Endocrine Journal 2006, **53** (5), 609–613

A Case of Preclinical Cushing's Syndrome Associated with Diurnal Rhythms of ACTH and Cortisol in Blood: Correlation with Histological Findings

TOMOO IKARASHI, KYUZI KAMOI, KATSUAKI ASAKAWA, MIDORI TANAKA, MASASHI MIYAKOSHI, TAKESHI KOMEYAMA*, HIDEO MORISHITA*, HIROYUKI USUDA**, IWAO EMURA** AND HIRONOBU SASANO***

Department of Internal Medicine, Division of Endocrine and Metabolism, Nagaoka Red Cross Hospital, Nagaoka, Niigata 940-2085, Japan
**Department of Urology, Nagaoka Red Cross Hospital, Nagaoka, Niigata 940-2085, Japan*
***Department of Pathology, Nagaoka Red Cross Hospital, Nagaoka, Niigata 940-2085, Japan*
****Department of Pathology, Tohoku University School of Medicine, Sendai, 980-8575, Japan*

Abstract. We describe a case of adrenocortical adenoma with preclinical Cushing's syndrome demonstrating diurnal rhythms of ACTH and cortisol in blood. A 50-year-old man was admitted to the hospital for the evaluation of incidental right adrenal mass with hyperglycemia and hypertension. On admission, there were no signs of clinical manifestation of hypercortisolism. The basal levels of cortisol (9.3 μg/dl) and ACTH (9.4 pg/ml) at 0800 h were not elevated and these diurnal rhythms were maintained. One or 8 mg of dexamethasone given orally overnight suppressed the plasma ACTH but not serum cortisol. Ultrasonogram, CT and scintiscan of ^{131}I adosterol all demonstrated an enlarged adrenal mass in the right adrenal gland. The right adrenal gland was subsequently resected by laparoscopic surgery. Histopathological findings of resected adrenal tumor were consistent with adrenocortical adenoma. Adjacent non-neoplastic adrenal tissue demonstrated adrenocortical atrophy but DHEA-sulfotransferase immunoreactivity in the zona reticularis was detected.

Key words: Preclinical Cushing's syndrome, Diurnal rhythms of cortisol and ACTH, Histology of adrenal gland
(*Endocrine Journal* **53**: 609–613, 2006)

SINCE Charbonnel *et al*. [1] reported the entity of preclinical Cushing's syndrome in 1981, numerous cases have been reported in the literature [2–9]. Some of them [2–4, 7] demonstrated a normal diurnal rhythm of cortisol in blood as shown in healthy persons [10], but the histopathological features of adrenocortical neoplasms in these patients have not been reported in the literature to the best of our knowledge.

We report a case of preclinical Cushing's syndrome associated with diurnal rhythms of ACTH and cortisol in blood including histopathological findings and immunohistochemical evaluation of steroidogenic enzymes.

Received: May 29, 2006
Accepted: June 6, 2006
Correspondence to Dr. Kyuzi KAMOI, Department of Internal Medicine, Nagaoka Red Cross Hospital, 297-1 Terajima-machi, Nagaoka, Niigata 940-2085, Japan

Case Report and Methods

A 50-year-old man was admitted to our hospital for evaluation of incidental right adrenal tumor associated with hyperglycemia and hypertension. Hypertension had been pointed out since he was 30 years old. At 44 years of age, he received lithectomy for ureterolithiasis. At 45 years of age, hyperglycemia was detected by regular health check up. His mother had hypertension and diabetes mellitus. Prior to admission, hypertension (176/107 mmHg) and hyperglycemia with 191 mg/dl in fasting blood glucose and 9.2% in HbA1c were noted. In addition, a right adrenal mass was detected by an abdominal echogram, which was performed by regular health check up. His chief complaints at the time of admission were polydipsia, polyuria and thirst. He was 162 cm tall and weighed 62.5 kg, and had BMI of 23.4 kg/m^2. His pulse was 68/min and regular, with blood pressure of 182/

100 mmHg. There were no signs of moon face, buffalo hump, central obesity, hirsutism, subcutaneous bleeding or striae cutis. Also, there was no edema in legs.

On this admission, sustained hyperglycemia was noted with normokalemia (Table 1). He had nonproliferative retinopathy and mild arteriosclerosis.

Table 1. General laboratory data obtained at the time of admission

Urinalysis	
Glucose	(3+)
Protein	(–)
Ketones	(–)
Sediment	
Red blood cells	(–)
Hematological analysis	
Red blood cells	$517\times10^4/mm^3$
Hemoglobin	15.4 g/dl
Hematocrit	44.9%
White blood cells	$3{,}500/mm^3$
Eosinophils	1%
Stab	0%
Segmental neutrophils	68%
Monocytes	4%
Lymphocytes	27%
Atypical lymphocytes	0%
Platelets	$22.7\times10^4/mm^3$
Blood chemistry analysis	
GOT	21 IU/l
GPT	44 IU/l
LDH	215 IU/l
γGTP	43 IU/l
ALP	277 IU/l
TBiI	0.8 mg/dl
TP	6.6 g/dl
Alb	4.6 g/dl
TC	182 mg/dl
HDL-C	52 mg/dl
LDL-C	111 mg/dl
TG	102 mg/dl
CRP	0.01 mg/dl
Electrolytes	
BUN	13.3 mg/dl
Uric acid	4.1 mg/dl
Na	141 mEq/l
K	4.4 mEq/l
Creatinine	0.8 mg/dl
Cl	104 mEq/l
Ca	9.8 mg/dl
P	2.0 mg/dl
Diabetes mellitus-related data	
FBG	179 mg/dl
Blood glucose 2 h after dinner	305 mg/dl
Fasting IRI	2.2 μIU/ml
HbA1c	9.7%
Urinary CPR	67.6–82.2 μg/day

As shown in Table 2, the adrenomedullary function was within normal limits. Concentrations of aldosterone and dehydroepiandrosterone sulfate (DHEA-S) in blood were also within normal range. The basal levels of ACTH (9.4 pg/ml) and cortisol (9.3 μg/dl) measured at 0800 h (Table 2) were not much elevated and decreased to 5.9 pg/ml and 4.0 μg/dl at 2200 h, respectively. This indicated that the levels of both ACTH and cortisol in this patient were elevated in the morning and declined throughout the daytime (Fig. 1). Excretion of urinary 17-OHCS and 17-KS were within normal range, but urinary excretion of free cortisol was elevated. One or 8 mg of dexamethasone given orally at 2300 h suppressed the basal levels of plasma ACTH at 0800 h, respectively, while they did not completely suppress the serum cortisol level. These findings were

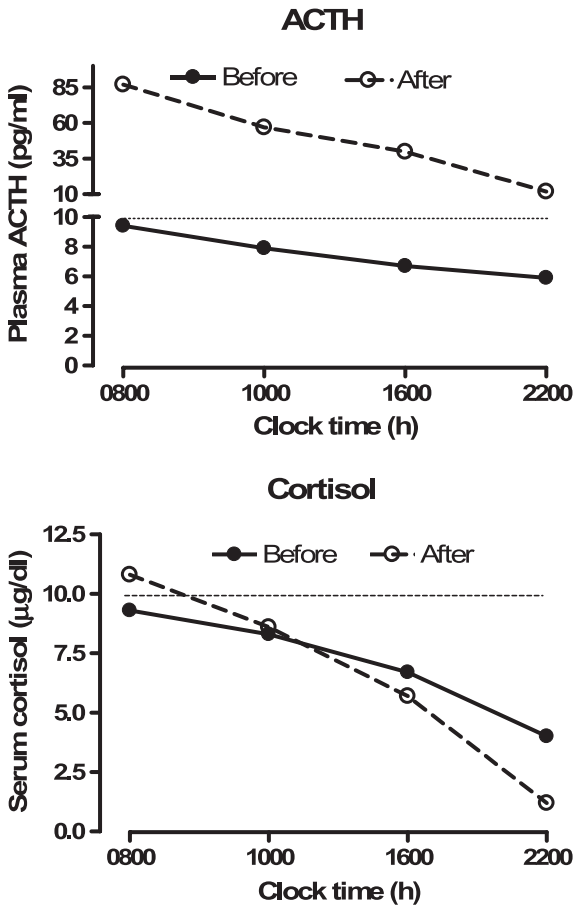

Fig. 1. Diurnal patterns of plasma ACTH (upper figure) and serum cortisol (lower figure) before (•–•) and after (○–○) the resection of right adrenal gland, respectively. The dotted lines represent the lower limits of normal in plasma ACTH and serum cortisol concentrations at 0800 h, respectively.

consistent with hypercortisolism.

As ultrasonogram of the abdomen demonstrated an enlarged and hypoechoic mass (1.8 cm in greatest dimension) in his right adrenal gland, CT scan was performed to further characterize its features. CT demonstrated the same enlarged mass in the right adrenal gland. Scintiscan of ^{131}I adosterol also displayed strong uptake of ^{131}cholesterol in the same region, while there was reduced uptake of ^{131}cholesterol in the left adrenal gland.

The right adrenal gland was subsequently resected by laparoscopic surgery. The resected adrenal tissue weighed 12 gm and the cut surface of the tumor appeared brown-yellow in color. Light microscopic ex-

Table 2. Adrenal-related laboratory data before and after the resection of right adrenal tumor

		Before	After	Normal range
Urinary catecholamine excretion				
Adrenaline	(μg/day)	16	11	1–29
Noradrenaline	(μg/day)	91	100	26–230
Dopamine	(μg/day)	762	824	310–1140
Metanephrine	(mg/day)	0.11	0.05	0.04–0.19
Normetanephrine	(mg/day)	0.18	0.16	0.09–0.33
Plasma renin	(pg/ml)	4.1		2.5–21.4
aldosterone	(pg/ml)	68	45.5	55.9–150
Blood DHEA-S	(ng/ml)	739	501	480–2860
Urinary free cortisol	(μg/day)	102	19	11–80
17-OHCS	(mg/day)	7.42	3.96	3.40–12.00
17-KS	(mg/day)	6.58	2.70	4.60–18.00
Overnight dexamethasone suppression test				
1 mg; ACTH*	(pg/ml)	9.4 → 4.0	86.8 → 4.0	4.4–48.0
Cortisol*	(μg/dl)	9.3 → 3.4	10.8 → 0.4	10.0–25.0
8 mg; ACTH*	(pg/ml)	9.4 → 4.7		
Cortisol*	(μg/dl)	9.3 → 2.9		

* Values were obtained at 0800 h.

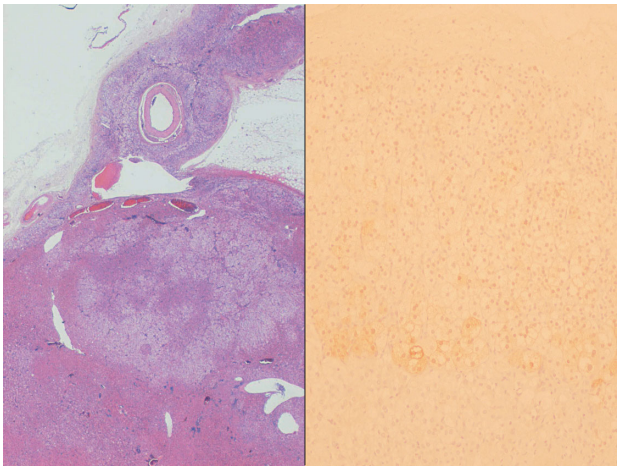

Fig. 2. Histological findings of the tumor. The tumor located in the right adrenal gland showed well-circumscribed but not encapsulated adrenocortical adenoma (A). Coexistence of compact (70%) and clear cells (30%) and all enzymes of steroid synthesis including DHEA-sulfotransferase were expressed by the immunohistochemical method (B).

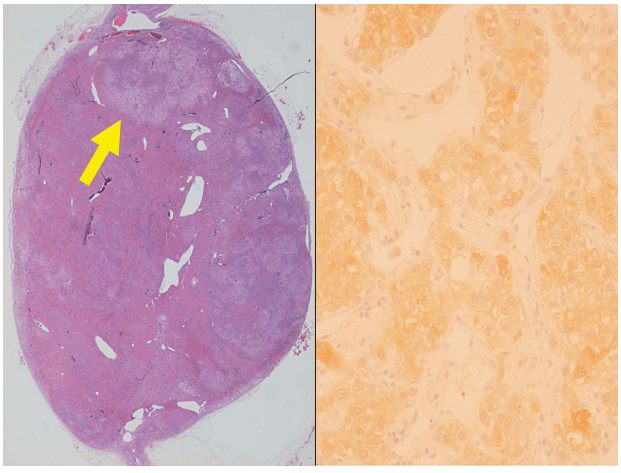

Fig. 3. Histological findings of the tissue adjacent to the tumor. Adjacent adrenal tissue was atrophic and had adrenocortical nodules that occurred secondarily (A). However, DHEA-sulfotransferase in the zona reticularis was abundantly expressed (B). The tumor is indicated by the yellow arrowhead.

amination of the resected specimen demonstrated the mass was well-circumscribed but not encapsulated. The mass was diagnosed as adrenocortical adenoma. Histological coexistence of compact (70%) and clear cells (30%) was noted (Fig. 2A) and all enzymes of adrenocortical hormone synthesis including DHEA-sulfotransferase were detected by an immunohistochemical method reported previously [5] (Fig. 2B), which is consistent with cortisol production in this adrenocortical adenoma. In contrast, tissue adjacent to the tumor was atrophic and had adrenocortical nodules that occurred secondarily (Fig. 3A). However, DHEA-sulfotransferase in the adjacent zona reticularis was abundantly detected (Fig. 3B).

After the resection, plasma level of ACTH at 0800 h increased markedly, while serum cortisol level and urinary excretion of free cortisol returned to normal level. One mg of dexamethasone given orally at 2300 h suppressed both the levels of serum cortisol and plasma ACTH at 0800 h completely (Table 2). These diurnal patterns were also maintained throughout the clinical course (Fig. 1).

At 3 to 4 weeks after the resection, blood glucose levels before breakfast and 2 h after dinner improved to 103 mg/dl and 160 mg/dl, respectively. HbA1c decreased to 7.0%. In addition, clinic blood pressure normalized to 130/70 mmHg.

Discussion

The patient had no specific clinical manifestation of Cushing's syndrome, although he had hyperglycemia and hypertension. Further, the blood cortisol level at 0800 h was not so high and was observed to have a circadian pattern. Nevertheless, urinary excretion of free cortisol was increased. In addition, serum cortisol levels at 0800 h were not completely suppressed by either 1 mg or 8 mg of dexamethasone administration, while plasma ACTH levels were low. Imaging methods suggested the presence of a functional adrenal tumor in this patient. These abnormalities improved after the resection of right adrenal tumor. All findings indicate that this patient had hypercortisolism. However, at least few questions were raised regarding his pathophysiological status: first, are the circadian patterns of ACTH and cortisol in blood related to diurnal rhythms as those in healthy subjects [10], and second, how did the histopathological finding with immunohistochemical evaluation of steroidogenic enzymes in this case change.

Van Cauter [11] defines diurnal rhythms as following: (1) The patterns of ACTH and cortisol in blood are associated with an early morning maximum, gradual decrement throughout daytime, a quiescent period of minimal secretory activity and an abrupt elevation during late sleep. (2) The amplitude of circadian rhythm may be estimated as 50% of the difference between maximum and minimum values of the best-fit curve. In this case, an abrupt elevation during late sleep could not necessarily be determined, but both his ACTH and cortisol levels in blood were elevated in the morning and decreased throughout the daytime. We could not evaluate his levels of minimal secretory activity, but estimated that the levels in blood was 4.0 pg/ml in ACTH and 2.9 μg/dl in cortisol on the basis of results by dexamethasone suppression test. Accordingly, the differences between maximum value at the morning and minimum value at the quiescent period may be higher than 50%, which indicated that this patient had diurnal rhythms.

Interestingly, adjacent non-neoplastic adrenal tissue, especially the zona reticularis was associated with abundant expression of DHEA-sulfotransferase, although it was morphologically atrophic and had adrenocortical nodules that occurred secondarily. In general, DHEA-sulfotransferase in the zona reticularis is not detected in patients with preclinical Cushing's syndrome without diurnal rhythms or full blown Cushing's syndrome, because DHEA-sulfotransferase production is directly influenced by the ACTH via negative feedback system of hypothalamus, pituitary and adrenal axis (HPA axis) [5, 12]. However, the findings of this case suggest that the ACTH-derived stimulation of DHEA-sulfotransferase expression may be controlled by the pulsatile-like secretion of ACTH rather than the absolute value of plasma ACTH in the low concentration of plasma ACTH. Usually, the serum concentration of DHEA-S in patients with preclinical Cushing's syndrome as well as Cushing's syndrome is decreased [5]. In this case, the normal level of DHEA-S may be explained by this morphological finding, but better investigations are required for further characterization of this unique finding. Moreover, the absence of specific histopathological findings in the cortical adenoma in this case, compared to those of preclinical Cushing's syndrome associated with no evidence of diurnal cortisol rhythm may be explained by the less

pronounced hypersecretion of cortisol in the tumor [5, 12].

All findings suggest that it is difficult to discriminate among nonfunctioning adenoma, preclinical Cushing's syndrome and overt Cushing's syndrome on the basis of steroidogenesis in the tumor, and that the analysis of adjacent non-neoplastic adrenal glands including the evaluation of histological atrophy of the zona fasciculata and reticularis as well as DHEA-sulfotransferase expression in the zona reticularis is of help in understanding the status of HPA axis in patients with these adrenocortical neoplasms.

In 1996, the "disorders of adrenal hormones" research committee of the Japanese Ministry of Health and Welfare [13] reported the diagnostic guidelines of preclinical Cushing's syndrome. In this case, all findings fulfilled the criteria of preclinical Cushing's syndrome except for the presence of both diurnal rhythms of ACTH and cortisol in blood, and normal level of blood DHEA-S. Since there is no report on the histopathological finding of DHEA-sulfotransferase activity in the zona reticularis in a case of preclinical Cushing's syndrome with diurnal rhythms of ACTH and cortisol in blood, our finding may contribute to better understanding the degree of ACTH suppression due to hypercortisolism.

In conclusion, we describe potentially significant pathohistological findings in a case of preclinical Cushing's syndrome with diurnal rhythms of ACTH and cortisol in blood.

This work was presented in part at the 6th Meeting of the Japan Endocrine Society, Kanto-Koshinetsu Block, Tokyo, March 5, 2006.

References

1. Charbonnel B, Chatal JF, Ozanne P (1981) Does the corticoadrenal adenoma with "pre-Cushing's syndrome" exist? *J Nuc Med* 22: 1059–1061.
2. Reincke M, Nieke J, Krestin GP, Saeger W, Allolio B, Winkelmann W (1992) Preclinical Cushing's syndrome in adrenal "incidentalomas": comparison with adrenal Cushing's syndrome. *J Clin Endocrinol Metab* 75: 826–832.
3. Ambrosi B, Peverelli S, Passini E, Re T, Ferrario R, Colombo P, Sartorio A, Faglia G (1995) Abnormalities of endocrine function in patients with clinically "silent" adrenal masses. *Eur J Endocrinol* 132: 422–428.
4. Mantero F, Masini AM, Opocher G, Giovagnetti M, Arnaldi G (1997) Adrenal incidentalomas: an overview of hormonal data from the National Italian Study Group. *Horm Res* 47: 284–289.
5. Morio H, Terano T, Yamamoto K, Tomizuka T, Oeda T, Saito Y, Tamura Y, Sasano H (1996) Serum levels of dehydroepiandrosterone sulfate in patients with asymptomatic cortisol producing adrenal adenoma: comparison with adrenal Cushing's syndrome and nonfunctional adrenal tumor. *Endocr J* 43: 387–396.
6. Morioka M, Tanaka H, Ohashi Y, Jin TX, Komatsu F, Watanabe H (1997) The analysis of steroidogenic activity in non-hyperfunctioning adrenocortical adenoma. *Endocr J* 44: 647–653.
7. Morioka M, Fujii T, Matsuki T, Jo Y, Kobayashi T, Tanaka H, Ohashi T, Kondo K (2000) Preclinical Cushing's syndrome: report of seven cases and a review of the literature. *Int J Urol* 7: 126–132.
8. Mune T, Morita H, Suzuki T, Takahashi Y, Isomura Y, Tanahashi T, Daido H, Yamakita N, Deguchi T, Sasano H, White PC, Yasuda K (2003) Role of local 11 beta-hydroxysteroid dehydrogenase type 2 expression in determining the phenotype of adrenal adenomas. *J Clin Endocrinol Metab* 88: 864–870.
9. Tokunagaa H, Miyamura N, Sasaki K, Yoshizato K, Itasaka M, Nakamaru K, Suefuji M, Kawashima J, Matsumoto K, Sasano H, Araki E (2004) Preclinical Cushing's syndrome resulting from black adrenal adenoma. A case report. *Horm Res* 62: 60–66.
10. Krieger DT, Allen W, Rizzo F, Krieger HP (1971) Characterization of the normal temporal pattern of plasma corticosteroid levels. *J Clin Endocrinol Metab* 32: 266–284.
11. Van Cauter E (2001) Endocrine rhythms. In: Becker KL (ed) Principles and Practice of Endocrinology and Metabolism. Third edition, Lippincott Williams & Wilkins, New York, 57–64.
12. Midorikawa S, Sanada H, Hashimoto S, Suzuki T, Watanabe T, Sasano H (2001) Analysis of cortisol secretion in hormonally inactive adrenocortical incidentalomas: study of in vitro steroid secretion and immunohistochemical localization of steroidogenic enzymes. *Endocr J* 48: 167–174.
13. Nawata H, Demura H, Suda T, Takayanagi R (1996) Adrenal preclinical Cushing syndrome. Annual report of the Ministry of Health and Welfare "Disorders of Adrenal Hormones" Research Committee, Japan, 1995–1996, pp. 223–225.