Adrenal Venous Sampling Is Useful for a Definitive Diagnosis in Cushing's Syndrome with Bilateral Adrenal Tumors

Toshiro SEKI[*1], Atsushi YASUDA[*1], Natsumi KITAJIMA[*1], Masayuki OKI[*2], Atsushi TAKAGI[*2], Naoya NAKAMURA[*3], Kazuya HANAI[*4], Toshiro TERACHI[*4] and Masafumi FUKAGAWA[*1]

[*1]*Division of Nephrology, Endocrinology and Metabolism,*
[*2]*Division of General Internal Medicine, Department of Internal Medicine,*
[*3]*Department of Pathology,* [*4]*Department of Urology, Tokai University School of Medicine*

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We report three cases of Cushing's syndrome (CS) with bilateral adrenal tumors. When bilateral adrenal tumors are encountered, a differential diagnosis is difficult to make, especially in the case of functioning bilateral adrenocortical adenoma. Adrenal scintigraphy has become a standard technique to determine the laterality of excessive hormone secretion; however, this examination results in bilateral adrenal activity in the functioning bilateral adrenocortical adenoma. Our three patients were diagnosed with adrenocorticotropic hormone (ACTH)-independent CS based on biochemical testing, and an abdominal computed tomography (CT) scan detected bilateral adrenal tumors. Adrenal scintigraphy showed bilateral adrenal activity in all cases. However, adrenal venous sampling (AVS) demonstrated three different hormone-excess patterns (case 1: bilateral cortisol-excess secretions; case 2: unilateral cortisol-excess secretion and bilateral aldosterone-excess secretions; and case 3: bilateral cortisol-excess secretions and bilateral aldosterone-excess secretions). Based on these findings, we could select optimal treatment for each case. Therefore, AVS is useful to obtain a definitive diagnosis and adequate therapy for CS with bilateral adrenal tumors.

Key words: bilateral adrenal tumors, adrenal venous sampling, Cushing's syndrome, primary aldosteronism

INTRODUCTION

Cushing's syndrome (CS) can be divided into two general types: adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent [1]. ACTH-independent CS is associated with autonomous adrenal cortisol production [1], and the majority of ACTH-independent CS cases are caused by unilateral adrenocortical lesions [2]. In general, the diagnosis of CS is based on endocrinological results and an abdominal computed tomography (CT) scan. Additionally, adrenal scintigraphy has become a standard technique to determine the laterality of excessive hormone secretion, although bilateral cortisol-secreting tumors are rarely observed in CS [2]. Thus, there remains no consensus regarding the optimal diagnosis of bilateral adrenal lesions.

When bilateral adrenal tumors are encountered, three possibilities can explain these findings: (a) bilateral cortisol hyper-functioning tumors, (b) bilateral macronodular adrenal hyperplasia, or (c) a unilateral cortisol-secreting tumor and a contralateral nonfunctioning or functioning tumor (secreting hormones such as aldosterone) [3]. To adequately distinguish these conditions, adrenal scintigraphy, confirmatory tests for primary aldosteronism (PA) and adrenal venous sampling (AVS) are performed. Adrenal scintigraphy has been used to differentiate between unilateral and bilateral causes of CS and PA [4]. However, a differential diagnosis cannot be established with scintigraphy in the case of possibility (c). Confirmatory tests should be highly sensitive to avoid missing PA; otherwise, a false-positive result may be obtained [4]. AVS provides important information concerning the laterality of excessive aldosterone secretion [4], although the use of AVS in patients with CS due to bilateral adrenal adenomas has rarely been reported [3–8].

We present three cases of CS with bilateral adrenal tumors. Adrenal scintigraphy showed bilateral adrenal activity in all cases, and AVS was used to distinguish three unique hormone-excess patterns. Based on these findings, we could select optimal treatment for each case.

CASE REPORT

Case 1

The patient was a 63-year-old man. He was referred to our hospital for the evaluation of bilateral adrenal tumors. His past medical history included diagnoses of diabetes mellitus and hypertension 10 years prior. There was no known family history of endocrine disease or malignant tumors.

The patient was 167 cm tall and weighed 65 kg. His blood pressure was 169/101 mmHg, with a pulse of 95 beats per minute, and he was taking 5 mg/day of amlodipine and 40 mg/day of telmisartan. He exhibited central obesity, moon face, thin skin, easy bruising, striae cutis, buffalo hump and pitting edema of the lower extremities. Osteoporosis was not present. Before evaluating the adrenal tumors and hyperten-

Toshiro SEKI, Division of Nephrology, Endocrinology and Metabolism, Department of Internal Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan Tel: +81-463-93-1121 Fax: +81-463-91-3350 E-mail: tsekimdpdd@tokai-u.jp

Table 1 Results of circadian variation in serum cortisol levels

Case 1		
Time	8 am	11 pm
Cortisol (μg/dL)	19.9	14.6
ACTH (pg/mL)	< 2.0	< 2.0

Case 2		
Time	8 am	11 pm
Cortisol (μg/dL)	10.8	11.8
ACTH (pg/mL)	< 2.0	< 2.0

Case 3		
Time	8 am	11 pm
Cortisol (μg/dL)	11.7	3.3
ACTH (pg/mL)	< 2.0	11.3

Abbreviations: ACTH, adrenocorticotropic hormone.

Table 2 Results of the overnight dexamethasone suppression test

Case 1		
Dexamethasone	1 mg	8 mg
Cortisol (μg/dL)	13.7	20.0
ACTH (pg/mL)	< 2.0	< 2.0

Case 2		
Dexamethasone	1 mg	8 mg
Cortisol (μg/dL)	13.4	16.9
ACTH (pg/mL)	< 2.0	< 2.0

Case 3		
Dexamethasone	1 mg	8 mg
Cortisol (μg/dL)	1.8	1.7
ACTH (pg/mL)	< 2.0	< 2.0

Abbreviations: ACTH, adrenocorticotropic hormone.

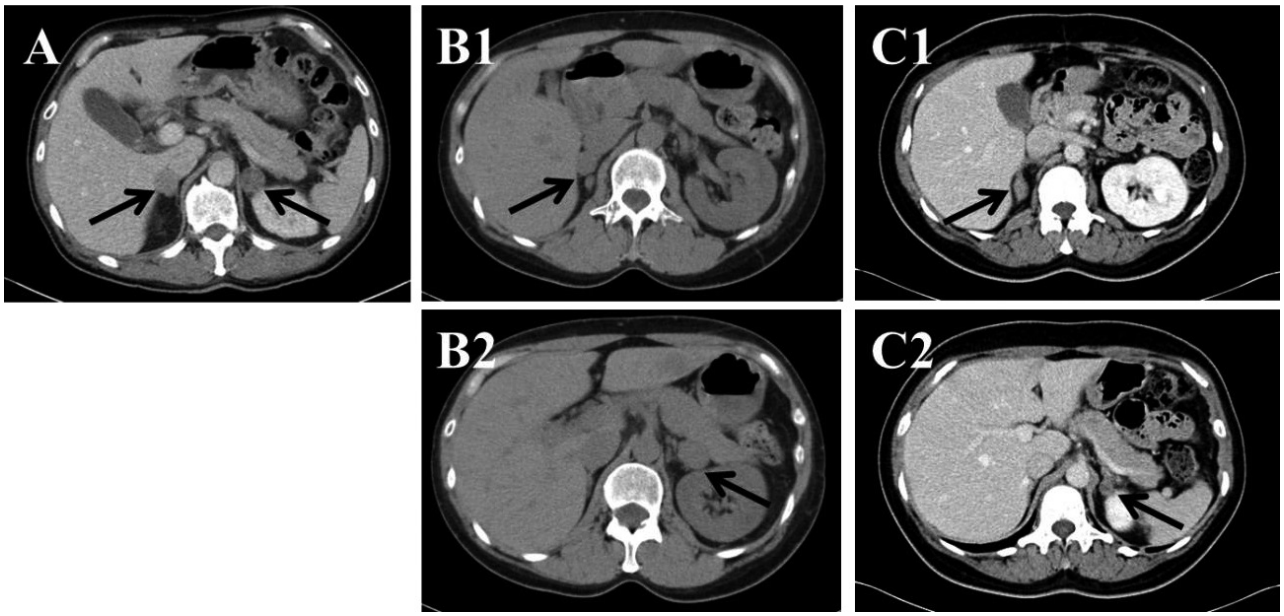

Fig. 1 Abdominal computed tomography (CT) scans of the abdomen. Bilateral adrenal tumors (arrows) and atrophied glands were observed. Each tumor was composed of well-circumambulated masses, without apparent irregularity on the surface or inside. (A: case 1, B1 and B2: case 2, C1 and C2: case 3).

sion, telmisartan was discontinued, and 5 mg/day of amlodipine was added for blood pressure control. We intentionally limited the use of antihypertensive agents to calcium channel blockers to minimize any effects on the renin-angiotensin-aldosterone system during the diagnostic process [9].

Routine laboratory examinations were within the normal ranges except for a mildly elevated neutrophil count and a decreased eosinophil count. The hormonal examination revealed high levels of urinary free cortisol (90.0 μg/day; normal range, 11.2–80.3 μg/day). There was no circadian variation in serum cortisol levels (19.9 μg/dL at 8 am and 14.6 μg/dL at 11 pm; normal range, < 5 μg/dL at 11 pm, Table 1), and plasma ACTH levels were repeatedly undetectable (< 2.0 pg/mL; normal range, 7.2–63.3 pg/mL). Plasma cortisol levels were not suppressed after the low- and high-dose overnight dexamethasone suppression tests (13.7 μg/dL after 1 mg of dexamethasone and 20.0 μg/dL after 8 mg; normal range, < 5 μg/dL, Table 2). The plasma aldosterone concentration (PAC, 15.6 pg/mL, normal range, 30–159 pg/mL) and plasma renin activity (PRA, 0.3 ng/mL/hr, normal range, 0.3–5.4 ng/mL/hr) were suppressed. CT scan of the abdomen showed bilateral adrenal tumors (Fig. 1A). Adrenal scintigraphy revealed bilateral adrenal activity (Fig. 2A). We performed confirmatory tests for PA to avoid missing this disease. The furosemide-upright test was positive, and the other tests were negative (Table 3). Thus, AVS was performed to determine the laterality of excessive cortisol or aldosterone secretion. As shown in Table 4, the cortisol levels before and after ACTH stimulation

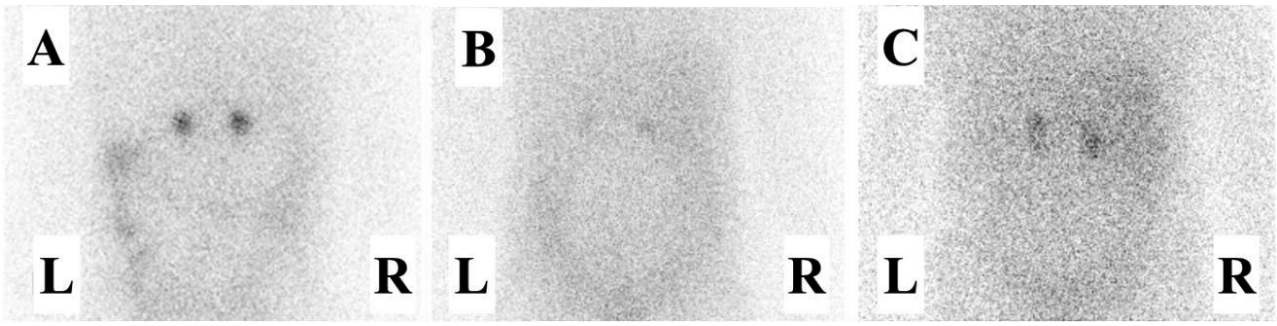

Fig. 2 Adrenal scintigraphies of bilateral adrenal tumors. Each adrenal scintigraphy using ^{131}I-adosterol showed bilateral uptake of the tracer (A: case 1, B: case 2, C: case 3).

(an iv bolus injection of 0.25 mg ACTH) were 238.3 μg/dL and 485.9 μg/dL, respectively, from the right adrenal vein and 298.1 μg/dL and 1523 μg/dL, respectively, from the left adrenal vein. The aldosterone levels obtained from the right and left adrenal veins were 1,340 and 441 pg/mL (normal range, < 2,000 pg/mL, [10]), respectively, at baseline and 4,910 and 3,930 pg/mL (normal range, < 14,000 pg/mL, [10]), respectively, after ACTH stimulation (Table 4). These results indicated that excessive aldosterone secretion from both adrenal glands was absent. Additionally, there was no laterality of the cortisol secretion. Based on these findings, we made a preoperative diagnosis of CS due to bilateral adrenal tumors.

To preserve adrenal function, the left adrenal gland was totally resected, whereas the right adrenal gland was partially resected laparoscopically. Immunohistochemistry of both tumors revealed strong positive staining for 3β-HSD and P450c17 (Fig. 3). The surrounding cortical cells in both adrenal glands were atrophic. These pathological data led to a definitive diagnosis of CS due to bilateral adrenocortical adenomas. After adrenalectomy, our patient continued to take maintenance doses of hydrocortisone (15–30 mg/day). He became normotensive in the postoperative period, and antihypertensive drugs were gradually tapered. In addition, his Cushingoid features disappeared. He is being followed by the nephrology, endocrinology and metabolism outpatient division and currently receives 15 mg/day of hydrocortisone.

Case 2

A 44-year-old woman was referred to our hospital for an evaluation of bilateral adrenal tumors. Her past medical history included a myoma of the uterus, and she had undergone uterectomy at 32 years of age. She had no family history of endocrine disease or malignant tumors.

The patient was 163 cm tall and weighed 55 kg. Her blood pressure was 154/110 mmHg, with a pulse of 67 beats per minute. She had central obesity, moon face, buffalo hump and pitting edema of the lower extremities. Osteoporosis was not present.

Routine laboratory examinations were within the normal ranges, including serum electrolytes. Hormonal examination revealed high levels of urinary free cortisol (113 μg/day; normal range, 11.2–80.3 μg/day). There was no circadian variation in serum cortisol levels (10.8 μg/dL at 8 am and 11.8 μg/dL at 11 pm; normal range, < 5 μg/dL at 11 pm, Table 1), and plasma ACTH levels were repeatedly undetectable (< 2.0 pg/mL; normal range, 7.2–63.3 pg/mL). Plasma cortisol levels were not suppressed after the low- and high-dose overnight dexamethasone suppression tests (13.4 μg/dL after 1 mg of dexamethasone and 16.9 μg/dL after 8 mg; normal range, < 5 μg/dL, Table 2). The PAC (185 pg/mL, normal range, 30–159 pg/mL) was elevated and the PRA (0.3 ng/mL/hr, normal range, 0.3–5.4 ng/mL/hr) was suppressed, which gave an aldosterone-to-renin ratio (ARR) of 617 (normal range, < 200) suggestive of excessive secretion of aldosterone. Accordingly, we performed confirmatory tests for PA. The captopril test and furosemide-upright test were positive, whereas the saline-loading test was negative (Table 3). CT scans of the abdomen showed bilateral adrenal tumors (Fig. 1B). Adrenal scintigraphy revealed bilateral adrenal activity (Fig. 2B). Thus, AVS was performed to determine the laterality of excessive cortisol or aldosterone secretion. As shown in Table 4, the cortisol levels before and after ACTH stimulation (an iv bolus injection of 0.25 mg ACTH) were 100.3 μg/dL and 577.7 μg/dL, respectively, from the right adrenal vein and 49.0 μg/dL and 299.9 μg/dL, respectively, from the left adrenal vein. The aldosterone levels obtained from the right and left adrenal veins were 6,100 and 1,310 pg/mL (normal range, < 2,000 pg/mL, [10]), respectively, at baseline and 1,820 and 81,700 pg/mL (normal range, < 14,000 pg/mL, [10]), respectively, after ACTH stimulation (Table 4). These results suggested that the right adrenal tumor, compared with the left adrenal tumor, produced excessive cortisol. In addition, both adrenal glands were considered to be secreting excessive aldosterone. Based on these findings, we made a preoperative diagnosis of CS caused by the right adrenal tumor and PA caused by both adrenal glands.

We performed a laparoscopic right total adrenalectomy for the treatment of CS and offered medical therapy for the treatment of PA. Immunohistochemistry of the right tumor revealed strong positive staining for 3β-HSD and P450c17 (Fig. 4). The surrounding cortical cells in both adrenal glands were atrophic. However, there was no pathological finding of PA. These pathological data led to a definitive diagnosis of CS caused by the right adrenocortical adenoma and PA caused by the left adrenal gland. After adrenalectomy, our patient continued to take maintenance doses of hydrocortisone (15–30 mg/day) and eplerenone

Table 3 Results of confirmatory tests for primary aldosteronism

Case 1					
Captopril test					
min	0	60	90		
PAC (pg/mL)	45.9	39.3	32.6		
PRA (ng/mL/hr)	0.4	0.3	0.3		
ARR	114.8	131.0	108.7		
Furosemide-upright test					
min	0			120	
PAC (pg/mL)	15.6			41.2	
PRA (ng/mL/hr)	0.3			0.4	
Saline-loading test					
min	0				240
PAC (pg/mL)	34.1				30.2
PRA (ng/mL/hr)	0.3				0.2
Case 2					
Captopril test					
min	0	60	90		
PAC (pg/mL)	127	68.8	67.5		
PRA (ng/mL/hr)	0.2	0.2	0.2		
ARR	635	344	337.5		
Furosemide-upright test					
min	0			120	
PAC (pg/mL)	185			341	
PRA (ng/mL/hr)	0.3			0.7	
Saline-loading test					
min	0				240
PAC (pg/mL)	78.0				34.1
PRA (ng/mL/hr)	0.2				0.2
Case 3					
Captopril test					
min	0	60	90		
PAC (pg/mL)	90.7	76.1	84.7		
PRA (ng/mL/hr)	2.5	0.4	0.2		
ARR	36.3	190.3	423.5		
Furosemide-upright test					
min	0			120	
PAC (pg/mL)	61.6			166	
PRA (ng/mL/hr)	< 0.1			0.3	
Saline-loading test					
min	0				240
PAC (pg/mL)	44.4				35.3
PRA (ng/mL/hr)	0.5				0.3

Abbreviations: PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, PAC/PRA ratio.

Table 4 Results of adrenal venous sampling

Case 1

	Cortisol (μg/dL)		Aldosterone (pg/mL)	
	RAV	LAV	RAV	LAV
Baseline	238.3	298.1	1340	441
After ACTH 250 μg	485.9	1523	4910	3930

Case 2

	Cortisol (μg/dL)		Aldosterone (pg/mL)	
	RAV	LAV	RAV	LAV
Baseline	100.3	49.0	6100	1310
After ACTH 250 μg	577.7	299.9	1820	81700

Case 3

	Cortisol (μg/dL)		Aldosterone (pg/mL)	
	RAV	LAV	RAV	LAV
Baseline	20.8	25.8	3550	1570
After ACTH 250 μg	1102	894.0	27600	28600

Abbreviations: RAV, right adrenal vein; LAV, left adrenal vein; ACTH, adrenocorticotropic hormone.

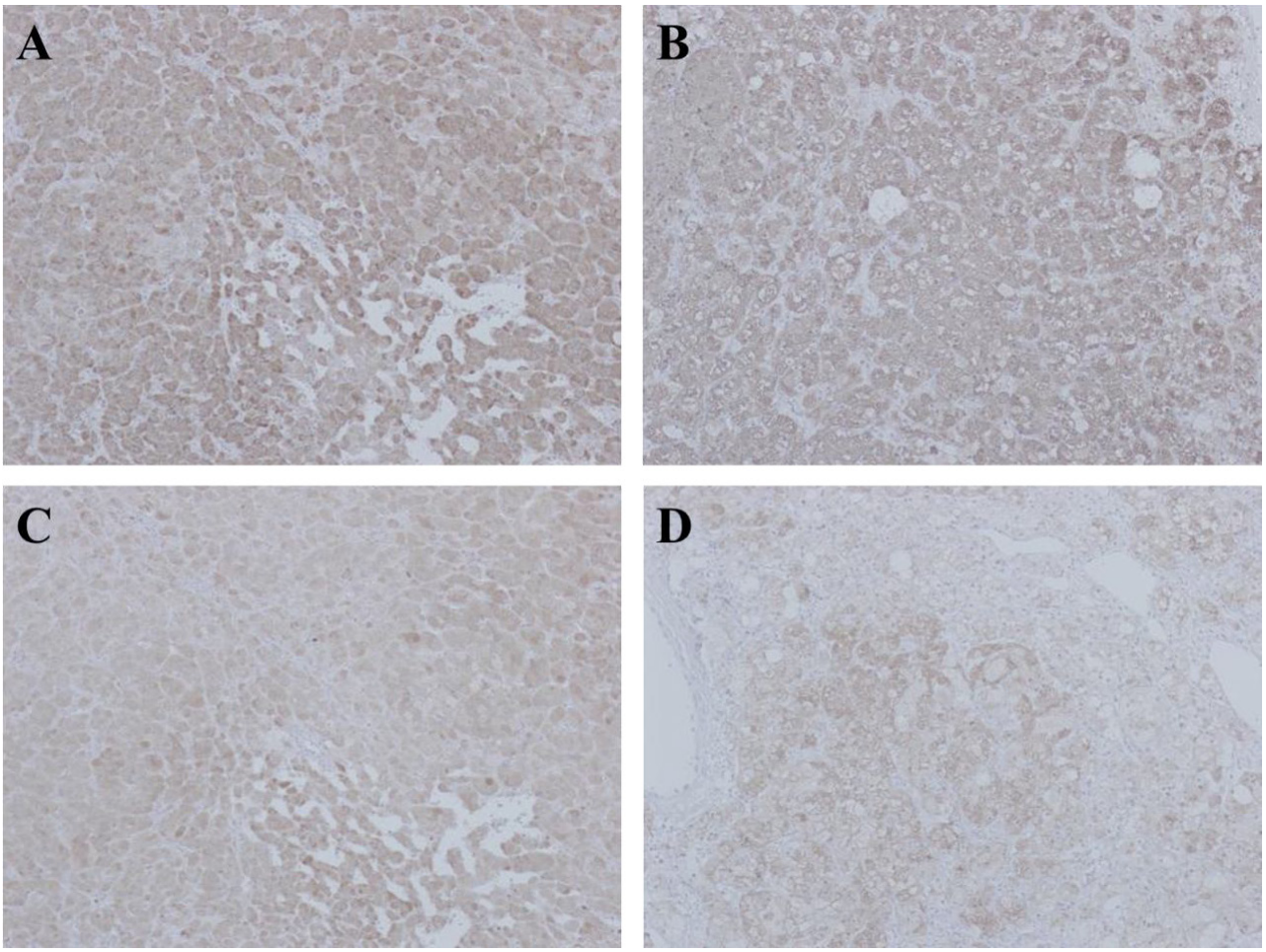

Fig. 3 Immunohistochemistry of the adrenal adenomas in case 1 (×100). The right and left adenomas were strongly positive for 3β-HSD (A and B) and P450c17 (C and D).

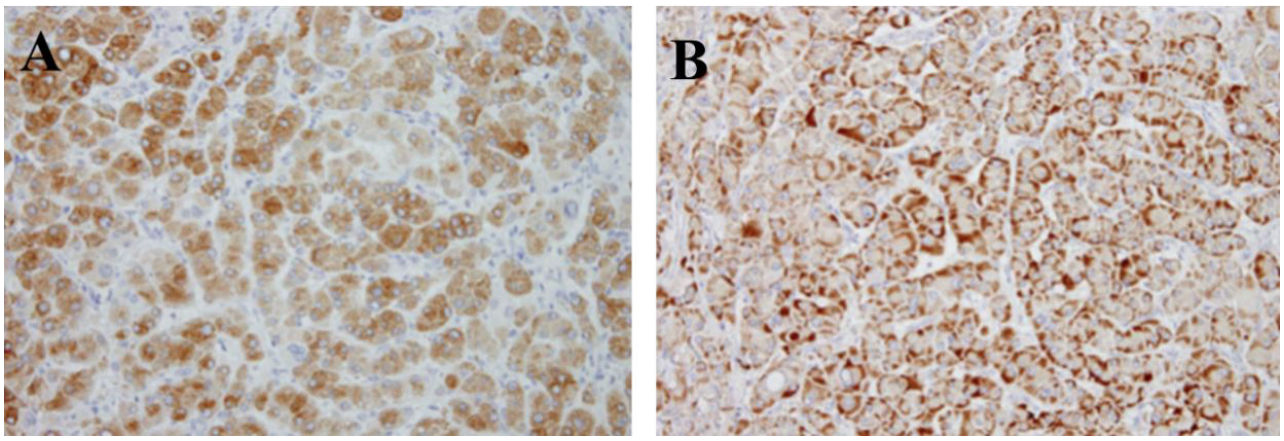

Fig. 4 Immunohistochemistry of the adrenal adenoma in case 2 (×100). The right adenoma was strongly positive for 3β-HSD (A) and P450c17 (B).

(100 mg/day). Hydrocortisone was gradually tapered, and the Cushingoid features disappeared. She is being followed by the nephrology, endocrinology and metabolism outpatient division.

Case 3

A 49-year-old woman was referred to our hospital for the evaluation of bilateral adrenal tumors. She had no past medical history and no family history of endocrine disease or malignant tumors.

The patient was 150 cm tall and weighed 60 kg. Her blood pressure was 103/60 mmHg, with a pulse of 72 beats per minute. She had no Cushingoid features. Osteoporosis was absent.

Routine laboratory examinations were within the normal ranges including serum electrolytes. The hormonal examination revealed normal circadian variation in serum cortisol levels (11.7 μg/dL at 8 am and 3.3 μg/dL at 11 pm; normal range, < 5 μg/dL at 11 pm, Table 1), and plasma ACTH level was undetectable (< 2.0 pg/mL; normal range, 7.2–63.3 pg/mL). Plasma cortisol level was suppressed after the low-dose overnight dexamethasone suppression test for subclinical CS (1.8 μg/dL after 1 mg of dexamethasone; normal range, < 3 μg/dL, Table 2) but was not suppressed after the high-dose test (1.7 μg/dL after 8 mg of dexamethasone; normal range, < 1 μg/dL, Table 2). The PAC level was normal (110 pg/mL, normal range, 30–159 pg/mL) and the PRA was suppressed (0.1 ng/mL/hr, normal range, 0.3–5.4 ng/mL/hr). CT scans of the abdomen showed bilateral adrenal tumors (Fig. 1C). Adrenal scintigraphy revealed bilateral adrenal activity (Fig. 2C). We performed confirmatory tests for PA to avoid missing this disease. The captopril test and furosemide-upright test were positive, whereas the saline-loading test was negative (Table 3). Therefore, to determine the laterality of the excessive cortisol or aldosterone secretion, we performed AVS. As shown in Table 4, the cortisol levels before and after ACTH stimulation (an iv bolus injection of 0.25 mg ACTH) were 20.8 μg/dL and 1102 μg/dL, respectively, from the right adrenal vein and 25.8 μg/dL and 894.0 μg/dL, respectively, from the left adrenal vein. The respective aldosterone levels obtained from the right and left adrenal veins were 3,550 and 1,570 pg/mL (normal range, < 2,000 pg/mL, [10]), respectively, at baseline and 27,600 and 28,600 pg/mL (normal range, < 14,000 pg/mL, [10]), respectively, after ACTH stimulation (Table 4). These results indicated that both adrenal glands produced excessive aldosterone. However, laterality of the cortisol secretion was not present. Based on above findings, we made a diagnosis of PA caused by both adrenal glands and subclinical CS caused by both adrenal tumors.

Interestingly, this patient has no risk factors for cardiovascular disease such as hypertension, obesity, diabetes mellitus or dyslipidemia. Thus, we are observing her without medical therapy. She is being followed by the nephrology, endocrinology and metabolism outpatient division.

DISCUSSION

Adrenal scintigraphy has become a standard technique to determine the laterality of excessive hormone secretion in ACTH-independent CS because most of these cases are caused by unilateral adrenocortical lesions [2]. However, when bilateral adrenal tumors are encountered, it is impossible to establish a differential diagnosis using adrenal scintigraphy, because this examination results in bilateral adrenal activity in the functioning bilateral adrenocortical adenoma (as shown in our cases). It is well known that AVS provides important information concerning the laterality of excessive aldosterone secretion [4, 10]. However, the use of AVS in patients with CS due to bilateral adrenal adenomas has rarely been reported [3–8]. Moreover, there is no consensus regarding the optimal determination of the laterality of excessive hormone secretion. Thus, standardized criteria for AVS in CS with bilateral adrenal tumors needed to obtain the optimal determination of the laterality of excessive hormone secretion. In this regard, our cases demonstrated detailed data including CT scans, adrenal scintigraphy, confirmatory tests for PA and pathological findings in addition to AVS. Therefore, our report may be used to formulate standard criteria for AVS in CS with bilateral adrenal tumors in the near future.

As shown in Table 4, the results of AVS indicated that the laterality of the cortisol secretion was not present in case 1 and case 3, and the right adrenal tumor,

compared with the left adrenal tumor, was secreting excessive cortisol in case 2. The pathological findings of case 1 and case 2 confirmed our preoperative diagnosis using AVS. Recently, Omura *et al*. reported that super-selective ACTH-stimulated AVS, by which AVS samples were collected from tributary veins of the adrenal glands, was useful to determine the laterality of excessive aldosterone secretion in PA [11, 12]. These authors also demonstrated the criterion of super-selective ACTH-stimulated AVS for the laterality of cortisol secretion in CS [13]. This report indicated that the cortisol levels were > 380 μg/dL in both central veins or the tributary veins from the adenomas, and the cortisol levels were < 380 μg/dL in other tributary veins in the case of bilateral cortisol-secreting adenomas. Although we could not prove that the cortisol levels were < 380 μg/dL in the tributary veins, this criterion may have applied to all of our cases (Table 4) and may therefore serve as a standard criterion for AVS in CS with bilateral adrenal tumors.

Adrenal scintigraphy showed bilateral adrenal activity in all our cases. However, AVS demonstrated three different hormone-excess patterns (case 1: bilateral cortisol-excess secretions, case 2: a unilateral cortisol-excess secretion and bilateral aldosterone-excess secretions, and case 3: bilateral cortisol excessive secretions and bilateral aldosterone-excess secretions). Based on these findings, we selected a different treatment approach for each case. In the treatment of bilateral adrenal cortical functioning tumors, a unilateral or bilateral partial adrenalectomy is a better choice if functional adrenal tissue can be preserved, because a bilateral total adrenalectomy causes acute adrenal insufficiency necessitating lifelong steroid replacement. Indeed, partial adrenalectomies for bilateral adrenal cortical functioning tumors causing CS or PA have been reported [3, 6, 14, 15]. In our case 1, the left adrenal gland was totally resected, whereas the right adrenal gland was partially resected laparoscopically. Additionally, we performed a laparoscopic right total adrenalectomy for the treatment of CS and provided medical therapy for the treatment of PA in case 2. Once again, a standard criterion of AVS in CS is necessary to select the adequate treatment of CS with bilateral adrenal tumors.

We made a diagnosis of PA caused by both adrenal glands and subclinical CS caused by both adrenal tumors for the patient in case 3, even though she had no risk factors of cardiovascular disease such as hypertension, obesity, diabetes mellitus or dyslipidemia. Thus, we are continuing to observe this patient without medical therapy. Mineralocorticoid receptors have been expressed in various cell types obtained from vascular and cardiac tissues, and long-term exposure to inappropriately elevated plasma aldosterone concentrations causes renal and cardiac dysfunction independent of blood pressure levels [16]. In general, adrenalectomy or the administration of mineralocorticoid receptor antagonists is effective in patients with PA, and recent studies have shown that both treatments improve renal and cardiovascular outcomes [17]. However, patients with subclinical CS lack specific symptoms of CS but commonly suffer from diabetes mellitus and hypertension; thus, whether adrenalectomy should be performed in these patients remains controversial [18]. One important remaining question is why excessive hormone secretion did not affect the cardiovascular status of the patient from case 3. In this regard, there may be a group of patients with a genetic difference that causes susceptibility to the effects of mineralocorticoid [19, 20]. Further study will be required to resolve this issue.

In summary, we presented three cases of CS with bilateral adrenal tumors. When bilateral adrenal tumors are encountered, the differential diagnosis is difficult, especially in the functioning bilateral adrenocortical adenoma. Adrenal scintigraphy, which has become a standard technique to determine the laterality of excessive hormone secretion, showed bilateral adrenal activity in all cases. However, AVS distinguished three unique hormone-excess patterns. Based on these findings, we could select adequate treatment for each case. Thus, AVS is necessary to obtain a definitive diagnosis and optimal therapy in CS with bilateral adrenal tumors.

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