

ORIGINAL

Long-term study of subclinical Cushing's syndrome shows high prevalence of extra-adrenal malignancy in patients with functioning bilateral adrenal tumors

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Abstract. Subclinical Cushing's syndrome (SCS) is characterized by subtle autonomous cortisol secretion from adrenal tumors without specific signs and symptoms of hypercortisolism. Patients with SCS have a high prevalence of "lifestyle-related diseases," such as hypertension, diabetes mellitus, dyslipidemia, and osteoporosis. Long-term follow-up of SCS patients is reportedly indispensable for establishing indications for surgical treatment of SCS. We performed a follow-up survey of 27 patients with SCS (median: 5.3 years) and compared those who had undergone surgical treatment (n=15) with those who had not (n=12). The mean diameter of tumors was 31 mm; 16 (59%) patients had unilateral lesions and 11 (41%) carried bilateral ones. In 67% and 60% of the treatment group, respectively, hypertension and diabetes mellitus improved. We also noticed that eight of 11 (73%) SCS patients with bilateral adrenal tumors had extra-adrenal malignancies in various tissues. Interestingly, among nine SCS patients who had malignancies, eight showed bilateral adrenal uptake in ¹³¹I-aldosterol scintigraphy. The results imply that surgical treatment can reduce cardiovascular risks in SCS patients. Screening for malignancy may be necessary in patients with bilateral adrenal tumors suspected of autonomous hypersecretion of cortisol from both sides.

Keywords: Subclinical Cushing's syndrome, Adrenal tumor, Cortisol, Extra-adrenal cancer

SUBCLINICAL CUSHING'S SYNDROME (SCS) is defined as adrenocorticotrophic hormone (ACTH)-independent cortisol secretion by adrenal adenoma or hyperplasia in the absence of specific signs and symptoms of overt cortisol excess, such as moon face, buffalo hump and central obesity [1, 2]. SCS is estimated to be present in 5–30% of patients with incidentally discovered adrenal mass (adrenal incidentaloma [AI]) [3]. SCS patients are also known to have a high prevalence of "life style-related diseases", such as hypertension (HT), diabetes mellitus (DM), dyslipidemia and osteoporosis.

Diagnostic criteria for SCS have not been fully

established because of variability of autonomous cortisol secretion among patients [3, 4]. Although the dexamethasone suppression test (DST) has been recommended as a screening test for SCS, cortisol cut-off values differ by current criteria. Because SCS is equivocally defined and no large prospective randomized trial has been performed, the comparative efficacy of surgical and non-surgical treatments for these patients is unclear [1, 2].

Several long-term studies of SCS patients have shown that surgical treatment improved cardiovascular risks, such as HT, DM and dyslipidemia in SCS

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Abbreviations: ACTH, adrenocorticotrophic hormone; AI, adrenal incidentaloma; AIMAH, ACTH-independent bilateral adrenocortical hyperplasia; AT, adrenal tumor; DHEA-S, dehydroepiandrosterone sulfate; DM, diabetes mellitus; DST, dexamethasone suppression test; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HT, hypertension; IGT, impaired glucose tolerance; PKA, protein kinase A; SCS, subclinical Cushing's syndrome.

patients, whereas these risk factors deteriorated in the conservative treatment group [2, 5–8].

AIs have often been found during detailed examinations of extra-adrenal malignancy. SCS is the most frequent hormonal dysfunction detected in AI. Here, we performed a follow-up survey of 27 SCS patients, of whom 15 (56%) had undergone surgery and 12 (44%) had not, and who all had been hospitalized in Kyushu University Hospital or Fukuoka University Hospital in the past 18 years. Most patients who underwent adrenalectomies showed no worsening of cardiovascular risk factors. During this follow-up survey, we realized that SCS patients with bilateral adrenal tumors (ATs) frequently had extra-adrenal malignancy. More detailed analysis indicated that patients who showed bilateral adrenal uptake in ^{131}I -adosterol scintigraphy had high rates of malignancy.

Subjects and Methods

Patients

We performed a follow-up survey among 27 SCS patients, of whom 15 had undergone surgery and 12 had not, and who all had been treated at Kyushu University Hospital or Fukuoka University Hospital between 1995 and 2013. These patients were diagnosed with SCS based on the Japanese criteria for SCS [4], and had unilateral or bilateral adrenal masses without typical characteristics of Cushing's syndrome (CS) such as central obesity, moon face or buffalo hump. Their basal serum cortisol levels were in the normal range. Autonomous cortisol secretion was confirmed by overnight 1-mg DSTs, low early-morning plasma ACTH levels, increased uptake on adrenal scintigraphy, no diurnal changes in serum cortisol level, and low serum DHEA-S. For this study, we considered ≥ 1.8 mg/dL after 1-mg DST to be the threshold level for low cortisol.

Among cardiovascular risk factors, we defined HT as the presence of a mean systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or current use of antihypertensive drugs; DM as a fasting blood sugar (FBS) ≥ 126 mg/dL and/or a random blood sugar ≥ 200 mg/dL and/or hemoglobin A1c (HbA1c) $\geq 6.5\%$ and/or use of antidiabetic agents; impaired glucose tolerance (IGT) as FBS ≥ 110 mg/dL and/or random blood sugar 140–199 mg/dL; dyslipidemia as total cholesterol level ≥ 220 mg/dL and/or low-density lipoprotein-cholesterol ≥ 140 mg/dL and/or high-density lipoprotein-cholesterol < 40 mg/dL and/or triglyceride ≥ 150

mg/dL or treatment with lipid-lowering medication; and obesity as body mass index (BMI) ≥ 25 kg/m².

Long-term follow-up study

Long-term clinical outcome of cardiovascular risks in SCS patients was evaluated as follows: Improvement of HT was defined as normalization of BP, or dose reduction or cessation of antihypertensive drugs. Worsening of HT was defined as deviation from the BP reference range, or dose increase or commencement of antihypertensive drugs. Improvement of DM and dyslipidemia was defined as normalization or amelioration of laboratory data, or dose reduction or cessation of therapeutic drugs. Worsening of DM and dyslipidemia was defined as deviation of laboratory data from reference ranges, or dose increase or commencement of therapeutic drugs.

Biochemical assays

Serum concentrations of cholesterol, triglyceride, fasting blood glucose and HbA1c were measured by standard procedures. Serum cortisol and ACTH levels were measured by radioimmunoassay or electrochemoluminescence immunoassay.

Imaging analyses

Computed tomography (CT) was used to detect ATs. Functional localization of adrenal adenoma or hyperplasia was evaluated using ^{131}I -adosterol scintigraphy. Patients were pretreated with iodine to block uptake of the isotope by thyroid glands. Imaging was performed 5 days after injection of ^{131}I -adosterol.

Statistical analyses

Results are presented as mean \pm standard deviation. Unpaired *t*-tests and χ^2 -tests were used to compare the means of the two patient subgroups. All statistical analyses were performed using Microsoft Excel 2010 software. *P* < .05 was considered statistically significant.

Results

The median follow-up period at the time of this study was 5.3 years. Mean diameter of ATs was 31 mm; 16 (59%) patients had unilateral lesions and 11 (41%) carried bilateral ones. Baseline characteristics of the surgical and non-surgical groups are summarized in Table 1. The surgical group was significantly younger and had a higher percentage of women than the non-surgical group. Most patients (13 of 15; 87%) who underwent

Table 1 Baseline clinical characteristics of surgical and non-surgical patients with SCS

	Surgical (n=15)	Non-surgical (n=12)	P
Age (years)	55.3±9.4	66.3±8.8	0.002
Sex (female/male)	13/2	6/6	0.04
BMI (kg/m ²)	23.3±4.0	24.2±1.9	0.27
Mass size (mm)	32.9±17.6	27.8±5.7	0.17
unilateral/bilateral	13/2	3/9	0.001
Morning cortisol (µg/dL)	12.9±4.1	13.1±2.8	0.46
Midnight cortisol (µg/dL)	8.4±4.3	7.8±3.4	0.35
1-mg DST (cortisol: µg/dL)	6.6±4.2	5.9±3.5	0.34
Hypertension	10 (67%)	6 (50%)	0.39
Diabetes mellitus	5 (33%)	5 (42%)	0.63
Dyslipidemia	8 (53%)	10 (83%)	0.10
Obesity	5 (33%)	5 (42%)	0.63

BMI, body mass index; DST, dexamethasone suppression test

Table 2 Long term outcome of surgical and non-surgical SCS patients

Cardiovascular risk factor at baseline	Surgical (n=15)				Non-surgical (n=12)			
	n	Improvement	No change	Worsening	n	Improvement	No change	Worsening
Hypertension								
Normotensive	6	-	5 (83%)	1 (17%)	6	-	1 (17%)	5 (83%)
Hypertensive	9	6 (67%)	3 (33%)	0	6	0	2 (33%)	4 (67%)
Diabetes mellitus								
DM, IGT (-)	10	-	10 (100%)	0	7	-	5 (71%)	2 (29%)
DM or IGT (+)	5	3 (60%)	2 (40%)	0	5	1 (20%)	2 (40%)	2 (40%)
Dyslipidemia								
Dyslipidemia (-)	7	-	7 (100%)	0	2	-	2 (100%)	0
Dyslipidemia (+)	8	1 (13%)	7 (87%)	0	10	1 (10%)	7 (70%)	2 (20%)

adrenalectomies had unilateral ATs. On the other hand, in the non-surgical group, three patients (25%) had unilateral ATs and nine (75%) had bilateral ATs. The two groups did not significantly differ in prevalence of complications such as HT, DM, dyslipidemia and obesity.

In the surgically treated group, 6 among 9 (67%) showed improvement of HT and 3 among 5 (60%) showed improved DM-IGT (Table 2). Only one patient who underwent adrenalectomy had worse HT. However, in the non-surgical group, 9 among 12 (75%) had worse HT and 4 among 12 (33%) had worse DM or IGT (Table 2). These results indicate that adrenalectomy in SCS patients can improve cardiovascular risk factors such as HT and DM.

We also divided patients into those with unilateral or bilateral ATs. At baseline, a significantly larger percentage of the bilateral group were male than in the unilateral group (Table 3). As in the comparison of surgical and non-surgical groups, HT and DM worsened

in the bilateral patients, most of whom were not surgically treated (Table 4).

The patients showed no symptomatic cardiovascular events during the present study. However, we noticed that eight (73%) of the 11 patients with bilateral ATs also had extra-adrenal cancers from various tissues (Table 5). Based on imaging studies of CT scans, four patients (Cases 3 to 6) showed bilateral nodular enlargement of adrenal glands that were considered as ACTH-independent bilateral adrenocortical hyperplasia (AIMAH). Other patients seemed to have bilateral adrenal adenomas. Moreover, among the nine patients whose bilateral ATs showed bilateral adrenal uptake in ¹³¹I-adosterol scintigraphy, eight (89%) had malignant tumors (Fig. 1). Only one patient who carried bilateral functioning tumors had no malignancy (Table 5). Among 14 SCS patients who showed contralateral suppression of adrenal uptake on ¹³¹I-adosterol scintigraphy (12 unilateral and two bilateral), only one patient suf-

Table 3 Baseline clinical characteristics of SCS patients with unilateral or bilateral adrenal tumors

	Unilateral (n=16)	Bilateral (n=11)	P
Age (years)	57.8±11.8	63.8±7.6	0.07
Sex (female/male)	14/2	5/6	0.02
BMI (kg/m ²)	23.3±4.0	24.1±1.9	0.27
Mass size (mm)	31.3±15.4	29.6±11.3	0.38
Morning cortisol (µg/dL)	13.3±4.2	12.5±2.5	0.27
Midnight cortisol (µg/dL)	8.2±3.8	8.0±4.1	0.47
1-mg DST (cortisol: µg/dL)	6.2±3.8	6.4±4.2	0.47
Hypertension	9 (56%)	6 (64%)	0.94
Diabetes mellitus	7 (44%)	3 (27%)	0.38
Dyslipidemia	10 (63%)	8 (73%)	0.56
Obesity	6 (38%)	4 (36%)	0.94

BMI, body mass index; DST, dexamethasone suppression test

Table 4 Long term outcome of SCS patients with unilateral and bilateral adrenal tumors

Cardiovascular risk factor at baseline	Unilateral (n=16)				Bilateral (n=11)			
	n	Improvement	No change	Worsening	n	Improvement	No change	Worsening
Hypertension								
Normotensive	7	-	5 (71%)	2 (29%)	4	-	0	4 (100%)
Hypertensive	9	7 (78%)	2 (22%)	0	7	0	4 (57%)	3 (43%)
Diabetes mellitus								
DM, IGT (-)	9	-	9 (100%)	0	8	-	6 (75%)	2 (25%)
DM or IGT (+)	7	4 (57%)	3 (43%)	0	3	0	0	3 (100%)
Dyslipidemia								
Dyslipidemia (-)	6	-	5 (83%)	1 (17%)	3	-	2 (67%)	1 (33%)
Dyslipidemia (+)	10	3 (30%)	6 (60%)	1 (10%)	8	1 (13%)	4 (50%)	3 (38%)

Table 5 Analysis of the SCS patients with bilateral adrenal tumors

Case	Age	Sex	OP	Adrenal mass size (mm)	Uptake on adosterol scintigraphy	Complication of malignancy	Follow-up (Y)
Unilateral uptake							
1	55	M	No	R25, L15	Unilateral (R)	None	10.6
2	63	F	Yes	R60, L30	Unilateral (R)	None	6.2
Bilateral uptake							
3	56	F	No	Bilateral multiple (max. R13, L25)	Bilateral (R>L)	Endometrium	6.8
4	59	F	No	Bilateral multiple (max. R27, L20)	Bilateral	Breast, Thyroid	7.8
5	61	M	No	Bilateral multiple (max. 18)	Bilateral	Bladder, Ureter	4.3
6	61	M	No	Bilateral multiple (max. R34, L17)	Bilateral	Colon	6
7	61	M	No	R20, L10	Bilateral (R>L)	Basal Cell	8
8	63	M	Yes	R33, L31	Bilateral (L>R)	None	1
9	68	F	No	R17, L23	Bilateral	Breast	4.5
10	77	F	No	R17, L31	Bilateral (L>R)	Thyroid	1.6
11	78	M	No	R30, L24	Bilateral	Lung	1.8

OP, operation; R, right; L, left

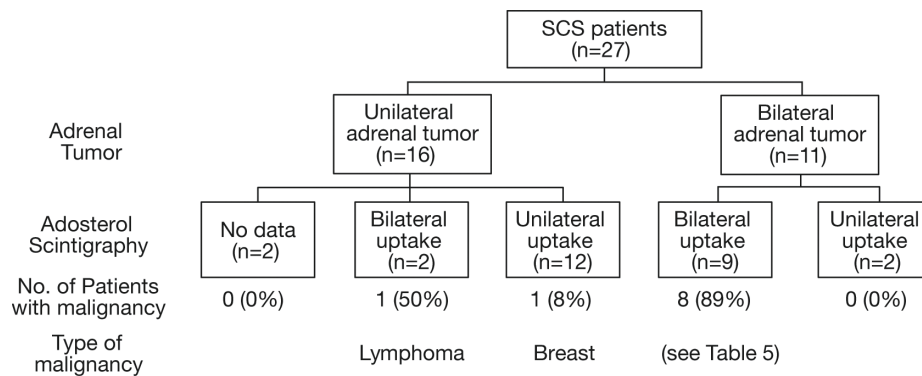


Fig. 1 Comparison of prevalence of extra-adrenal malignancy in each group. SCS patients (n=27) of the present study were divided into two groups, with unilateral or bilateral ATs. Each group was further divided by results of adosterol scintigraphy tests for unilateral or bilateral uptake. Two patients with unilateral tumors did not have the examination. The number and percentage of patients with extra-adrenal malignancy in each subgroup are shown. Malignancy types for patients with unilateral tumors are shown here; those for patients with bilateral tumors are shown in Table 5.

ferred extra-adrenal malignancy (Fig. 1). These results indicate that patients with functioning bilateral ATs tend to develop malignant tumors in extra-adrenal tissues.

Discussion

This long-term follow-up study of SCS patients indicates that surgical treatment in such patients can prevent increased cardiovascular risks. We also found that SCS patients with bilateral ATs who show bilateral uptake in adrenal scintigraphy have extremely high prevalence of extra-adrenal malignancy.

Previous long-term studies of SCS revealed that adrenalectomy significantly improved HT and DM, compared with non-surgical treatments [2, 5–8]. Our present results also demonstrated that surgical treatment for SCS can reduce cardiovascular risk factors. Although other reports indicated almost no worsening of HT after surgical treatment in SCS patients, only one patient in our study started to take antihypertensive drugs after adrenalectomy (Table 2). This patient did not show elevated blood pressure after surgical treatment, but medication for HT was started because her blood pressure remained in the high-normal range after adrenalectomy.

Incidentally discovered ATs are usually unilateral rather than bilateral [9, 10]. However, patients with bilateral ATs reportedly have higher prevalence of sub-clinical hypercortisolism than those with unilateral tumors [9]. Moreover, HT and vertebral fracture were more frequent in SCS patients with bilateral tumors than those with unilateral tumors, in spite of similar levels of cortisol secretion [10]. One reason for high

prevalence of these complications in SCS patients with bilateral ATs might be higher frequency of the N363S variant of the glucocorticoid receptor gene in patients with bilateral ATs [11]. This polymorphism is associated with increased sensitivity to glucocorticoids, and also related to HT and vertebral fracture [12].

Management of bilateral AI in SCS patients is quite controversial. For patients with overt CS due to AIMAH, unilateral adrenalectomy is an effective treatment to achieve long-term remission of CS, improvement of blood pressure values and glycemic control, and patient's quality of life [13, 14]. Also, in two SCS patients with AIMAH, unilateral adrenalectomy achieved satisfactory and prolonged control of cortisol secretion and remarkable improvement of HT and glycemic control [15]. The remaining contralateral ATs did not change after the surgery. To reduce risks of cardiovascular events, unilateral adrenalectomy might be considered for SCS patients with bilateral functioning tumors. Medical treatment with adrenal-blocking drugs such as metyrapone or ketoconazole could also be a therapeutic option to improve hypercortisolemia in patients with bilateral functioning ATs [16, 17].

¹³¹I-adosterol scintigraphy in SCS can help distinguish unilateral adenoma from bilateral cortical nodular hyperplasia. Adrenocortical adenomas usually display unilateral uptake on the scintigraphy as a result of suppressed ACTH and tracer accumulation in the contralateral, normal adrenal cortex. On the other hand, bilateral cortical nodular hyperplasia demonstrates bilateral uptake of the isotope [18, 19]. We obtained similar results in the present study. Most unilateral ATs showed

unilateral uptake on adosterol scintigraphy, whereas bilateral uptake was observed in patients with bilateral ATs. Adrenal scintigraphy is reportedly able to predict metabolic outcomes and occurrence of postoperative hypoadrenalism [19]. Here, we also showed the potential of scintigraphy in predicting cancer complications. Adrenal scintigraphy could be useful for both diagnosing SCS and predicting prognosis of SCS patients.

AIs are often found during preoperative examinations for cancer patients, which initially might appear to account for the high cancer frequency found in these patients. However, among six of the eight patients with both bilateral ATs and extra-adrenal malignancies, identification of bilateral ATs preceded diagnosis of their extra-adrenal cancers, which implies that functioning bilateral ATs are a risk factor for extra-adrenal carcinogenesis.

In the present study, many more cancers were detected in patients with bilateral ATs than in those with unilateral tumors. As the unilateral and bilateral groups did not significantly differ in cortisol secretion, genetic alterations in tissues of patients with bilateral tumors seem to affect tumorigenesis rather than exposure to subtle hypercortisolemia.

As shown in Table 1, there is a strong bias between patients with unilateral and bilateral ATs in the point of surgical treatment. Most patients with unilateral ATs were able to avoid hypercortisolism after surgery, whereas those with bilateral ATs were continuously exposed to hypercortisolism. People might think that the difference between the SCS patients with unilateral and bilateral ATs in the prevalence of extra-adrenal malignancy would be due to whether patients undergo surgery or not. However, in five of eight bilateral AT patients with extra-adrenal malignancy, cancer was diagnosed before or during their admission. On the other hand, no malignancy was detected in patients with unilateral ATs in spite of careful examination during their admission before adrenalectomy. These results indicated that SCS patients with both unilateral and bilateral ATs were similarly exposed to subtle hypercortisolism before their admission, however, the prevalence of extra-adrenal cancers was significantly higher in patients with bilateral functioning ATs than in those with unilateral ATs.

Moreover, in the previous long-term follow up studies of patients with overt CS, the main cause of death was cardiovascular diseases or infection, and the prevalence of extra-adrenal cancers was not increased [20, 21]. Also in the follow up study of AIs, there was no significant increase of cancers as complications in SCS

patients compared with patients with non-functioning ATs [22]. These results indicate that long-term exposure to hypercortisolism alone is not enough to increase the prevalence of extra-adrenal cancers. It seems that genetic alterations in SCS patients with bilateral functioning ATs might be more important in extra-adrenal carcinogenesis than the period of exposure to hypercortisolism.

In the previous follow-up studies for SCS or CS patients, bilateral functional ATs were not separated from other ATs. If only SCS patients with bilateral functioning ATs would be analyzed as we performed in the present study, the prevalence of extra-adrenal malignancy might be augmented.

Although the mechanism of AT formation with excess cortisol secretion is unclear, various genetic alterations of cAMP/protein kinase A (PKA) signaling pathways in adrenocortical tumors have been identified [23–25]. Aberrant expressions of various G-protein-coupled receptors for vasopressin, serotonin, glucose-dependent insulinotropic peptide, catecholamines, luteinizing hormone and human chorionic gonadotropin in adrenocortical tissue have been reported and ACTH-independent cortisol production can be driven *via* these ectopically expressed receptors. Mutations of genes that encode G-protein subunit α , type 1 α regulatory subunit of cAMP-dependent PKA, and phosphodiesterases type 11A and type 8B have also been identified in adrenocortical adenoma or hyperplasia [26–29]. Activation of the Wnt/ β -catenin signaling pathway has been shown to affect adrenocortical tumorigenesis [30–32]. Moreover, mutations of tumor suppressor genes such as *p53*, *MEN1* and *ARMC5* have been observed in adrenocortical adenoma or hyperplasia [24, 33, 34]. Interestingly, glucocorticoids are reported to decrease *p53* protein levels in mice and may promote tumorigenesis [35]. Some genes mutated in adrenal lesions are also altered in cancers derived from other organs. Our results suggest that patients with bilateral functioning ATs carry some genetic alteration and are exposed to prolonged hypercortisolism, leading to high susceptibility to cancer.

For management of chronic autoimmune and inflammatory diseases, long-term systemic glucocorticoid treatment is required. Many previous reports have shown that there is no increase of cancers as adverse events of the long-term pharmacological use of glucocorticoids [36–38]. In contrast, some other clinical studies suggested that systemic use of glucocorticoids

increased the risk of skin, bladder and prostate cancers and non-Hodgkin lymphomas [39-41]. Further clinical studies will be needed to clarify the relationship between systemic glucocorticoid therapy and cancer risk.

A major limitation of our study is the relatively small sample size. The relationship between SCS and extra-adrenal malignancy should be confirmed in a larger study. We also saw some biases (age, sex) at baseline between the unilateral and bilateral tumor groups. We cannot rule out the possibility that the improvement of cardiovascular risk factors after adrenalectomy might be related to whether SCS patients have unilateral or bilateral ATs.

In summary, the present study of patients with SCS showed that cardiovascular risk factors such as HT and DM can be improved by adrenalectomy. We also found quite high prevalence of extra-adrenal malignancy in SCS patients who showed bilateral adrenal uptake on

the adosterol scintigraphy, which indicates that SCS patients with bilateral adrenal mass, showing bilateral high uptake in adrenal scintigraphy, should be carefully monitored for cancers, including intensive whole-body examinations and careful follow-up.

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Disclosure

The authors have no conflict of interest.

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