Unique Cases of Unilateral Hyperaldosteronemia Due to Multiple Adrenocortical Micronodules, Which Can Only be Detected by Selective Adrenal Venous Sampling

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Primary aldosteronism is classified as aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), unilateral adrenal hyperplasia (UAH), primary adrenal hyperplasia (PAH), adrenal cancer, and glucocorticoid-remediable aldosteronism. We describe here 4 cases of primary aldosteronism due to unilateral hyperaldosteronemia, demonstrating unique histopathologic findings, such as unilateral multiple adrenocortical micronodules in the affected adrenals. Thirty-three patients with primary aldosteronism were consecutively admitted; 27 of them were treated by unilateral adrenalectomy. Four of them also had unilateral adrenal hypersecretion of aldosterone by selective adrenal venous sampling and adrenocortical multiple micronodules without an adenoma. These patients had hyporeninemic hyperaldosteronism with normokalemic hypertension. In these patients, furosemide plus upright test failed to increase plasma renin activity (PRA); the ratio of plasma aldosterone concentration (PAC) to PRA at 90 minutes after captopril administration was similar to that in patients with IHA and APA. Aldosterone concentrations were increased in each unilateral adrenal vein, and poorly encapsulated multiple adrenocortical micronodules from 2 to 3 mm in diameter were microscopically detected in the resected adrenal glands. Immunohistochemical analysis of steroidogenic enzymes, including cholesterol side chain cleavage, 3β-hydroxysteroid dehydrogenase, 21-hydroxylase, 17α-hydroxylase, and 11β-hydroxylase, indicated that the cortical cells within these micronodules were active in aldosterone production, while the non-nodular zona glomerulosa cells were inactive. We conclude that the clinical and pathologic characteristics of our 4 cases with unilateral multiple adrenocortical micronodules (UMN) are distinct from those of APA, IHA, UAH, and PAH. Furthermore, unilateral hyperaldosteronemia induced by UMN may be frequently misdiagnosed, because standard imaging tests, which cannot always detect tiny abnormalities of adrenals, showed "normal adrenal glands" in these patients. Thus, primary aldosteronism due to UMN should be carefully examined for differential diagnosis of each form of hyperaldosteronemia.

PRIMARY ALDOSTERONISM IS classified as aldosterone-producing adenoma (APA), idiopathic hyperaldosteronism (IHA), unilateral adrenal hyperplasia (UAH), primary adrenal hyperplasia (PAH), adrenal cancer, and glucocorticoid-remediable aldosteronism.[1] We have admitted and evaluated 33 patients with primary aldosteronism and have subjected 27 of them to unilateral adrenalectomy. Based on histopathologic findings, 20 patients were diagnosed with APA, 2 with IHA, and 1 with UAH. However, there were 4 patients whose histopathologic findings were different from those of APA, IHA, UAH, PAH, adrenal cancer, and glucocorticoid-remediable aldosteronism.

In this study we compared clinical, endocrinologic, and histopathologic findings in these cases with those of APA, IHA, and UAH. Based on the results, we would like to emphasize that the disorders in the adrenals showing unilateral multiple adrenocortical micronodules (UMN), which are only pathologically identified, can also induce hyperaldosteronism, and that UMN should be diagnosed when some cases are showing unilaterally overproduction of aldosterone.

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PATIENTS AND METHODS

Case Reports

Four patients were definitely diagnosed with primary aldosteronism because of hypertension associated with hyporeninemic hyperaldosteronemia. Characteristics of each case are shown in Table 1. Hypertension and hyporeninemic hyperaldosteronemia were completely improved after unilateral adrenalectomy, as shown in Table 1.

Comparison With Other Primary Aldosteronism and Essential Hypertension Patients

Endocrinologic, imaging, and pathologic findings of these 4 cases were compared with those of 20 patients with APA, 8 with IHA, and 1 with UAH. Twenty patients with APA, 2 with IHA, and 1 with UAH were diagnosed on the basis of endocrinologic tests, selective venous sampling, and surgery. Six patients with IHA were diagnosed on the basis of endocrinologic tests and venous sampling. Ten patients with hyporeninemic essential hypertension (EHT) were also evaluated for comparison to those with primary aldosteronism.

Endocrinologic Evaluation

Antihypertensive drugs, including calcium antagonists, β-blockers, and angiotensin-converting enzyme inhibitors, thought to interfere with the renin-angiotensin aldosterone system, were withdrawn at least 2 weeks before admission. Plasma aldosterone concentration (PAC), plasma concentrations of 11-deoxycorticosterone (DOC), corticosterone (B), cortisol,[2] 18-hydroxycorticosterone (18-OH-B),[3] 18-hydroxy-11-deoxycorticosterone (18-OH-DOC),[4] and plasma renin activity (PRA)[5] were measured by specific radioimmunoassays.

Furosemide Plus Upright Test

Furosemide plus upright test was performed in the morning after resting for at least 30 minutes in the supine position, as previously reported.[6] PAC and PRA were measured before and 120 minutes after intravenous injection of furosemide (40 mg) in the upright position.

Table 1. Profile of Four Patients With UMN

Patient No.	Age (yr)	Sex	Duration of HT (yr)	Before Adrenalectomy: Blood pressure (mm Hg)	Before Adrenalectomy: PAC (ng/dL)	Before Adrenalectomy: PRA (ng/mL/h)	Findings of CT Examination	Affected Site of Adrenal Gland Confirmed by Adrenal Venous Sampling	After Adrenalectomy: Blood pressure (mm Hg)	After Adrenalectomy: PAC (ng/dL)	After Adrenalectomy: PRA (ng/mL/h)
1	60	F	32	220/108	21.0	0.2	Normal	Right	124/68	8.7	0.8
2	46	F	16	196/98	19.9	<0.1	Normal	Left	124/64	9.3	1.0
3	50	F	4	180/100	20.8	0.1	Normal	Right	132/88	10.7	1.1
4	46	F	2	168/94	13.6	0.7	Left adrenal tumor	Right	136/70	7.2	1.8

Abbreviations: UMN, unilateral multiple adrenocortical micronodules; HT, hypertension; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

Captopril Loading Test

PAC and PRA were measured before and 90 minutes after oral administration of captopril (50 mg). The patients were asked to rest in the supine position during the test.[7]

Adrenal Venography and Adrenal Venous Sampling

Adrenal venography and selective venous sampling were performed to clarify the laterality of aldosterone hypersecretion. Adrenal venography was performed by femoral approach. Adrenal venous blood was selectively sampled before and 30 minutes after intravenous injection of 250 μg synthetic ACTH (1-24) via the median cubital vein. The proper placement of the catheter tip was confirmed just before and after sampling by injecting a small amount of contrast medium.

Adrenal Computed Tomography

Thin-section high-resolution computed tomography (CT) scans of the adrenal glands were obtained using an X force system (Toshiba Medical, Tokyo, Japan). We obtained thin-section scans with a 2-mm collimation at 2-mm intervals after intravenous injection of contrast medium. The scanning parameters were 120 kVp, 100 mA, and 30-sec scanning time. The scans were reconstructed with a standard reconstruction algorithm.

Pathologic Studies of Resected Adrenal Glands

Immunohistochemical analysis of steroidogenic enzymes, including cholesterol side chain cleavage (P-450$_{SCC}$), 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase (P-450$_{C21}$), 17α-hydroxylase (P-450$_{C17}$), and 11β-hydroxylase (P-450$_{C11}$) was performed on routinely processed formalin-fixed paraffin-embedded specimens by using the biotin-streptavidin amplified method with the StrAvi-Gen B-SA immunostaining system (Biogenex, Dublin, CA). For control immunostaining, 0.01mol/L phosphate-buffered saline (PBS) and normal rabbit or mouse immunoglobulin G (IgG) were used instead of primary antibod-

Table 2. Effects of Various Clinical Tests on PAC and PRA in Patients with Primary Aldosteronism Due to UMN, APA, IHA, UAH, and EHT

	PAC (ng/dL)/PRA (ng/mL/h)		PAC/PRA Ratio	
	0 Minutes	120 Minutes	0 Minutes	120 Minutes
Effect of furosemide plus upright				
UMN (n = 4)	16.6 ± 1.8*/0.3 ± 0.1*	37.7 ± 8.3/0.7 ± 0.2*	105.0 ± 44.4*	77.0 ± 29.3†
APA (n = 20)	21.0 ± 2.1*/0.2 ± 0.1†	41.5 ± 3.8/0.5 ± 0.1†	172.1 ± 27.5†	171.4 ± 33.6†
IHA (n = 8)	18.6 ± 0.6*/0.3 ± 0.1*	36.0 ± 3.5/1.0 ± 0.1†	103.6 ± 19.8†	38.5 ± 8.1
UAH (n = 1)	18.2/0.2	30.8/0.4	91.0	77.0
EHT (n = 10)	13.8 ± 1.6‡/0.7 ± 0.2‡	33.5 ± 4.5/3.2 ± 0.9‡	35.3 ± 8.8‡	20.9 ± 5.8§
	PAC (ng/dL)/PRA (ng/mL/h)		PAC/PRA ratio	
	0 Minutes	90 Minutes	0 Minutes	90 Minutes
Effect of captopril				
UMN (n = 4)	17.0 ± 0.2/0.1 ± 0.1†	8.4 ± 0.6/0.2 ± 0.1†	120.0 ± 39.8*	38.1 ± 3.5†
APA (n = 20)	21.9 ± 2.1*/0.2 ± 0.1†	21.3 ± 3.1†/0.3 ± 0.1†	137.9 ± 19.4†	100.7 ± 12.0†‡
IHA (n = 8)	16.8 ± 0.6/0.2 ± 0.1†	11.1 ± 1.2/0.3 ± 0.1†	106.1 ± 20.3†	37.1 ± 4.5†
UAH (n = 1)	ND	ND	ND	ND
EHT (n = 10)	12.7 ± 2.0/0.7 ± 0.1§	8.3 ± 1.4/1.8 ± 0.4§	28.2 ± 12.2‡	5.6 ± 1.2§

NOTE. Clinical tests by administration of furosemide and captopril were performed as described in the text. Each parameter was also determined as described in Patients and Methods. The parameters obtained from UMN, APA, IHA, and EHT were statistically calculated and the results are expressed as mean ± SE. Statistical analysis was performed as unpaired Student's *t* test.

Abbreviations: PAC, plasma aldosterone concentration; PRA, plasma renin activity; UMN, unilateral multiple adrenocortical micronodules; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; UAH, unilateral adrenal hyperplasia; EHT, essential hypertension; ND, not determined.

*$P < .05$ compared with EHT.

†$P < .01$ compared with EHT.

‡$P < .05$ compared with UMN.

§$P < .01$ compared with UMN.

Table 3. Plasma Concentrations of DOC, B, 18-OH-B, and 18-OH-DOC in Patients With UMN, APA, IHA, and UAH

	DOC (ng/mL)	B (ng/mL)	18-OH-B (ng/mL)	18-OH-DOC (ng/mL)
UMN (n = 4)	0.11 ± 0.02 (0.06–0.21)	2.97 ± 1.38 (1.59–4.35)	0.30 ± 0.01 (0.29–0.32)	0.06 ± 0.05 (0.01–0.11)
APA (n = 20)	0.17 ± 0.04 (0.04–0.46)	3.92 ± 0.78 (0.38–13.3)	0.36 ± 0.08 (0.08–1.51)	0.09 ± 0.02 (0.01–0.45)
IHA (n = 8)	0.20 ± 0.09 (0.09–0.37)	2.93 ± 1.08 (0.73–5.56)	0.24 ± 0.06 (0.05–0.44)	0.09 ± 0.04 (0.02–0.17)
UAH (n = 1)	0.17	1.20	ND	0.07
Normal range	0.08–0.28	0.38–8.42	0.09–0.58	0.01–0.07

NOTE. Plasma concentrations of DOC, B, 18-OH-B, and 18-OH-DOC were determined as described in Patients and Methods. The parameters obtained from UMN, APA, and IHA were statistically calculated and the results are expressed as mean ± SE. Statistical analysis was performed as unpaired Student's *t* test. Range of each value in UMN, APA, and IHA is shown in parentheses.

Abbreviations: DOC, 11-deoxycorticosterone; B, corticosterone; 18-OH-B, 18-hydroxycorticosterone; 18-OH-DOC, 18-hydroxy-11-deoxycorticosterone; UMN, unilateral multiple adrenocortical micronodules; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; UAH, unilateral adrenal hyperplasia; ND, not determined.

ies. Immunostaining procedures and properties of the primary antibodies used in these studies have been described in detail elsewhere.[8]

Statistical Analyses

Statistical analyses were performed by Student's *t* test. Results are expressed as the mean ± SE.

RESULTS

Laboratory examinations showed that serum cholesterol levels of 4 patients with UMN were elevated at 255 to 298 mg/dL (6.59 to 7.71 mmol/L). Other biochemical parameters, including serum potassium, however, were within the normal range. PAC was elevated, and PRA was suppressed in all patients after 30 minutes in the supine position (Table 1). Results of the furosemide plus upright test and captopril test for the 4 UMN cases, 20 cases of APA, 8 of IHA, 1 of UAH, and 10 of EHT are shown in Table 2. PAC increased in each group when stimulated by furosemide plus upright posture and PRA increased in IHA and EHT, although PRA remained suppressed to be less than 1.0 ng/mL/h in the cases with UMN, as well as in patients with APA and UAH. The ratio of PAC to PRA at 90 minutes after 50 mg of captopril administration in those patients with UMN, APA, and IHA was more than 20 ng/dL per ng/mL/h.

Peripheral blood concentrations of mineralocorticoids such as DOC, B, 18-OH-B, and 18-OH-DOC are shown in Table 3. There were no significant differences in the concentrations of these steroids among UMN, APA, and IHA patients.

PAC and plasma cortisol levels of adrenal venous effluent obtained by selective adrenal venous sampling technique are shown in Table 4. The aldosterone secretion from the affected adrenal glands before adrenocorticotropic hormone (ACTH) administration in patients with primary aldosteronism due to UMN, APA, and UAH increased significantly higher than that of the mean value from each adrenal gland in EHT. Mean value of PAC in each adrenal gland in IHA was also significantly higher than that in EHT before and after ACTH administration. Moreover, patients with primary aldosteronism due to UMN and those with APA showed hyper-responsiveness of aldosterone to ACTH stimulation in venous effluent from the affected adrenal glands.

From 2 to 3 years after unilateral adrenalectomy, blood pressure, PAC, and PRA values were improved in all patients with UMN, APA, and UAH; they were not improved in 2 patients with IHA who had been treated by unilateral adrenalectomy (Table 5).

The resected right adrenal gland of patient 1 measured 4.5 × 3.0 × 0.8 cm, the left adrenal gland of patient 2 measured

Table 4. Aldosterone and Cortisol Concentrations in the Adrenal Venous Effluent of Four Patients With Primary Aldosteronism Due to UMN and in Patients With APA, IHA, UAH, and EHT

	Aldosterone Contents in Adrenal Venous Effluent Before and 30 Minutes After ACTH Administration						Cortisol Contents in Adrenal Venous Effluent Before and 30 Minutes After ACTH Administration					
	Affected adrenal vein		Unaffected adrenal vein		Mean value in IHA or EHT		Affected adrenal vein		Unaffected adrenal vein		Mean value in IHA or EHT	
	Aldosterone (ng/dL)		Aldosterone (ng/dL)		Aldosterone (ng/dL)		Cortisol (μg/dL)		Cortisol (μg/dL)		Cortisol (μg/dL)	
	0 Minutes	30 Minutes	0 Minutes	30 Minutes	0 Minutes	30 Minutes	0 Minutes	30 Minutes	0 Minutes	30 Minutes	0 Minutes	30 Minutes
UMN (n = 4)	711 ± 332*	2,608 ± 378†	75 ± 43	830 ± 176			123 ± 68	793 ± 195	141 ± 97	313 ± 63		
APA (n = 20)	815 ± 140‡	3,681 ± 626†	69 ± 13	537 ± 106			100 ± 26	731 ± 105	103 ± 24	433 ± 46		
IHA (n = 8)					487 ± 123*	3002 ± 422†					241 ± 37‡	622 ± 43
UAH (n = 1)	397	ND	125	ND			23	ND	40	ND		
EHT (n = 10)					129 ± 60	498 ± 84					26 ± 8	478 ± 83

NOTE. Selective adrenal venous sampling was performed as described in the text. Each parameter was also determined as described in Patients and Methods. The parameters obtained from UMN, APA, IHA, and EHT were statistically calculated and the results are expressed as means ± SE. Statistical analysis was performed as Student's *t* test. The results of aldosterone and cortisol contents in each adrenal vein of IHA and EHT were described differently because there was no difference between right and left adrenal veins.

Abbreviations: UMN, unilateral multiple adrenocortical micronodules; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; UAH, hyperaldosteronism due to unilateral adrenal hyperplasia; EHT, essential hypertension; ND, not determined.

*$P < .05$ compared with EHT before ACTH stimulation.

†$P < .01$ compared with EHT 30 minutes after ACTH stimulation.

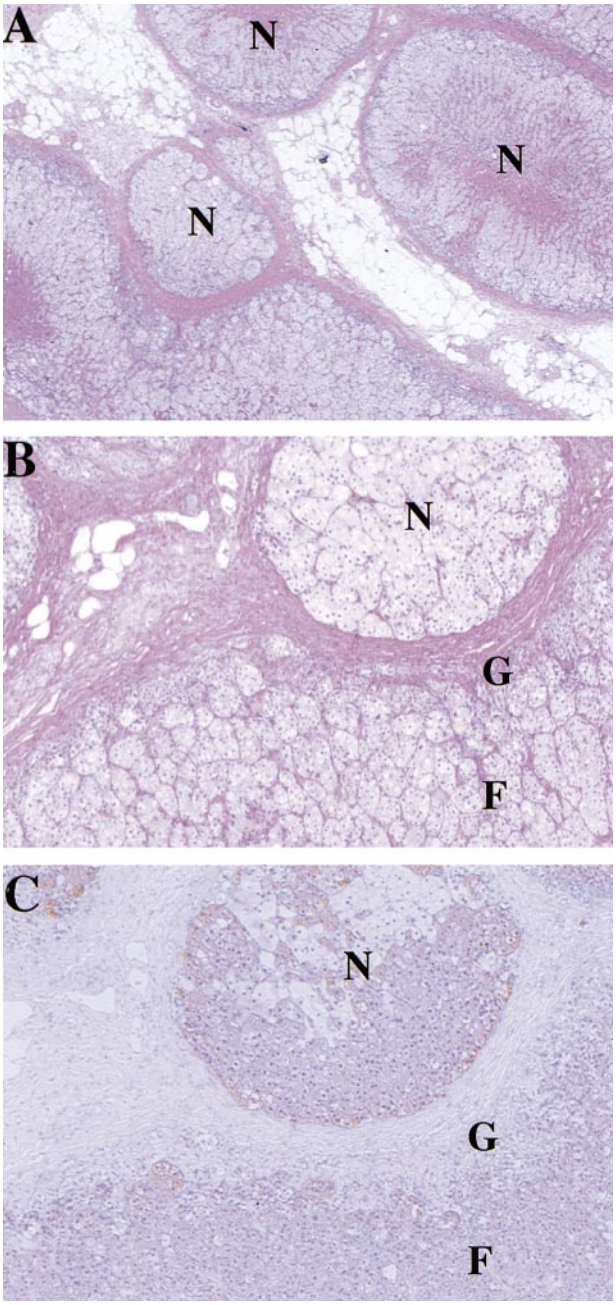
‡$P < .01$ compared with EHT before ACTH stimulation.

Table 5. Follow-up Studies on Plasma Aldosterone Concentration, PRA, and blood pressure of Four Patients With Primary Aldosteronism Due to UMN, 20 Patients With APA, Two Patients With IHA, and One With Hyperaldosteronism Due to UAH

	Plasma Aldosterone Concentration (ng/dL)			PRA (ng/mL/h)			Blood Pressure (mm Hg)					
							Preop		Postop		*P* Value	
	Preop	Postop	*P* Value	Preop	Postop	*P* Value	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
UMN (n = 4)	18.2 ± 0.8	8.4 ± 0.7	<.001	0.3 ± 0.1	1.1 ± 0.2	<.001	180 ± 6	99 ± 2	134 ± 2	74 ± 2	<.001	<.001
APA (n = 20)	22.5 ± 1.7	5.8 ± 0.6	<.001	0.2 ± 0.1	1.0 ± 0.1	<.001	181 ± 4	101 ± 3	129 ± 3	77 ± 2	<.001	<.001
IHA (n = 2)	18.1 ± 1.6	17.6 ± 0.3	NS	0.3 ± 0.1	0.3 ± 0.2	NS	162 ± 8	102 ± 2	161 ± 9	95 ± 5	NS	NS
UAH (n = 1)	16.3	7.3		0.2	1.1		164	98	120	82		

NOTE. The data obtained from UMN, APA, and IHA were statistically calculated and the results are expressed as mean ± SE. *P* values of each parameter between preoperative stage and postoperative phase were determined by paired Student's *t* test.

Abbreviations: NS, not significant, UMN, unilateral multiple adrenocortical micronodules; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; UAH, unilateral adrenal hyperplasia; Preop, before; Postop, after unilateral adrenalectomy; PRA, plasma renin activity.



6.5 × 2.0 × 0.8 cm, the right adrenal gland of patient 3 measured 4.5 × 3.5 × 0.7 cm and the right adrenal gland in patient 4 measured 4.5 × 3.2 × 0.8 cm. Histologic sections of these adrenal glands demonstrated the presence of poorly encapsulated multiple adrenocortical micronodules ranging from 2 to 3 mm in greatest dimension, which were composed of both compact and clear cells, predominantly the latter (Fig 1A). The non-nodular adrenals demonstrated hyperplasia of the zona glomerulosa, whereas the zona fasciculata-reticularis was unremarkable (Fig 1B). The results of immunohistochemical analysis of steroidogenic enzymes including P-450$_{SCC}$, 3β-HSD, P-450$_{C21}$, P-450$_{C11}$, and P 450$_{C17}$ are summarized in Table 6. Immunohistochemical analysis of steroidogenic enzymes showed immunoreactivity for P-450$_{SCC}$, 3β-HSD (Fig 1C), P-450$_{C21}$, and P-450$_{C11}$ in cortical cells of the nodules; but P-450$_{C17}$, which was positive in cortical cells of both aldosterone-producing adenomas and nodular lesions of UAH, was negative. The distribution for immunoreactivity of steroidogenic enzymes in the non-nodular adrenal cortex was similar to those of the normal adrenal gland, including immunolocalization of P-450$_{C17}$ in the zona fasciculata-reticularis, whereas immunoreactivity for 3β-HSD in the hyperplastic zona glomerulosa, which was positive in IHA and UAH, was negative in UMN, similar to APA (Fig 1C). Patients 2, 3, and 4 also demonstrated similar microscopic and immunohistochemical features of the adrenal cortex as those in patient 1.

DISCUSSION

We present here 4 patients with a unilateral adrenal disorder causing primary aldosteronism. Our cases had characteristics similar to APA, IHA, and UAH in some respects, such as hypertension and hyporeninemic hyperaldoster-

Fig 1. (A) Histopathologic features of adrenocortical nodules in patient 1. Multiple nodules were observed. N, nodule (×40) hematoxylin-eosin stain. (B) Histopathologic features of a representative adrenocortical nodule (N) and non-nodular adrenal cortex in patient 1. The zona glomerulosa of the non-nodular adrenal cortex demonstrated hyperplasia. G, zona glomerulosa; F, zona fasciculata (×100) hematoxylin & eosin stain. (C) Immunohistochemistry of 3β-HSD in one of the adrenocortical nodules and non-nodular adrenal cortex in patient 1. 3β-HSD immunoreactivity, demonstrated as brown reaction products of diaminobenzidine reaction, was detected in both cortical N cells and F, but not in G (×100).

Table 6. Histologic Findings in UMN, APA, IHA, and UAH

	UMN	APA	IHA	UAH
Pathologic findings	Poorly encapsulated multiple nodules	Adrenocortical adenoma	Adrenocortical hyperplasia	Nodules and adrenocortical hyperplasia
Immunohistochemical distribution of cytochrome P-450 in nodular lesions of the affected adrenal gland				
$P\text{-}450_{SCC}$	+	+		+
3β-HSD	+	+		+
$P\text{-}450_{c21}$	+	+		+
$P\text{-}450_{c11}$	+	+		+
$P\text{-}450_{c17}$	−	+		+
Immunohistochemical distribution of cytochrome P-450 in zona glomerulosa of the affected adrenal gland				
$P\text{-}450_{scc}$	±	±	+	+
3β-HSD	−	−	+	+
$P\text{-}450_{c21}$	+	+	+	+
$P\text{-}450_{c11}$	±	±	+	+
$P\text{-}450_{c17}$	−	−	−	−

Abbreviations: UMN, unilateral multiple adrenocortical micronodules; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; UAH, hyperaldosteronism due to unilateral adrenal hyperplasia; −, no immunoreactivity; ±, weak immunoreactivity; +, strong immunoreactivity.

onism, suppression of PRA when stimulated by furosemide infusion plus the upright position, and an elevated ratio of PAC to PRA at 90 minutes after captopril administration.[7] Moreover, the peripheral blood concentrations of other mineralocorticoids, including DOC, B, 18-OH-B, and 18-OH-DOC in UMN were not significantly different from those in APA, IHA, and UAH. Therefore, it appears to be impossible to differentiate our 4 cases from other causes of primary aldosteronism on endocrinologic findings.

The clinical features of UMN resemble those of APA and UAH in the hypersecretion of aldosterone from one of the

Table 7. Summary of Clinical, Endocrinologic, and Histopathologic Findings in UMN, APA, IHA, UAH, and PAH

	UMN	APA	IHA	UAH	PAH*
Response of PRA to furosemide plus upright test	Suppressed	Suppressed	Moderately suppressed	Suppressed	
PAC/PRA 90 min after administration of 50 mg captopril	20<	20<	20<	ND	
Findings of adrenal computed tomography	No abnormality	Unilateral tumor	Bilateral adrenal enlargement	Unilateral adrenal enlargement	No abnormality
Aldosterone concentration in adrenal vein	Increased unilaterally	Increased unilaterally	Increased bilaterally	Increased unilaterally	Increased bilaterally
Pathologic findings	Poorly encapsulated multiple nodules	Adrenocortical adenoma	Adrenocortical hyperplasia	Nodules and adrenocortical hyperplasia	Adrenocortical hyperplasia
Immunohistochemical distribution of cytochrome $P\text{-}450_{c17}$ in nodular lesions of the affected adrenal gland	−	+		+	
Immunohistochemical distribution of cytochrome P-450 in zona glomerulosa of the affected adrenal gland					
$P\text{-}450_{scc}$	±	±	+	+	
3β-HSD	−	−	+	+	
$P\text{-}450_{c11}$	±	±	+	+	
Effect of unilateral adrenalectomy					
Blood pressure	Improved	Improved	Not improved	Improved	
Plasma aldosterone concentration	Decreased	Decreased	Not changed	Decreased	

Abbreviations: PAC, plasma aldosterone concentration; PRA, plasma renin activity; UMN, unilateral multiple adrenocortical micronodules; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; UAH, hyperaldosteronism due to unilateral adrenal hyperplasia; PAH, primary adrenal hyperplasia; ND, not determined; −, no immunoreactivity; ±, weak immunoreactivity; +, strong immunoreactivity.

*Characteristics of PAH are summarized according to the report described by Biglieri et al[9] and Banks et al.[10]

adrenal glands and in the improvement of hypertension and hyporeninemic hyperaldosteronism after unilateral adrenalectomy. UMN is, however, different from IHA and PAH in the following ways: (1) PAC was bilaterally high by selective adrenal venous sampling in the cases with IHA and PAH,[9,10] while laterality of PAC was observed in UMN, as well as in APA and UAH. (2) Hypertension and hyporeninemic hyperaldosteronism were not completely improved after unilateral adrenalectomy in IHA, although those were cured after resection of affected adrenals in UMN, APA, and UAH. Thus, it is possible to conclude that UMN is a completely different cause of primary aldosteronism from that of IHA and PAH.

Findings from the histopathologic examinations and immunohistochemical analysis of steroidogenic enzymes of resected adrenal glands were useful to differentiate UMN from other types of primary aldosteronism. Microscopic analysis of the resected adrenal gland in our 4 patients demonstrated small, poorly encapsulated multiple cortical nodules, while the residual adrenal tissue showed no evidence of hyperplasia in the outer portions of the zona fasciculata. These findings were completely different from those in APA, in which a well-encapsulated adenoma is present, from those of IHA, in which there is hyperplasia of both the zona glomerulosa and the outer portions of the zona fasciculata.

The immunohistochemical analysis of steroidogenic enzymes such as P-450$_{SCC}$, 3β-HSD, P-450$_{C21}$, P450$_{C11}$, and P-450$_{C17}$ clearly showed differences between our cases with UMN and those with APA. In UMN, immunoreactivity of P-450$_{SCC}$, 3β-HSD, P-450$_{C21}$, and P450$_{C11}$ was marked, but that of P-450$_{C17}$ was absent, especially in internodular cells. The pattern of these enzymes immunolocalization in internodular cells in the adrenals of our UMN patients was similar to that observed in the hyperplastic glomerulosa cells in IHA,[11] but different from that in the adenoma cells of APA patients, because P-450$_{C17}$ was usually expressed in the latter. Moreover, the immunohistochemical distribution of these enzymes in the zona glomerulosa cells adjacent to the micronodules resembled that observed in the zona glomerulosa cells adjacent to aldosteronoma, suggesting that the biosynthesis of steroids in the residual tissues associated with micronodules in UMN may be reduced by the hypersecretion of aldosterone from the cortical micronodules, because the same pattern of expression of steroidogenic enzymes including P-450$_{SCC}$, 3β-HSD, and P-450$_{C11}$ was detected in the adjacent tissues of APA. Furthermore, the pattern of these enzymes immunolocalization in the zona glomerulosa cells in UMN suggested that UMN may be different from UAH, because the immunohistochemical localization of these enzymes in the zona glomerulosa cells adjacent to nodules in UAH indicated the presence of active steroidogenesis in these cells.

Furthermore, we would like to emphasize that the diagnosis of UMN is clinically very important. Endocrinologic findings in UMN were quite similar to those in APA, IHA, and UAH (Table 7), but roentgenologic examinations showed no abnormality in adrenal glands in UMN. Therefore, patients with UMN may be misdiagnosed as hyporeninemic EHT or IHA and treated with antihypertensive drugs, although hypertension caused by UMN apparently responds to unilateral adrenalectomy. We recommend here that hypersecretion of aldosterone from unilateral adrenal gland must be confirmed by selective adrenal venous sampling in patients with hyperaldosteronism who demonstrate "normal" adrenal glands in standard imaging and also that unilateral adrenalectomy should be performed in those patients, because long follow-up studies demonstrated a complete improvement in blood pressure and hormonal abnormalities in patients with UMN (Table 5).

In conclusion, as summarized in Table 7, our findings suggest that the multiple adrenocortical nodules found in patients with UMN are different from APA, IHA, and UAH and hypersecrete aldosterone, resulting in primary aldosteronism. We, therefore, would like to propose hyperaldosteronism due to UMN as a cause of primary aldosteronism, which has not yet been reported.

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