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The Recovery of Hypothalamic-Pituitary-Adrenal Axis Is Rapid in Subclinical Cushing Syndrome

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Background: In subclinical Cushing syndrome (SC), it is assumed that glucocorticoid production is insufficient to cause a clinically recognizable syndrome. Differences in hormonal levels or recovery time of the hypothalamic-pituitary-adrenocortical (HPA) axis after adrenalectomy between patients with overt Cushing syndrome (OC) and SC remain unknown.

Methods: Thirty-six patients (10 with OC and 26 with SC) with adrenal Cushing syndrome who underwent adrenalectomy from 2004 to 2014 were reviewed retrospectively. Patients were treated with glucocorticoid after adrenalectomy and were reevaluated every 1 to 6 months using a rapid adrenocorticotropic hormone (ACTH) stimulation test.

Results: Levels of basal 24-hour urine free cortisol (UFC), serum cortisol after an overnight dexamethasone suppression test (DST), and serum cortisol and 24-hour UFC after low-dose DST and high-dose DST were all significantly lower in patients with SC compared with OC. Basal ACTH levels showed significantly higher in patients with SC compared with OC. The probability of recovering adrenal function during follow-up differed significantly between patients with OC and SC (P=0.001), with significant correlations with the degree of preoperative cortisol excess. Patients with OC required a longer duration of glucocorticoid replacement to recover a normal ACTH stimulation test compared with patients with SC (median 17.0 months vs. 4.0 months, P<0.001).

Conclusion: The HPA axis recovery time after adrenalectomy in patients with SC is rapid and is dependent on the degree of cortisol excess. More precise definition of SC is necessary to achieve a better management of patients and to avoid the risk of under- or overtreatment of SC patients.

Keywords: Subclinical Cushing syndrome; Overt Cushing syndrome; Adrenal incidentaloma; Hydrocortisone; Hypothalamic-pituitary-adrenocortical axis recovery

INTRODUCTION

With the increasing use of ultrasonography and computed tomography, incidentally discovered adrenal tumors are common, with a prevalence of at least 3% among the general population over the age of 50 years [1]. Although most of these tumors are nonfunctioning adrenocortical adenomas [2], 5% to 30% of patients with adrenal incidentaloma show some degree of hypercortisolism without typical signs or symptoms of overt Cushing syndrome (OC) [3,4]; this is called 'subclinical Cushing syndrome (SC).' Although SC and OC have been considered different diseases with different genetic alterations [5], in SC, it is assumed that cortisol production is insufficient to cause a clinically recognizable syndrome and to suppress the release of cortico-

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tropin releasing hormone and adrenocorticotropic hormone (ACTH). Temporary adrenal insufficiency lasting several months ensues from surgical resection of an autonomously functioning adrenal tumor in OC due to suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis, during which glucocorticoid replacement is mandatory. However, it is still unclear whether all adrenalectomized patients with SC also exhibit the HPA axis impairment observed in OC.

The aims of this study were to evaluate the different degrees of cortisol excess and the HPA axis recovery time after adrenalectomy in patients with SC and OC. Moreover, we wanted to determine the relationship between the degree of cortisol excess and the HPA axis recovery time after surgery.

METHODS

Patients

Six hundred and seventy-four patients with incidentally detected adrenal masses underwent an overnight dexamethasone suppression test (DST) and 24-hour urine collection for measurement of free cortisol as a screening test from April 2004 to December 2014 at the Chonnam National University Hwasun Hospital. Patients who failed to suppress serum cortisol below 1.8 μg/dL (50 nmol/L) after administration of 1 mg dexamethasone or excreted 24-hour urine free cortisol (UFC) greater than 50 μg/day underwent a 4 mg low-dose DST (LDST) and 16 mg high-dose DST (HDST) for confirmative diagnosis [6]. Thirty-eight patients without typical signs or symptoms of OC by three experienced endocrinologists were finally identified as having cortisol-producing tumors (SC). Among them, 12 patients did not opt for adrenalectomy. Finally, the findings in 26 patients with SC were compared with those of 10 patients with OC who underwent unilateral adrenalectomy at our hospital during the same period. OC was defined by the presence of one of clinical signs of cortisol excess such as easy bruising, moon face, dorsocervical or supraclavicular fat pads, central obesity with proximal muscle weakness or thin skin. The presence of diabetes and hypertension was defined as being on medication. The mean follow-up period was 23.5 months (range, 4 to 109).

Diagnostic criteria for adrenal insufficiency after adrenalectomy

The protocol for postoperative glucocorticoid replacement was 50 mg hydrocortisone intravenously just before adrenalectomy and 50 mg intravenously every 8 hours on the day of surgery, followed by a gradual tapering according to the signs and symptoms of adrenal insufficiency. Morning cortisol was checked after discontinuation of hydrocortisone for at least 3 days, at the time of discharge and every month. In the patients with over the 10 μg/dL of morning cortisol, recovery of adrenal function was analyzed using the sequential cosyntropin (ACTH, 250 μg) stimulation test, defined as a peak cortisol level of at least 18 μg/dL (500 mmol/L) [7]. At discharge, patients usually received 15 mg hydrocortisone per day, and this dose was tapered by 2.5 to 5 mg/day every 4 weeks according to patients' complaints and the results of the cosyntropin stimulation test. Hydrocortisone replacement was discontinued when the test showed a normal response, which defined as the time to recovery from adrenal insufficiency. If the patients showed a normal response at discharge, it was determined that adrenal insufficiency did not occur.

Biochemical measurements

Serum cortisol was measured by radioimmunoassay using RIAZENco CORTISOL (R-JG-100, ZenTech, Liege, Belgium). The measurement of plasma ACTH was performed by immunoradiometric assay using ELSA-ACTH (Cisbio Bioassays International, Codolet, France). Urine cortisol measurements were performed by radioimmunoassay using a gamma counter (Wizard 1470, Wallac Oy, Turku, Finland).

Ethics statement

This study was reviewed and approved by the Institutional Review Board of the Chonnam National University Hwasun Hospital, Hwasun, Korea (IRB No. CNUHH-2015-144).

Statistical analysis

All statistical analyses were performed using SPSS version 21.0 (IBM Co., Armonk, NY, USA). Differences in non-categorical and categorical factors between patients with SC and OC were compared using the Mann-Whitney *U* test and chi-square or Fisher exact test, respectively. The correlation coefficient between the preoperative cortisol levels and the recovery time was calculated by Spearman correlation test. R^2 was calculated by linear regression analysis. Kaplan-Meier analysis was performed to elucidate the cumulative probability of recovery of adrenal function. If no recovery appeared until the last known patient contact at our center, the patient was considered censored. The Cox proportional-hazard model was used to analyze factors associated with adrenal function recovery. A value of $P<0.05$ was taken to indicate statistical significance.

RESULTS

Clinical characteristics and differential degree of cortisol excess between patients with subclinical and overt Cushing syndrome

The mean age of the 36 patients was 53.6±10.8 years (range, 21 to 75). Patients with SC were older than patients with OC (P=0.028). Gender, body mass index (BMI), concurrent diabetes and hypertension, and tumor size did not differ significantly between patients with SC and OC. Biochemical parameters of cortisol excess were more severe in patients with OC, compared with SC. Plasma ACTH levels were significantly lower in patients with OC compared with SC (Table 1).

HPA axis recovery associated with preoperative cortisol levels

Statistically significant positive correlations were observed between the recovery time and preoperative cortisol levels in all subjects (Table 2). However, the significance disappeared in

Table 2. Relationship between Recovery Time and Preoperative Cortisol Levels

Variable	Median (range)	ρ[a]	R^2[b]
Recovery time, mo	5 (0–30)	-	-
Basal UFC, μg/day	74.0 (26–1,450.0)	0.485[c]	0.197[c]
P-cortisol after ODST, μg/dL	7.4 (2.1–24.1)	0.645[d]	0.465[d]
UFC after LDST, μg/day	48.3 (5.0–830.0)	0.729[d]	0.598[d]
P-cortisol after LDST, μg/dL	11.1 (2.7–29.7)	0.688[d]	0.472[d]
UFC after HDST, μg/day	57.0 (9.0–963.0)	0.697[d]	0.495[d]
P-cortisol after HDST, μg/dL	10.3 (2.2–39.1)	0.606[d]	0.303[c]

UFC, urine free cortisol; P-cortisol, plasma cortisol; ODST, overnight dexamethasone suppression test (1 mg); LDST, low-dose dexamethasone suppression test (4 mg); HDST, high-dose dexamethasone suppression test (16 mg).
[a]ρ (Spearman's rho) represents the correlation coefficient between recovery time and the clinical parameters of cortisol excretion; [b]R^2 was calculated by linear regression analysis; [c]P<0.05; [d]P<0.001.

Table 1. Baseline Characteristics of Patients with Subclinical and Overt Cushing Syndrome

Characteristic	Overt (n=10)	Subclinical (n=26)	P value
Age, yr	47.3±12.3 (21–65)	56.0±9.3 (37–75)	0.028
Female sex	9 (90.0)	19 (73.1)	0.274
BMI, kg/m^2	23.7±3.2	24.6±3.9	0.391
Hypertension	7 (70.0)	11 (42.3)	0.137
Diabetes mellitus	5 (50.0)	7 (26.9)	0.188
Tumor size, cm	2.7±0.7	2.4±0.9	0.348
Tumor location (right)	2 (20.0)	7 (26.9)	0.667
ACTH, pg/mL	11.7±6.0	24.3±16.1	0.023
Basal UFC, μg/day	520.1±432.0	71.9±33.1	0.010
Cortisol after ODST, μg/dL	18.1±6.0	7.8±5.9	0.001
UFC after LDST, μg/day	472.8±208.4	56.8±77.7	<0.001
Cortisol after LDST, μg/dL	22.2±6.2	8.7±6.1	<0.001
UFC after HDST, μg/day	487.8±284.2	74.1±113.5	0.001
Cortisol after HDST, μg/dL	21.1±5.8	10.2±9.0	0.001
DHEA-S, μg/dL	20.3±14.1	43.5±71.7	0.490
Postoperative ACTH at discharge, pg/mL	23.8±12.2	85.25±112.9	0.035
Postoperative cortisol at discharge, pg/mL[a]	2.9±1.8	10.9±8.3	<0.001
Recovery time, mo	17.0 (5–30)	4.0 (0–14)	<0.001
Follow-up duration, mo	23.5 (10–109)	23.5 (4–50)	0.422

Values are expressed as mean±SD (range), number (%), or median (range).
BMI, body mass index; ACTH, adrenocorticotropic hormone; UFC, urine free cortisol; ODST, overnight dexamethasone suppression test; LDST, low-dose dexamethasone suppression test; HDST, high-dose dexamethasone suppression test; DHEA-S, dehydroepiandrosterone sulfate.
[a]Peak cortisol level after cosyntropin stimulation test at discharge.

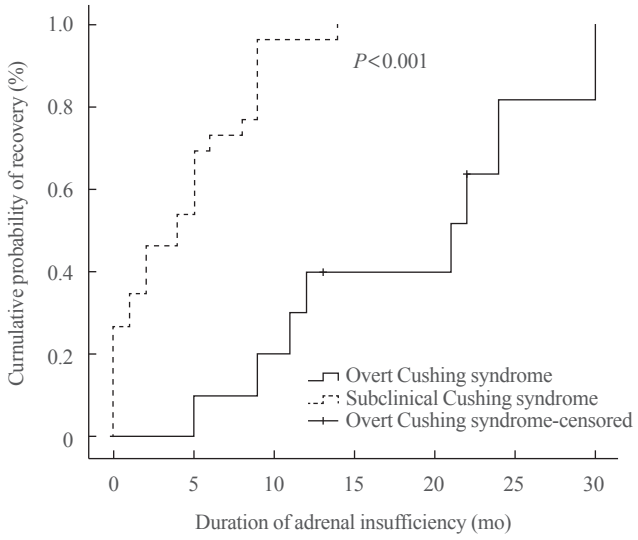

Fig. 1. Cumulative probability of adrenal function recovery in patients with overt and subclinical Cushing syndrome.

subgroup analyses of each patient group with OC or SC.

Differential recovery time between patients with subclinical and overt Cushing syndrome

The recovery time was shorter in patients with SC than those with OC (4.0 months vs. 17.0 months, $P<0.001$) (Fig. 1). None of these patients required steroid replacement after the discontinuation of glucocorticoids. The probability of adrenal function recovery was associated with the clinical phenotype with a hazard ratio of 24.3 (95% confidence interval, 3.04 to 194.29; $P=0.003$) in patients with SC compared with OC, independently of age, sex, BMI, presentation with diabetes or hypertension, basal 24-hour UFC, and ACTH levels, by the Cox proportional-hazards model. Adrenal insufficiency did not occur in 26.9% of patients with SC (seven of 26), whereas all OC patients required postoperative glucocorticoid replacement. The function of the contralateral adrenal gland recovered completely in all SC patients and in eight patients with OC. In two of 10 OC patients, the plasma cortisol response to ACTH stimulation was still blunted at the last follow-up (13 and 22 months). The median recovery time was 5 months (range, 0 to 30), and the cumulative probability of recovery of adrenal function at 6 months and 1 year were 52.82% and 77.8% in all patients with Cushing syndrome.

DISCUSSION

According to our results, patients with SC showed a lower degree of cortisol excess than did those with OC. The recovery time was shorter in patients with SC than that of OC. The degree of cortisol excess was accompanied by a difference in the recovery time of the HPA axis after surgery in all subjects.

SC is a status of cortisol excess defined by dynamic hormonal tests in patients with adrenal incidentalomas who have no overt symptoms of Cushing syndrome [8,9]. SC was initially defined as preclinical Cushing syndrome [10]; however, it has been considered a different disease category than OC. Different molecular pathogenetic mechanisms have been reported in SC compared with OC [5], and about two-third of SC remain subclinical during long-term clinical follow-up of SC [11]. Comparison of cortisol levels showed clear differences in clinical characteristics: patients with OC had more severe biochemical activity parameters than did those with SC [5]. These findings are similar to those of our study.

To date, the definition of SC is still controversial, and there is no gold standard diagnostic test because of the low reliability of the available hormonal assays (24-hour UFC, ACTH levels, and DST) [12]. DST is the most important diagnostic method to evaluate cortisol excess, but there has been no consensus on the cut-off level to use for diagnosis of SC. We used a low cut-off value (1.8 μg/dL) for excellent sensitivity of the test, despite the decline in specificity, and 2-day LDSTs (2 mg) and HDSTs (16 mg) were performed as confirmative tests, also with a low cut-off value (1.8 μg/dL) [12].

Untreated Cushing syndrome has an increased mortality rate due to cardiovascular, thromboembolic, and infectious complications [13,14]. Although patients with SC show subtle cortisol excess, they usually exhibit one or more symptoms from cortisol excess, including hypertension, diabetes, dyslipidemia, and osteoporosis [15-17]. In our results, there were no differences in the frequencies of hypertension, diabetes, and obesity between patients with SC and OC. Although the most appropriate treatment for SC remains controversial, surgical resection of adrenal tumors has shown beneficial effects on cardiovascular risk factors in most studies, with low surgical morbidity rates [18,19].

Cortisol oversecretion from autonomously hyperfunctioning adenomas of the adrenal gland leads to suppression of the HPA axis, resulting in hypofunctioning of the contralateral adrenal gland. The recovery of the HPA axis may be delayed; therefore, the patient often requires glucocorticoid replacement therapy for several months to a year and sometimes even longer after adrenalectomy. However, adrenal insufficiency did not occur in 27% of the patients with SC, whereas all OC patients showed adrenal insufficiency in our study. HPA axis recovery time was

shorter in patients with SC than those with OC (4.0 months vs. 17.0 months, $P<0.001$), and it correlated well with preoperative cortisol levels. Few studies have compared the recovery time between patients with OC and SC. Di Dalmazi et al. [20] reported that only 65% of SC patients had postsurgical adrenal insufficiency, compared with 99.7% of OC patients. They also reported that the mean recovery time was 6.5 months (range, 1 to 50) in SC patients and 11.2 months (range, 1 to 60) in those with OC, and they observed a tight relationship between the degree of hypercortisolism and the time to recovery, similar to our data. Some studies reported that old age at surgery is a critical factor influencing recovery time in patients with adrenal CS [21,22], but the clinical features were only explained as a factor influencing recovery time in patients with adrenal CS independently of age in our study by multivariate analysis. These results support that SC patients have a lower degree of cortisol excess that is not sufficient to fully suppress the HPA axis. However, Berr et al. [22] reported that ectopic CS, which had the highest cortisol excess, showed the shortest recovery time, compared with CS due to adrenal or pituitary etiologies. In general, ectopic CS shows rapid progression of the disease compared with adrenal CS; therefore, the recovery time might be influenced not only by the degree of cortisol excess but also by the period of exposure to cortisol excess. This is explained by findings of adrenal insufficiency after exposure to exogenous steroids. Adrenal insufficiency is frequently seen in patients treated with long-term glucocorticoids and is related to the duration and the cumulative dose of steroids [23].

There were several limitations in this study. First, the clinical features defining OC may vary among patients treated by different physicians. The clinical symptoms and signs of CS overlap those of many other common diseases, but CS displays more specific signs, such as muscle weakness, ecchymoses, and central obesity. In our study, three experienced endocrinologists confirmed whether the patient had OC or SC. Seconds, the correlation between the recovery time and preoperative cortisol levels showed statistically significant in all subjects, but the significance disappeared in the subgroup analyses of each patient with OC or SC. This finding is limited by the small sample size. Last, the dosage of steroid replacement and the time to evaluate the patient using a postoperative cortisol stimulation test (rapid ACTH stimulation test) were not controlled, because this study was retrospective. The intervals between tests were variable, ranging from 1 to 6 months, but this should not have been a significant issue, since we decided the timing of the test while decreasing the steroid dosages, levels of morning cortisol and watching the signs and symptoms of patients during follow-up at 1-month intervals.

In conclusion, the degree of cortisol excess differs between the two disease categories, and the time to adrenal function recovery was associated with the degree of cortisol excess. Some of the patients with SC showed no suppression of the HPA axis, and the majority of patients with SC recovered rapidly from HPA suppression within several months. More precise definition of SC is necessary to achieve a better management of patients and to avoid the risk of under- or over-treatment of SC patients.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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