A Case of Preclinical Cushing's Disease, Accompanied with Thyroid Papillary Carcinoma and Adrenal Incidentaloma

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Abstract. A 75-year-old woman had tumors in her pituitary, thyroid and left adrenal gland. Plasma ACTH and cortisol levels were both mildly elevated. Both plasma ACTH and cortisol concentrations were partially suppressed by 1 mg of overnight dexamethasone suppression test, while both were inhibited with a dosage of 8 mg dexamethasone. Plasma ACTH and cortisol levels were increased in response to human CRH and desmopressin. Together with the observation of pituitary microadenoma, the patient had a pituitary ACTH-producing tumor. The patient, however, had no typical Cushingoid features, hypertension, or impaired glucose tolerance, suggesting that the tumor had an autonomic ACTH secretion that was insufficient for expressing clinical symptoms, the so-called preclinical Cushing's disease. A case of preclinical Cushing's disease is extremely rare. Further, the patient had thyroid papillary carcinoma and non-functioning adrenal tumor. Molecular genetic analysis demonstrated a polymorphism of the *menin* gene in the patient. Even without Cushingoid features in pituitary incidentaloma, we concluded that the elevated ACTH and cortisol levels should be followed up by CRH, desmopressin and dexamethasone suppression tests. This patient with preclinical Cushing's disease would be observed whether the physical conditions in the patient develop to overt Cushing's disease.

Key words: ACTH, Preclinical Cushing's disease, Thyroid carcinoma, Incidentaloma, Pituitary tumor
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CUSHING'S syndrome results from chronic glucocorticoid excess with clinically characteristic signs and symptoms. Recently, absence of typically clinical features in adrenal Cushing's syndrome has been defined as preclinical Cushing's syndrome [1]. Cushing's disease is primarily caused from pituitary adrenocorticotrophic hormone (ACTH)-secreting tumor. A case of a pituitary adenoma with ACTH production, causing "preclinical Cushing's disease", is extremely rare. Only a few cases of preclinical Cushing's disease have been reported [2–4]. We describe another case of preclinical Cushing's disease, interestingly, accompanied with thyroid carcinoma and adrenal incidentaloma.

Case report

Clinical summary

In June, 2002, a 75-year-old woman was referred to our department for further evaluation of a left adrenal incidentaloma. The patient had been diagnosed with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). On admission to our hospital, her height was 160 cm, body weight 59 kg, with a body mass index of 23.0. Blood pressure measured in the supine position was 120/70 mmHg. She had no typical Cushingoid features in that the characteristic features of central obesity, moon face, skin atrophy, purple striae and buffalo hump were absent.

The remarks of biochemical analysis are summarized in Table 1 and show that creatinine clearance (64.5 ml/min) was slightly decreased. The results of the hormonal data are listed in Table 2 and show that

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Table 1. General laboratory data

<i>Peripheral blood</i>					
White blood cells	5490/ μ l	(3500–9000)			
Red blood cells	418×10^4 / μ l	(380–500 $\times 10^4$)			
Hemoglobin	13.0 g/dl	(11.1–15.0)			
Hematocrit	39.2%	(33.3–45.0)			
Platelets	237×10^3 /l	(150–350 $\times 10^3$)			
<i>Blood biochemistry</i>					
Total protein	6.1 g/dl	(6.7–8.3)	Blood urea nitrogen	20 mg/dl	(8–20)
Albumin	3.8 g/dl	(3.8–5.3)	Creatinine	0.7 mg/dl	(0.4–0.7)
Aspartate aminotransferase	27 U/l	(8–38)	Uric acid	5.0 mg/dl	(2.6–6.0)
Alanine aminotransferase	35 U/l	(4–44)	Sodium	138 mmol/l	(138–149)
Lactate dehydrogenase	167 U/l	(80–200)	Chloride	106 mmol/l	(99–110)
Alkaline phosphatase	224 U/l	(104–338)	Potassium	4.6 mmol/l	(3.5–5.0)
r-glutamyltranspeptidase	29 U/l	(6–42)	Calcium	8.5 mg/dl	(8.2–10.2)
Total bilirubin	0.4 mg/dl	(0.2–1.2)	Phosphorus	3.8 mg/dl	(2.5–4.5)
Triglycerides	121 mg/dl	(51–167)	Plasma glucose (fasting)	104 mg/dl	(70–110)
Total cholesterol	187 mg/dl	(129–247)	Hemoglobin A1c	4.8%	(4.3–5.8)
Creatine kinase	33 U/l	(12–144)	Creatinine clearance	64.5 ml/min	(70–130)

Normal basal ranges are indicated in parentheses.

Table 2. Hormonal laboratory data

<i>Hormonal data</i>					
ACTH	59 pg/ml	(<60)	5-HIAA	6.5 ng/ml	(1.8–6.1)
GH	0.6 ng/ml	(<3.1)	DHEA-S	55 μ g/dl	(13–154)
LH	18.1 mIU/ml	(0.3–7.1)	Gastrin	600 pg/ml	(<200)
FSH	47.7 mIU/ml	(1.6–10.6)	Glucagon	83 pg/ml	(40–180)
TSH	1.65 μ IU/ml	(0.38–3.64)	Intact PTH	41 pg/ml	(10–65)
PRL	10.4 ng/ml	(3.4–16.2)	NTX	111 nM/mMCr	(<40)
AVP	0.41 pg/ml	(0.3–4.2)	Ca ²⁺	4.8 mg/dl	(4.4–4.9)
Cortisol	24.7 μ g/dl	(4.5–21.1)	IRI	11 mIU/ml	(4–25)
IGF-I	124 ng/ml	(–346)	GAD Ab	Negative	
Free T ₃	2.84 pg/ml	(2.13–4.07)	IRI Ab	4.6%	(<7)
Free T ₄	1.53 ng/dl	(0.95–1.74)	CPR	3.8 ng/ml	(1.0–2.0)
Thyroglobulin	26 ng/ml	(<30)	PRA	0.7 ng/ml/h	(0.2–2.7)
Tg Ab, TPO Ab	Negative		PAC	3.5 ng/dl	(2–13)
UFC	211, 151, 162 μ g/day	(40–140)	Met-U	0.05 mg/day	(0.05–0.20)
17-OHCS	6.3 mg/day	(1.6–8.8)	NM-U	0.10 mg/day	(0.10–0.28)
17-KS	7.3 mg/day	(2.4–11.3)	CPR-U	112, 58, 52 μ g/day	(42–79)
Ald-U	2.0 μ g/day	(0.6–9.0)	5-HIAA-U	1.1, 1.5 mg/day	(0.6–4.1)

Normal basal ranges are indicated in parentheses. AVP, arginine vasopressin; IGF-1, insulin growth factor-1; Tg Ab, thyroglobulin antibody; TPO Ab, thyroid peroxidase antibody; 5-HIAA, 5-hydroxyindole acetic acid; DHEA-S, dehydro-epiandrosterone sulfate; PTH, parathyroid hormone; NTX, urine N-telopeptide cross-linked collagen type 1; IRI, insulin; CPR, C-peptide; PRA, plasma renin activity; PAC, plasma aldosterone concentration; UFC, urinary free cortisol; 17-OHCS, 17-hydroxycorticosteroid; 17-KS, 17-ketogenic steroid; Ald-U, urinary aldosterone excretion; Met, metanephthrine; NM, normetanephthrine.

Table 3. Endocrinological examinations

(a) GHRH/TRH/CRH/LHRH test							
Time (min)	0	30	60	90	120		
TSH (μ IU/ml)	4.21	41.90	34.97	26.57	23.96		
PRL (ng/ml)	8.8	69.0	52.2	38.5	33.0		
GH (ng/ml)	6.8	17.6	9.8	5.3	2.8		
ACTH (pg/ml)	29	69	76	37	25		
F (μ g/dl)	11.1	18.8	21.5	16.6	14.4		
LH (mIU/ml)	15.3	57.3	93.6	94.2	80.9		
FSH (mIU/ml)	42.3	68.2	82.8	76.7	95.7		
(b) DDAVP test							
Time (min)	0	30	60	90	120		
ACTH (pg/ml)	23	42	40	38	29		
F (μ g/dl)	9.8	11.3	12.9	12.2	12.1		
(c) Dex test							
Dose (mg)	0	1	8				
ACTH (pg/ml)		59	11	<3			
F (μ g/dl)	24.7	6.6	3.4				
(d) Circadian rhythm							
Time	6:00	12:00	18:00	0:00			
ACTH (pg/ml)	34	43	26	26			
F (μ g/dl)	17.5	16.0	7.6	10.5			
(e) 75 g oGTT							
Time (min)	0	30	60	90	120	180	240
PG (mg/dl)	95	183	150	116	77	82	88
IRI (μ IU/ml)	10	95	137	69	19	8	9
CPR (ng/ml)	3.5	9.4	15.7	14.8	9.6	5.3	4.2
F (μ g/dl)	11.5	11.7	20.0	21.9	16.7	15.5	11.1
Glucagon (pg/ml)	60	66	64	66	76	89	63
PA (mg/dl)	0.88				2.34		0.91
Lact A (mM/l)	1.1	1.6	2.1	1.9	1.7	1.5	1.5
(f) PG daily profile							
	meal 7:30	9:30	meal 11:30	13:30	meal 17:30	19:30	21:00
PG (mg/dl)	104	114	108	139	126	119	110
(g) Glucagon							
Time (min)	0	3	6	10	15	30	60
PG (mg/dl)	92	107	127	130	141	142	92
CPR (ng/ml)	3.0	8.4	9.9	8.9	8.9	6.7	4.1
IRI (μ IU/ml)	7	76	74	75	52	24	8
Gastrin (pg/ml)	780	650	690	550	580	420	380

F, cortisol; PG, plasma glucose; IRI, insulin; CPR, C-peptide; PA, pyruvic acid; Lact A, lactic acid.

(a) GHRH/TRH/CRH/LHRH test (GHRH 100 μ g, TRH 500 μ g, CRH 100 μ g, and LHRH 100 μ g, intravenous bolus)

(b) DDAVP test (desmopressin 4 μ g, intravenous bolus) (c) Dex test (dexamethasone per os at 23:00) (d) Circadian rhythm

(e) 75 g oGTT (75 g glucose per os) (f) PG daily profile (h) Glucagon test (glucagon 1 mg, intravenous bolus)

the concentrations of plasma cortisol (24.7 µg/dl; normal range: 4.5–21.1 µg/dl, chemiluminescent immunoassay) and the urinary excretion of free cortisol (211, 151, and 162 µg/day; normal range: 40–140 µg/dl, chemiluminescent immunoassay) were mildly increased, while ACTH levels (59 pg/ml; normal range: <60 pg/ml, immunoradiometric assay) were within upper normal ranges. Both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were increased (LH, 18.1 mIU/ml; FSH, 47.7 mIU/ml). In addition, 5-hydroxyindole acetic acid (5-HIAA, 6.5 ng/day; normal range: 1.8–6.1 ng/ml), gastrin (600 mg/dl; normal range: <200 mg/day), and C-peptide levels (3.8 ng/ml; normal range: 1.0–2.0 ng/ml) were elevated, while urinary excretions of 5-HIAA (1.1, 1.5 mg/day; normal range: 0.6–4.1 µg/day) were within normal range.

As shown in Table 3, the plasma levels of ACTH and cortisol were increased in response to the administration of 100 µg of human CRH (a) and 4 µg of desmopressin (b). Both plasma ACTH and cortisol concentrations were partially suppressed by 1 mg of overnight dexamethasone suppression test, while both were inhibited with a dosage of 8 mg dexamethasone (c). The circadian rhythms for plasma ACTH and cortisol levels were disturbed (d). Oral administration of glucose increased both serum insulin and C-peptide levels (e), while the daily profile of plasma glucose showed normal changes (f). Serum insulin levels were increased in response to an intravenous administration

of 1 mg of glucagon, while serum gastrin levels were gradually decreased (g).

Magnetic resonance imaging (MRI) of the head revealed the presence of a pituitary microadenoma (7 × 6 × 7 mm), which was not enhanced with gadolinium diethylenetriamine penta-acetic acid (Fig. 1). Computerized tomography (CT) dynamic scanings or MRI of the abdomen showed a left adrenal nodule (18 × 13 × 17 mm) (Fig. 2A). ¹³¹I-adosterol scintigram revealed equal accumulations of the radioisotope in both adrenal glands (Fig. 2B). Ultrasonography of the thyroid showed a low and heterogenous mass (7.9 × 6.4 × 5.7 mm) in the left lobe (Fig. 3) and the cytology suggested papillary carcinoma. The left lobe in the thyroid and attached lymph nodes were then resected, and the histological examinations confirmed the papillary carcinoma. Endoscopical examinations of the stomach and intestines revealed the atrophic gastritis, no gastric ulcer, and no polyps.

Molecular genetic analysis

In order to establish whether a mutation of the menin gene was present in the patient, molecular genetic analysis was commercially performed by Mitsubishi Kagaku MBC (Tokyo, Japan). Briefly, genomic DNA was isolated from peripheral leukocytes by standard procedures and exons 1–10 of the menin gene individually amplified by PCR, and then analyzed directly by Taq DyeDeoxy Terminator Cycle sequence method.

Fig. 1. Magnetic resonance imaging of the head. The coronal and sagittal T1-weighted image enhanced with gadolinium diethylenetriamine penta-acetic acid demonstrates the presence of a microadenoma (7 × 6 × 7 mm) in the pituitary.

Fig. 2. (A) Computerized tomography scan of the abdomen. The scan shows a left adrenal nodule ($18 \times 13 \times 17$ mm). (B) ^{125}I -adosterol scintigram of the adrenal glands. The scintigram reveals equal accumulations of the radioisotope in both adrenal glands.

Fig. 3. Ultrasonography of the thyroid. The echogram shows a low and heterogeneous mass ($7.9 \times 6.4 \times 5.7$ mm) in the left lobe.

Only a polymorphic change (GCA→ACA) in the exon 10 codon 541 of the *menin* gene was detected by this method [5].

Discussion

In our case, plasma ACTH and cortisol levels were both mildly elevated, and both were insufficiently suppressed by 1 mg of overnight dexamethasone test, suggesting autonomic secretion of ACTH. Plasma ACTH and cortisol levels increased in response to CRH and desmopressin. Glucocorticoid receptor abnormality or resistance syndrome would have no response to desmopressin. Further, the circadian rhythms for plasma ACTH levels were disturbed. Together with the observation of pituitary microadenoma, it was diagnosed that the patient had a pituitary ACTH-producing tumor. The case still might have other rare problems such as the bioactivity of ACTH or the sensitivity to ACTH in the adrenal glands. To further confirm the diagnosis, sinus venous sampling or histological examination would be useful [6].

The patient had no typical Cushingoid features, hypertension, or impaired glucose tolerance, suggesting that the tumor had an autonomic ACTH secretion that was insufficient for causing clinical symptoms in Cushing's disease, the so-called preclinical Cushing's disease. Adrenal preclinical Cushing's syndrome has been well-recognized. Adrenal tumor has an autonomic cortisol secretion that is insufficient for producing a clinically manifested Cushing's syndrome, so the patients do not present the clinical features of Cushing's syndrome [1]. Patients with preclinical Cushing's syn-

Table 4. Endocrinological data from reference [5] (adopted by authors' permission)

(a) CRH test					
Time (min)	0	30	60	90	120
ACTH (pg/ml)	26	254	111	57	33
F (μg/dl)	13	38	43	36	33
(b) DDAVP test					
Time (min)	0	30	60	90	120
ACTH (pg/ml)	27	113	49	21	17
F (μg/dl)	10	21	18	11	11
(c) Dex test					
Dose (mg)	0	0.5	1	2	4
ACTH (pg/ml)	35	30	20	19	5
F (μg/dl)	18	14	13	13	2
(d) Circadian rhythm					
Time	8:00	20:00			
ACTH (pg/ml)	52	35			
F (μg/dl)	18	17			

(a) CRH test (CRH 100 μg, intravenous bolus) (b) DDAVP test (desmopressin 4 μg, intravenous bolus) (c) Dex test (dexamethasone per os at 23:00) (d) Circadian rhythm

drome are frequently found as adrenal incidentaloma, especially in middle-aged women. However, preclinical Cushing's disease due to pituitary adenoma is rarely reported.

A case of preclinical Cushing's disease was first reported by Suda's group in Japan [2]. In comparison with the present case, plasma ACTH levels were extremely elevated in response to CRF and desmopressin in the first case, while both levels were normally increased to both tests in the present case (Table 3 and 4). Both plasma ACTH and cortisol levels failed to be sufficiently reduced by a low-dose of overnight dexamethasone suppression test in both cases. The circadian rhythms for plasma ACTH and cortisol were also disturbed in both cases. In their case, transsphenoidal resection of the pituitary adenoma was performed, and the immunohistochemistry revealed an ACTH-producing tumor. A recent review of the English medical literature revealed two more cases of preclinical Cushing's disease [3, 4]. Another case of preclinical Cushing's disease was reported by Takao *et al.* [3]. The other case of preclinical Cushing's disease, reported by

Nagai *et al.* [4], was accompanied by malignant hypertension and diabetes. They reported that the blood pressure and glucose levels were improved after the resection of the pituitary adenoma even in the state of preclinical Cushing's disease.

The diagnostic criteria for preclinical Cushing's disease have yet to be established. The following endocrinological findings may be considered as diagnostic criteria: 1) the absence of Cushingoid appearance, 2) evidence of autonomic or abnormal secretion of ACTH such as (a) normal plasma ACTH and cortisol levels in the morning, and high ACTH and cortisol levels during the night, (b) normal or high plasma dehydroepiandrosterone sulfate (DHEA-S) level and urinary excretion of free cortisol, (c) incomplete suppression of ACTH and cortisol by low-dose dexamethasone, (d) normal or exaggerated response of plasma ACTH levels to CRF, and/or (e) response of plasma ACTH levels to desmopressin [7], and 3) presence of pituitary adenoma.

Our patient had unilateral adrenal incidentaloma. It is well-known that overt hypersecretion of ACTH could develop into adrenal nodular hyperplasia. However, it is rarely reported in the case of ACTH secretion such as preclinical Cushing's disease. Recently, gastric inhibitory polypeptide-dependent Cushing's syndrome also has been reported in unilateral adrenal adenoma [8]. In our case, oral administration of glucose increased cortisol levels, as well as both serum insulin and C-peptide levels. Our case, however, had no evidence of direct cortisol stimulation by food, independent of stress conditions. Further pathological or histological examination of the adrenal specimens might be required to confirm the nature of the adrenal lesion [9].

Multiple endocrine neoplasia type 1 is often associated with a pituitary ACTH-producing tumor, and caused by the mutation of a tumor suppressor gene, named the *menin* gene [10]. Molecular genetic analysis presented only a polymorphism of the *menin* gene in our case. Unknown tumor suppression gene might contribute to the endocrine neoplasia in this patient.

In our case, interestingly, abnormal levels of other hormones were also observed, due to hyperinsulinemia and hypergastrinemia. Following oral glucose tolerance test, serum insulin levels were excessively elevated. Generally, mitochondrial disease often develops into diabetes due to failure of insulin secretion [11, 12]. The early stage of diabetes may cause reactive

hyper-response of insulin. Otherwise, insulin resistance due to abnormal cortisol secretion would contribute to this phenomenon in our case. Serum gastrin levels were gradually decreased by glucagon injection, considered as a normal response. Together with the results of endoscopical examinations, dynamic CT, and MRI, there was no evidence suggesting functional pancreatic or any other tumor such as gastrinoma or insulinoma. Further examinations following abnormal secretions of these hormones might be required in the future.

In summary, we report a rare case of preclinical Cushing's disease. The patient was diagnosed as having Cushing's disease by endocrinological examina-

tions. Even without clinical Cushingoid features in pituitary incidentaloma, we concluded that the elevated ACTH and cortisol levels should be followed up by CRH, desmopressin and dexamethasone suppression tests. This patient with preclinical Cushing's disease would be observed whether the physical conditions in this patient develop to overt Cushing's disease.

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