

Bilateral Aldosterone-Producing Adenomas in Two Patients Diagnosed by Immunohistochemical Analysis of Steroidogenic Enzymes

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WATANABE, N., TSUNODA, K., SASANO, H., OMATA, K., IMAI, Y., ITO, S. and ABE, K. *Bilateral Aldosterone-Producing Adenomas in Two Patients Diagnosed by Immunohistochemical Analysis of Steroidogenic Enzymes*. Tohoku J. Exp. Med., 1996, 179 (2), 123-129 — Bilateral adrenal aldosterone-producing adenomas (APA) are rare. It is important to distinguish bilateral APA from idiopathic hyperaldosteronism (IHA), which is due to bilateral hyperplasia of the adrenal cortex. We present two patients with bilateral APA in whom the diagnosis was made histochemically by analyzing steroidogenic enzymes. They showed hypokalemia, high plasma aldosterone concentration (PAC) and suppressed plasma renin activity (PRA). Bilateral adrenal tumors were represented by computed tomography, and surgical resection was performed. In both cases, cytochrome P-450 and other enzymes that were involved in aldosterone synthesis were found mainly in tumor, but little in the zona glomerulosa of the adjacent adrenals, which showed paradoxical hyperplasia. Such cases are difficult to distinguish from IHA. The two disorders were differentiated by immunohistochemical analysis of steroidogenic enzymes. ————— bilateral aldosterone producing adenomas; idiopathic hyperaldosteronism; immunohistochemistry; enzymatic analysis; adrenal gland

In most cases, primary aldosteronism is due to a unilateral adrenal adenoma or to bilateral hyperplasia of the adrenal cortex (Conn et al. 1964). Bilateral adrenal adenomas are rare. Furthermore, the routine radiological, endocrinological and histological methods are not necessarily sufficient to distinguish them from idiopathic hyperaldosteronism (IHA) in the case with bilateral small tumors.

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Recent advances in the steroidogenic enzymology and immunohistochemistry have permitted the evaluation of adrenal disorders on an entirely new level. Thus, we documented two patients with bilateral aldosterone-producing adenomas (APA) who were diagnosed by specific immunohistochemical methods (Sasano 1992; Sasano et al. 1992).

CASE REPORTS

Case 1

A 31-year-old Japanese woman was admitted to our hospital with a 2-year history of hypertension and hypokalemia. She frequently suffered from muscle weakness, nausea and vomiting. Her blood pressure was 154/100 mmHg, and her pulse rate was 66 beats/min. Results of laboratory tests were as follows: serum sodium 142 mEq/liter, potassium 2.8 mEq/liter, chloride 100 mEq/liter, and fasting blood sugar 79 mg/100 ml. Blood gas analysis showed metabolic alkalosis (pH 7.465). Electrocardiography showed ST depression in II, III, _aV, V_{F5} and V₆. The cardiothoratic ratio was 45.5%. Plasma renin activity (PRA) was suppressed below the measurable range (normal range: 0.83–5.0 ng/ml/hr), and posture-related stimuli or the administration of furosemide did not alter the PRA. A high plasma concentration of aldosterone (PAC) was observed: 26.6 ng/100 ml (normal range: 2–12 ng/100 ml). PRA and PAC were measured by previously reported methods (Abe et al. 1975; Tsunoda et al. 1986). Abdominal CT scanning showed a mass measuring 10 mm in diameter in the right adrenal gland, with a smaller mass present in the left adrenal gland. ¹³¹I-aldosterol adrenal scintigraphy demonstrated bilateral uptake, which was not suppressed by dexamethasone administration (2 mg/day, for 10 days). A left total adrenalectomy and a right partial adrenalectomy, including resection of the mass lesion, was performed. Bilateral adenomas with pseudohyperplasia of the adjacent adrenal glands were observed by microscopic examination on hematoxylin and eosin-stained slides.

The patient's blood pressure returned to normal postoperatively without medical treatment. Her PRA was then 1.3 ng/ml/hr, and the PAC decreased to the normal range (3.7 ng/100 ml).

Case 2

A 43-year-old Japanese woman with hypertension and hypokalemia was admitted for a detailed diagnostic examination in 1990. Her high blood pressure had been diagnosed in 1989: antihypertensive treatment was then started with a calcium entry blocker, a β -adrenergic blocker and an angiotensin converting enzyme inhibitor. Her serum potassium level was low (3.2 mEq/liter) when she was referred to our hospital. On admission, her blood pressure was 188/98 mmHg and her serum potassium level was 3.0 mEq/liter. The cardiothoratic ratio was 48%, and there was no remarkable findings in the electrocardiography. PRA was not detectable when she was resting supine, and did not respond to administration

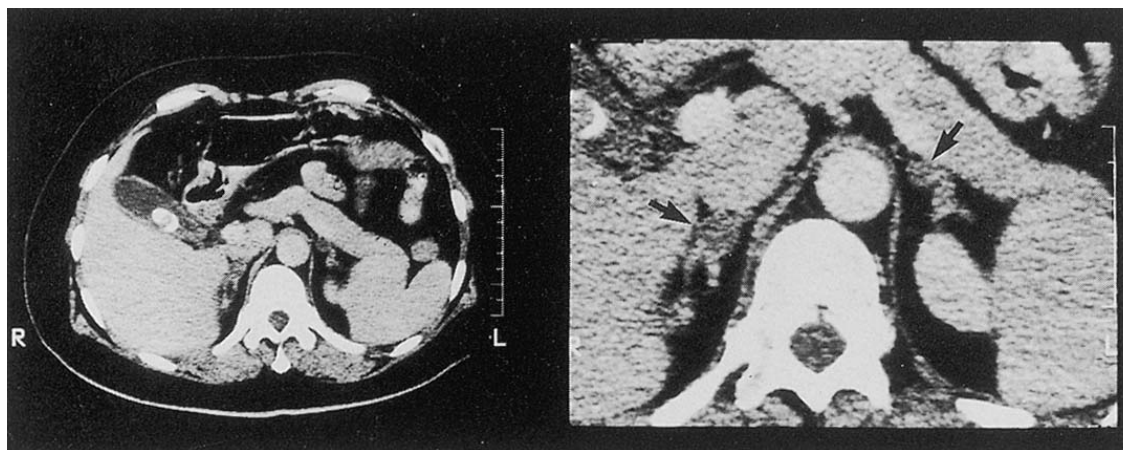


Fig. 1. Computed tomography scans showing bilateral adrenal tumors in Case 1. The right image shows a mass 1 cm in diameter in the left adrenal gland (arrow), and a mass less than 1 cm in diameter in the right adrenal gland (arrow).

of 50 mg furosemide or 50 mg captopril. The PAC was high (45.4 ng/100 ml at 8:00 a.m.) with a normal diurnal variation. A CT scanning of the abdomen showed adrenal mass lesions in the bilateral adrenal glands (Fig. 1, right: 10 mm in diameter, left: less than 10 mm in diameter). Primary aldosteronism with bilateral adenomas was diagnosed. A right total adrenalectomy and left partial adrenalectomy, including resection of the mass lesion were performed. Microscopic examination on hematoxylin and eosin-stained slides revealed that the adenomas consisted of clear cells and that the adjacent adrenal gland was hyperplastic (Fig. 2).

Immunolocalization of steroidogenic enzymes

The recent characterization of specific steroidogenic enzymes such as the cytochromes P-450, which are involved in adrenal steroid biosynthesis, makes it possible to generate specific antibodies against these enzymes. We used specific antibodies against the steroidogenic enzymes, including cholesterol side chain cleavage (P-450_{SCC}), 21-hydroxylase (P-450_{C21}), 11 β -hydroxylase (P-450_{11 β}), 3 β -hydroxysteroid dehydrogenase (3 β -HSD), 17 α -hydroxylase (P-450_{17 α}), to examine their localization in the adrenal glands. Resected adrenal tissues were fixed in 10% formalin solution after surgery and made into paraffin sections, 2.5 μ m thick. We employed the biotin-strept avidin (B-SA) amplified method using the StrAvi-Gen B-SA immunostaining system (Biogenex, Dublin, CA, USA). For control immunostaining, 0.01 M (phosphate-buffered saline PBS) and normal rabbit or mouse IgG were used instead of primary antibodies. (Sasano 1992; Sasano et al. 1992). In both patients, all the cytochromes P-450 and 3 β -HSD involved in corticosteroidogenesis were present in the adenomas except for P-450_{17 α} , which does not contribute to the biosynthesis of aldosterone. Immunoactivity of P-450_{11 β} and 3 β -HSD was demonstrated minimally in the hyper-

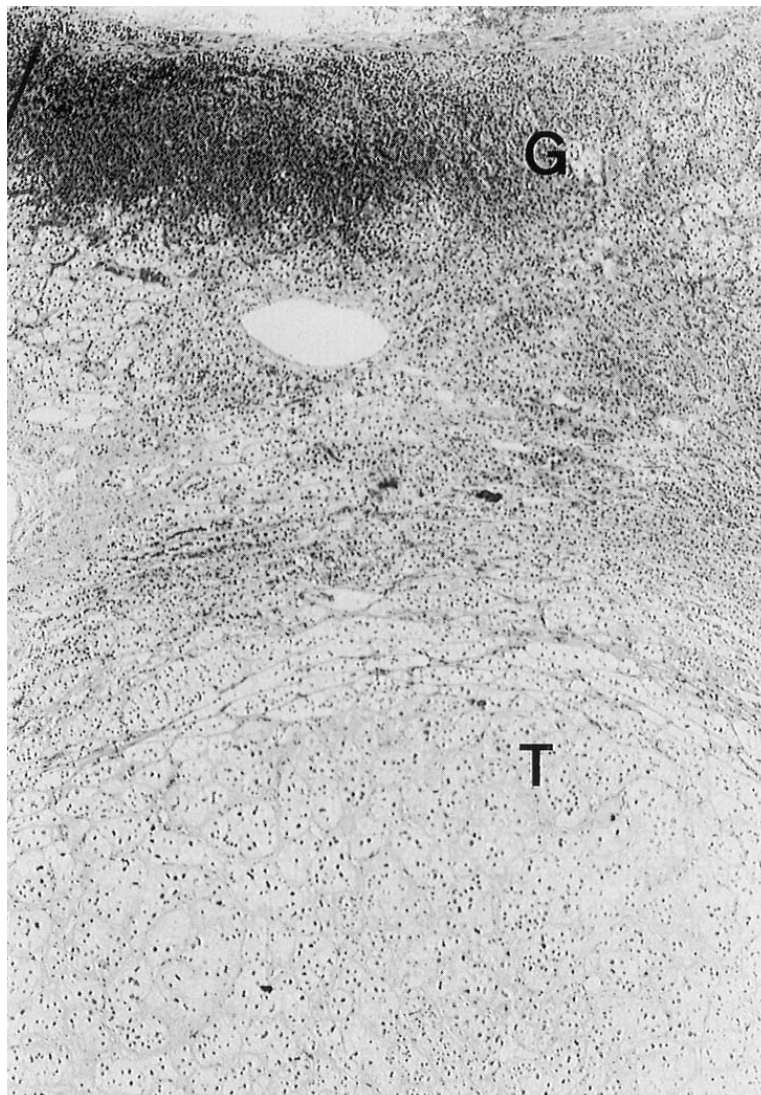


Fig. 2. Tumor (aldosteronoma) with adjacent adrenal gland which stained with haematoxylin and eosin (left adrenal in Case 2). Zona glomerulosa shows pseudohyperplasia.

G, zona glomerulosa; T, tumor

plastic zona glomerulosa of the adjacent adrenal glands in either patient (Figs. 3 and 4).

DISCUSSION

Primary aldosteronism due to bilateral APA is rare, accounting for only 1.4% of the cases of primary aldosteronism according to Conn et al in 1964. Bilateral APA must be differentiated from IHA because of the differences in treating the two diseases: APA is usually treated surgically whereas patients with IHA receive conservative medical treatment with agents such as spironolactone. Several methods have been suggested to distinguish bilateral APA from IHA. Hoefnagels et al (1980) found a normal diurnal rhythm under the influence of ACTH in patients with APA with high basal levels of PAC. In contrast, PAC in those

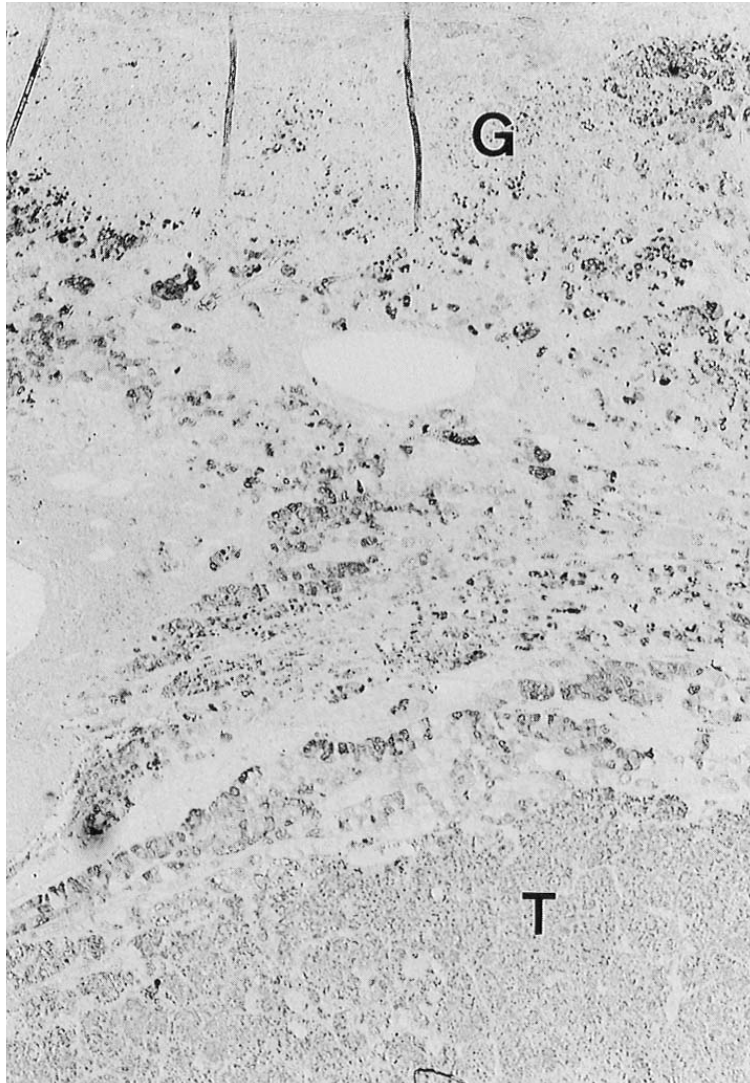


Fig. 3. Immunohistochemical distribution of 3β -HSD in the zona glomerulosa of the adrenal gland and the tumor (aldosteronoma) in Case 2. 3β -HSD stained weakly as compared to aldosteronoma. G, zona glomerulosa; T, tumor.

with IHA is modulated by the renin-angiotensin system, i.e., responsiveness to posture-related stimuli (Ganguly et al. 1973) or to exogenous angiotensin II (Wisgerhof et al. 1981). However, APA cannot be distinguished from IHA endocrinologically in all cases (Vetter et al. 1985). Computed tomography of the adrenal glands occasionally may be helpful, but it may not distinguish an adenoma from macronodular hyperplasia (Roberts et al. 1985).

Generally, in patients with APA, PAC and serum potassium are normalized and hypertension is cured or improved after adrenalectomy (Groth et al. 1985; Gleason et al. 1993). On the other hand, adrenalectomy is less effective in patients with IHA. Gleason et al. (1993) reported that hypertension was not cured or improved in 9 of 11 patients with IHA after unilateral adrenalectomy. Groth et al. (1985) evaluated the long-term effect of unilateral adrenalectomy in

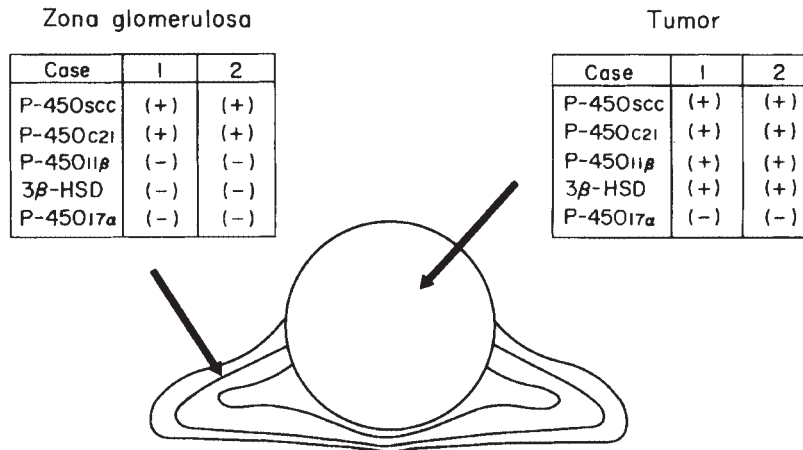


Fig. 4. Localization of steroidogenic enzymes in surgically resected right adrenal glands in Cases 1 and 2. The same findings were observed in the left adrenal gland of each patient.
(+), positive; (-), weak or negative.

51 patients with primary aldosteronism (39 with APA and 12 with IHA), and they reported that none of patients with IHA showed a long-lasting normalization of aldosterone secretion. The patients with IHA required antihypertensive agents including spironolactone, after surgery.

In the present study, we determined the localization of steroidogenic enzymes in surgically resected adrenal glands, employing specific antibodies against those enzymes. The zona glomerulosa of the adrenal glands adjacent to the aldosteronoma occasionally demonstrated hyperplasia (Conn 1955), which makes it difficult to differentiate APA from IHA. Recent immunohistochemical study of steroidogenic enzymes showed that overexpression of steroidogenic enzymes involved in the biosynthesis of aldosterone were absent in the zona glomerulosa of the adrenal glands adjacent to the aldosteronoma, while hyperplastic zona glomerulosa in the adrenal glands of IHA presented a marked immunoreactivity to steroidogenic enzymes, particularly P-450_{11 β} and 3 β -HSD (Sasano 1992).

We observed that the steroidogenic enzymes, i.e., P-450_{11 β} and 3 β -HSD, were minimally expressed in the zona glomerulosa of the adrenal glands adjacent to the aldosteronoma, strongly suggesting a diagnosis of APA in such cases. We conclude that the immunolocalization of steroidogenic enzymes can differentiate post operatively bilateral adrenocortical adenomas from IHA in patients with hyperaldosteronism.

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