			
		cortisol		ACTH		cortisol		ACTH	
		Δ $\mu\text{g/dl}$	$\Delta\%$	Δ pg/ml	$\Delta\%$	Δ $\mu\text{g/dl}$	$\Delta\%$	Δ pg/ml	$\Delta\%$
A	15	14.29 $\pm$	85.93 $\pm$	46.80 $\pm$	151.95 $\pm$	10.08 $\pm$	51.95 $\pm$	23.59 $\pm$	105.34 $\pm$
		2.46	14.83	6.51*	29.69	2.18*	10.66*	7.31*	34.87*
B	15	12.97 $\pm$	113.50 $\pm$	19.64 $\pm$	146.36 $\pm$	1.03 $\pm$	8.46 $\pm$	0.24 $\pm$	1.61 $\pm$
		1.14	12.49	2.15	20.55	0.18	1.50	0.12	0.88

Data presented as mean $\pm$ SEM

Δ – an increase (relatively to baseline value) in serum cortisol or ACTH level after CRH or desmopressin administration

$\Delta\%$ – an increase (relatively to baseline value) in serum cortisol or ACTH level after CRH or desmopressin administration given as the percentage of baseline level

* statistically significant differences between mean values in studied groups, $p < 0.01$

pressin binds with V3 receptors on pituitary corticotrophs. As corticotrophs in pituitary adenomas express more V3 receptors than the same cells in normal pituitaries [15], intravenous administration of desmopressin bolus in healthy people does not cause an increase in serum concentrations of either ACTH or cortisol. On the contrary, in 84–89% of patients with ACTH-dependent Cushing's syndrome of pituitary origin, desmopres-

sin administration results in an increased secretion of ACTH and cortisol [7,8,12,16-19].

The study was aimed at comparing a value of the desmopressin test with high-dose dexamethasone suppression test and CRH test in establishing the diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin. 15 patients with typical clinical symptoms of Cushing's syndrome, confirmed

by high serum cortisol (at least 2 abnormal results obtained before admission to the inpatient department) and inappropriately high urinary free cortisol entered the study. In all cases the diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin was confirmed intraoperatively (all patients underwent transsphenoidal pituitary microadenoma resection), histologically and immunohistochemically.

Morning serum ACTH level was normal in 14 out of 15 patients. In one case (patient nr 13 – table 1), ACTH value was marginally elevated (60,33 pg/ml, with the upper normal range <60 pg/ml). It is believed that serum morning ACTH value of less than 10 pg/ml, in a patient with proven high serum cortisol is highly suggestive of ACTH-independent Cushing's syndrome, whereas concentrations exceeding 10 pg/ml, but still within normal ranges, may be found in patients with ACTH-dependent Cushing's syndrome of pituitary origin. It is typically (but not only) observed in patients with pituitary adenoma that secretes ACTH episodically.

All 3 tests yielded positive results in 11 out of 15 patients. In 1 patient out of the remaining 4, the CRH test turned out to be negative with positive results of the remaining tests. In another patient the dexamethasone suppression test was negative, yet the remaining two tests were positive. In last 2 patients the negative results came from desmopressin tests, while CRH and dexamethasone tests were positive. Presented results may suggest that all 3 performed tests have similar diagnostic value and, to some degree, are complementary to one another. In the studied group of 15 patients with ACTH-dependent Cushing's syndrome of pituitary origin, the desmopressin tests were positive in 86% subjects, as confirmed by postoperative pathological examination. Among patients from the control group, results of the desmopressin test were normal in all cases, therefore there were no false-positive results. Similarly, the high-dose dexamethasone suppression test was positive in 93% of patients and yielded no false-positive results. The CRH test must be interpreted differently, as the positive response is present in healthy people and at the same time it is typical for Cushing's disease (Cushing's syndrome caused by pituitary adenoma). It is impossible to univocally estimate the risk of drawing a false-positive conclusion from the results of dynamic tests used in this study, since the number of participants was small.

It should be pointed out that the desmopressin test was unequivocally positive in 2 patients, among whom one had negative results in the dexamethasone suppression test and the other – negative CRH test. Therefore, in selected cases the desmopressin test may be helpful in establishing the final diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin and in qualifying the patient for transsphenoidal pituitary microadenoma resection (even though microadenoma is not confirmed by pituitary MRI). The results obtained by other authors comparing the CRH test with the desmopressin test [9] or the CRH test with the high-dose dexamethasone suppression test [14] were similar, still there are no studies comparing all three tests at the same time.

The second objective was to assess, if results of the desmopressin test are unequivocal enough to ultimately establish the final diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin, in patients with Cushing's syndrome but no pituitary adenoma recognizable on MRI scans. The results of this study indicate that the desmopressin test may be helpful in such cases, but not fully conclusive. It was positive in 4 out of 5 patients in whom MRI did not reveal pituitary microadenoma. However, it was univocally negative in all subjects, in whom Cushing's syndrome and other endocrinopathies were excluded. Therefore, the desmopressin test may be a useful tool for 1) selecting healthy subjects and 2) excluding the diagnosis of ACTH-dependent Cushing's syndrome of pituitary origin in people with pituitary tumor found on MRI, that is a pituitary incidentaloma that does not secrete ACTH.

Ectopic ACTH secretion was neither diagnosed nor suspected in studied patients with ACTH-dependent Cushing's syndrome, as indicated by not increased serum ACTH levels and long follow-up period after the neurosurgery. In one case the CRH test was negative, but it is well accepted that CRH test results can neither confirm nor exclude ectopic ACTH production. Assessment of the value of the desmopressin test in diagnosing ectopic ACTH secretion was not the aim of this study, which was carried out only in patients with confirmed ACTH-dependent Cushing's syndrome of pituitary origin.

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