A Case of Cortisol Producing Adrenal Adenoma Associated with a Latent Aldosteronoma: Usefulness of the ACTH Loading Test for the Detection of Covert Aldosteronism in Overt Cushing Syndrome

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Abstract

A 36-year-old woman with Cushing syndrome was evaluated for coexisting hyperaldosteronism, which was suggested by an abnormal response of the aldosterone-to-cortisol ratio in peripheral blood to the ACTH-administration despite a low basal aldosterone-to-renin ratio. Computed tomography revealed two independent tumors in the left adrenal gland, and adrenal venous sampling indicated hyperaldosteronism in addition to hypercortisolism in the same side. Postsurgical study including immunohistochemical analysis of steroidogenic enzymes suggested one adenoma to be cortisol-producing and the other, aldosterone-producing. The comorbidity of these different hormone-producing adenomas is not rare and careful pre-surgical evaluation is necessary to avoid post-surgical exacerbation of latent hyperaldosteronism.

Key words: Cushing syndrome with latent hyperaldosteronism, ACTH loading test

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Introduction

Once believed to be rare, primary aldosteronism (PA) is now known to be a more common disorder that represents the secondary hypertension (1-3). This epidemiological transition is a result of the advanced screening method using aldosterone-to-renin ratio (ARR) and the subsequent confirmatory tests, and also due to the detection of smaller aldosterone-producing adrenal adenoma (APA) and hyperplastic lesions of idiopathic hyperaldosteronism (IHA) that cannot be found by usual imaging tests, but which are found only by advanced technique of adrenal venous sampling (AVS). In addition, a radiologically detectable adrenal lesion in a PA patient may be a non-functioning adenoma or nodule combined with other occult APA(s) or IHA as previously

reported (4). Therefore, when a surgical treatment is planned in a patient with PA, a definite localization of the responsible lesion(s) using AVS should be performed to avoid an erroneous adrenalectomy (5-7). In contrast, AVS is not recommended as a diagnostic procedure for adrenal Cushing syndrome (CS), because most of the cortisol-producing lesions are large enough to be identified by CT or MRI. In addition, an adrenal scintigram is effective to detect a definite uptake of iodine-125-aldosterone into the lesion in CS patients, although it is not always effective in PA patients due to the generally smaller size of the aldosterone-producing lesion and a lower level of iodine-125-aldosterone uptake compared with CS patients.

It is well known that the two disorders described above, PA and CS (including overt and subclinical), sometimes develop concomitantly, and a clinical guideline for the preoperative correct diagnosis of such cases has been proposed in

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Table 1. Clinical Data on Admission

Blood			Urine		
		(normal range)			(normal range)
ACTH	1.00 pg/mL	(7.4-55.7)	U-free cortisol	99.6 µg/day	(11.2-80.3)
Cortisol	17.5 µg/dL	(6.5-25)	U-Aldosterone	13 µg/day	< 10 µg/day
DHEA-S	36 µg/dL	(23-266)	U-Ad	18 µg/day	(1-11)
Testosterone	33.3 ng/dL	(6-86)	U-MN	0.02 mg/day	(0.04-0.19)
PRA	0.3 ng/mL/h	(0.3-5.4)	U-NMN	0.02 mg/day	(0.09-0.33)
PAC	4.89 ng/dL	(2.2-15)	VMA	173 µg/day	(17.2-181.0)
ARR	16.3				
P-Ad	31 ng/mL	(<100)			
P-NAd	129 ng/mL	(100-450)			
P-DO	10 ng/mL	(<20)			
TSH	0.77 µU/mL	(0.5-5.0)			
F-T3	2.91 pg/mL	(2.3-4.0)			
F-T4	1.61 ng/dL	(0.9-1.7)			
PRL	7.4 ng/mL				
IRI	8.5 µU/mL				

ACTH, adrenocorticotrophic hormone; DHEA-S, dehydroepiandrosterone sulfate; PRA, plasma renin activity; PAC, plasma aldosterone concentration; P-Ad, plasma adrenaline; P-NAd, plasma noradrenaline; P-DO, plasma dopamine; TSH, thyroid-stimulating hormone; FT3, free T3; FT4, free T4; PRL, prolactin; IRI, immunoreactive insulin; U-Ad, urinary adrenaline; U-NAd, urinary noradrenaline; U-MN, urinary metanephrine; U-NMN, urinary normetanephrine; VMA, vanillylmandelic acid.

a literature (8). However the literature did not clarify the differences between PA cases with subclinical CS (SCS) and those with overt CS, probably the former with detectable levels and the latter with undetectable levels of ACTH suppressed by autonomic secretion of cortisol. This case report demonstrates the usefulness of the ACTH loading test and subsequent AVS for the correct diagnosis of covert hyperaldosteronism in patients with overt CS. The patient initially presented with typical CS, and was finally found to have two separate adrenal adenomas. The post operative histopathological analysis indicated that one of them was a cortisol-producing adenoma (CPA) and the other an APA.

Case Report

A 36-year-old woman was referred to our hospital under the suspicion of CS. She had suffered from schizophrenia since the age of 19 years old, and had also developed hypertension, hyperlipidemia and glucose intolerance at the age of 31. Although medication with amlodipine besylate (5 mg/day), valsartan (160 mg/day), carvedilol (20 mg/day) and simvastatin (10 mg/day) had been continued thereafter, control of hypertension and hyperlipidemia had remained insufficient (blood pressure was 140/90-172/118 mmHg, LDL-cholesterol was 142 mg/dL). A few months before referral to our hospital, an examination of her right hypochondrial pain by CT scan identified a gallstone, as well as a tumor of the left adrenal gland.

On admission, she was obese (body weight: 75.0 kg, BMI: 31.6) and hypertensive (176/128 mmHg). Physical examination revealed moon face and central obesity, although purple striae of abdominal skin were absent. Other routine

examinations showed no abnormality. An examination protocol for hypercortisolemic patients in our institution was undertaken. The levels of ACTH concentration and plasma renin activity (PRA) were low, PAC was within normal range, PAC to PRA rate (ARR) was also within normal range, and urinary excretions of both free cortisol and aldosterone were slightly elevated (Table 1). Impaired glucose tolerance was present on the basis of the 75 g oral glucose tolerance test (Table 2a). There was neither circadian variation in serum cortisol nor suppression of serum cortisol by 1 and 8 mg of dexamethasone administration (Table 2b, c). These results were consistent with CS. Three examinations were performed according to the guideline proposed by the Japanese Endocrine Society (JES) for PA diagnosis (9). First, PAC was completely suppressed in a saline-loading test (Table 2e), secondly ARR was suppressed to 1.56 in a captopril challenge test (Table 2f), and lastly PRA was elevated from 0.5 to 2.5 ng/mL/h in a furosemide-upright position challenge test (Table 2g). These results did not meet the JES guideline to confirm the interim diagnosis of PA. However, re-evaluation using thin slice CT revealed a small mass sized 1.0 cm in diameter in addition to the pre-recognized mass with 3.0 cm in diameter in left adrenal gland (Fig. 1a). Adrenal scintigraphy with ¹³¹I-6-β-iodomethylnorcholesterol showed a significant uptake by the left adrenal gland (Fig. 1b). Lastly, ACTH loading test (0.25 mg of cortrosyn[®]) was performed, in which the peak value of PAC to cortisol ratio was 1.19 (Table 2d). Since this value was regarded to indicate a high possibility of PA complication in the patient (8), AVS was performed.

Upon AVS, the levels of cortisol and PAC after ACTH loading were 969 µg/dL and 4,140 ng/dL, respectively in the

Table 2. Endocrinological Examinations

<u>a)75gOGTT</u>				
Time (min)	0	30	60	120
Glucose (mg/dL)	119	226	285	169
IRI (μU/mL)	15.9	26.9	51.5	59.2
<u>b) Circadian rhythm of cortisol and ACTH</u>				
Time (h)	7:30	14:00	17:00	23:00
Cortisol (μg/dL)	17.5	15.7	16.3	16.3
ACTH (pg/mL)	1.0	1.0	1.0	1.0
<u>c) 1-mg or 8-mg overnight dexamethasone suppression test</u>				
	ACTH (pg/mL)		Cortisol (μg/dL)	
1-mg DST	1.0		18.4	
8-mg DST	1.0		20.1	
<u>d) ACTH administration test</u>				
Time (min)	0	30	60	
PAC (ng/dL)	10.6	36.8	33.0	
Cortisol (μg/dL)	19.1	30.8	37.7	
PAC / Cortisol		1.19	0.88	
<u>e) Saline-loading test</u>				
Time (hr)	0	4		
PRA (ng/mL/hr)	0.7	0.5		
PAC (ng/dL)	3.27	2.30		
<u>f) Captopril challenge test</u>				
Time (min)	0	90		
PRA (ng/mL/hr)	0.3	0.8		
PAC (ng/dL)	4.89	1.25		
ARR	16.3	1.56		
<u>g) Furosemide-upright position challenge test</u>				
Time (min)	0	120		
PRA (ng/mL/hr)	0.5	2.5		
PAC (ng/dL)	4.74	33.72		

left adrenal vein (Table 3). The corresponding values in the right adrenal vein were 71 μ g/dL and 1,123 ng/dL, respectively. We postulated that the left adrenal gland was producing not only cortisol but also aldosterone because the PAC value in left adrenal vein was greater than 1,400 ng/dL, which met the criteria for PA of the JES guideline (9). The results of adrenal venous sampling strongly suggested hyperaldosteronism in addition to hypercortisolism in the left adrenal gland. Therefore, laparoscopic left adrenalectomy was performed.

After surgery, serum cortisol level decreased immediately to 0.4 μ g/dL and hydrocortisone replacement (15 mg/day) was continued. Eight months after surgery, her blood pressure was 142/82 mmHg under administration of nifedipine-retard (40 mg/day), PAC was 2.54 ng/dL and PRA was 0.6 ng/mL/h. The Cushingoid features disappeared, and the abnormality of lipid metabolism improved, such that no medication was necessary. These data suggested that the remaining right adrenal gland had normal function.

Macroscopic examination of the resected specimens revealed the presence of two different cortical lesions. Both of them were diagnosed as adrenocortical adenoma. The one

measuring 25 mm in greatest dimension was admixed with tumor cells with clear cytoplasm (60%) and those with eosinophilic cytoplasm (40%) (Fig. 2a). Immunohistochemical analysis of steroidogenic enzymes demonstrated relatively abundant presence of both 17 α -hydroxylase (P450c17) and 3-beta-hydroxysteroid dehydrogenase (3BHSD) immunoreactivity in this tumor (Fig. 2d, g). The other adrenocortical adenoma measuring 12 mm in greatest dimension was predominantly composed of tumor cells with abundant clear cytoplasm, and immunoreactivity of 3BHSD but not of P450c17 was detected in the tumor cells (Fig. 2c, f, i). Attached non-neoplastic adrenal gland was markedly atrophic with little DHEAST immunoreactivity in the zonae fasciculata and reticularis, and "paradoxical hyperplasia of the zona glomerulosa" (10, 11) with scarce 3BHSD immunoreactivity was also detected (Fig. 2b, e, h). All of these findings indicated that the large tumor was a CPA according to the expression of c17 and histological features, and the other tumor could represent an APA according to the absence of c17 and light microscopic findings.

Figure 1. Adrenal dynamic CT and ^{131}I -Adosterol scintigram without dexamethasone suppression: Adrenal CT showed (A) the left bigger adrenal adenoma (arrow head) and (B) the smaller adrenal adenoma (arrow). Adrenal scintigraphy with ^{131}I -6- β -iodomethylnorcholesterol showed (C, D) significant uptake by the left adrenal.

Discussion

A relatively high prevalence of concomitant PA and CS (including overt and subclinical) has recently been shown (8). Aldosterone- and cortisol-co-secreting adrenal tumors are now also of interest as a subtype of PA (12). Cardiovascular complications may be more severe in such cases (13). Most of the reported cases of the comorbidity can generally be classified into three groups. They comprise (i) both aldosterone and cortisol being simultaneously produced from a single, sometimes multiple, adrenocortical adenoma (A/CPA), (14-20) (ii) aldosterone produced from APA and cortisol from CPA, and these adenomas may exist unilaterally or separately in each other side, (21-27) (iii) aldosterone from both adrenal glands with bilateral hyperplasia (IHA) and cortisol from CPA (28, 29).

Clinical and pathological features of PA associated with SCS have recently been described (30). Most of these cases were analyzed in those primarily diagnosed as PA and afterward as complicated with SCS, with average values of aldosterone to cortisol ratio after ACTH stimulation of 2.4 and 1.2 in patients with simple-PA and PA/SCS, respectively (30). Aldosterone to cortisol ratio after ACTH stimulation in patients with simple-CPA (mean value: approximately 0.5) was significantly lower than that in those with CPA/PA (mean value: approximately 1.3), while no significant difference of aldosterone to cortisol ratio after ACTH

stimulation between those with simple-PA (mean value: approximately 1.6) and CPA/PA was observed (8). It is noteworthy that both groups of simple-CPA and CPA/PA included both overt CS and SCS in the analysis (8). More recently, a recommendation has been made to perform screening test using ACTH loading to calculate the aldosterone to cortisol ratio after ACTH stimulation for all the patients with suspicion of CPA (overt CS or SCS with adrenal tumor), also suggesting a cut-off of >0.85 for subsequent AVS to detect complication of PA (31).

Upon AVS, the aldosterone level obtained from the right adrenal vein was 585 ng/dL at baseline. This finding rendered the possibility of hyperaldosteronism in the right adrenal gland as doubtful. However, the level of aldosterone after ACTH loading was 1,123 ng/dL, which did not reach the criteria for PA of the JES guideline (9). Furthermore, the postoperative progress and ARR level ($=4.2$) suggested that the remaining right adrenal gland functioned normally.

We reported herein a case originally diagnosed as overt CS and later as complicated with PA, with an aldosterone to cortisol ratio after ACTH stimulation ($=1.19$) meeting the proposed cut-off criteria of 0.85. Our opinion is that not only absolute values of aldosterone to cortisol ratio after ACTH stimulation but also relative changes of ACR and/or PAC levels before and after ACTH stimulation should be taken into account when considering concurrent PA, especially in patients with overt CS with very low levels of ACTH. Further study, however, will be needed to establish

Figure 2. Pathological analysis of the surgically removed left adrenal gland: (A) Macroscopic features of the cut surface of the left adrenal tumors. The larger adrenal lesion appeared brown in color and the smaller one appeared golden yellow in color. Immunohistochemical analysis: (B) Hematoxylin and Eosin staining (a, b, c), 3β -HSD immunohistochemistry (d, e, f), c17 immunohistochemistry (g, i), and DHEAST immunohistochemistry (h), a, d and g: the larger adrenocortical adenoma, b, e and h: non-neoplastic adrenal and c, f and i: the smaller adrenocortical adenoma.

Table 3. Results of Adrenal Venous Sampling

	Baseline			ACTH 30min		
	PAC (ng/dL)	Cortisol (μ g/dL)	PAC/Cortisol	PAC (ng/dL)	Cortisol (μ g/dL)	PAC/Cortisol
①IVC	<1.0	16.2	<0.06	17.7	27.6	0.64
②lt adrenal vein	7	257	0.03	4140	969	4.27
③rt adrenal vein	585	8.4	10.01	1122	71.0	15.80

the solid criteria using ACTH loading test to detect patients with hyperaldosteronism, which may be hidden in overt adrenal CS.

It is also necessary to seek a new method to judge the cannulation success at AVS, because the current method using cortisol concentrations in adrenal veins cannot be the gold standard due to variable ACTH levels which regulate normal adrenal zona fasciculata in overt or subclinical adrenal CS. Recently a method utilizing chromogranin measurement has been studied for the substitution of selectivity index (SI) using cortisol in AVS (32). Although the sensitivity of chromogranin-SI was reportedly lower than that of cortisol-SI for patients with simple-PA, it may be available as one of the substituting standards in assessment or inter-

pretation of AVS results for patients with PA/CPA.

In conclusion, a case with cortisol producing adrenal adenoma was described. The patient was also affected with APA but hyperaldosteronism had been masked probably because of ACTH suppression induced by CS. ACTH loading test was effective to detect latent hyperaldosteronism, and two separate adenomas in the left adrenal gland were successfully resected with complete cure of the two disorders. As the comorbidity of the two disorders seems present more frequently than generally recognized, careful pre-treatment diagnosis is necessary, especially to avoid post-surgical exacerbation of the latent hyperaldosteronism.

The authors state that they have no Conflict of Interest (COI).

D.K. and N.M. contributed equally to this work.

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