Pathophysiology and treatment of subclinical Cushing's disease and pituitary silent corticotroph adenomas

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Abstract. Pituitary adrenocorticotropic hormone (ACTH)-secreting tumor presents with a variety of clinical features. We outlined the features of ACTH release and characteristics of corticotroph adenoma cells. We especially focused on the corticotroph adenomas in patients with no clinical features of Cushing's disease. Subclinical Cushing's disease is defined by ACTH-induced mild hypercortisolism without typical features of Cushing's disease. Silent corticotroph adenomas (SCAs) are defined by normal cortisol secretion and ACTH-immunopositive staining without autonomous ACTH secretion. Clinicians who are not well-informed about the disease may sometimes confuse SCAs (because of their clinically silent nature) with "subclinical Cushing's disease". The recent criteria for diagnosing subclinical Cushing's disease in Japan are presented. Cortisol measurement was recently standardized in Japan, so plasma cortisol cutoff level should be reconsidered for the diagnosis. In patients with uncontrolled diabetes and hypertension despite appropriate treatment, subclinical Cushing's disease may be efficiently detected. Subclinical Cushing's disease may be associated with metabolic change. In subclinical Cushing's disease, mild hypercortisolism due to autonomous secretion of ACTH contributes to metabolic change and treatment of subclinical hypercortisolism can reverse this change.

Key words: ACTH, Cortisol, Cushing's disease, Pituitary, Silent corticotroph adenoma

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CUSHING'S SYNDROME results from chronic glucocorticoid excess with characteristic accompanying clinical signs and symptoms [1]. Adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome includes Cushing's disease and ectopic ACTH syndrome. Cushing's disease is primarily caused by pituitary ACTH-secreting tumor. The diagnostic criteria for Cushing's disease were established by the working group of the Ministry of Health, Labour, and Welfare of Japan in 2003 and revised in 2007 and 2010 [2, 3]. Most cases of Cushing's disease can be recognized by their obvious Cushingoid appearance, namely a moon face, central obesity, dorsocervical fat pad (buffalo hump), purple striae, thin skin, easy bruising, and proximal myopathy. Moreover, children with Cushing's disease exhibit slow growth retardation. In general, patients can also have a number of non-typical features triggered by cortisol, including hypertension, menstrual abnormalities, acne, hirsutism, peripheral edema, impaired glucose tolerance, diabetes, osteoporosis, pigmentation, and mental abnormalities. Diagnostic guidelines for Cushing's disease require the presence of at least one typical and one non-typical feature [4]. Corticotroph adenomas are typically recognized when patients show excessive ACTH levels and have Cushingoid features. In the same way as for somatotroph adenoma [5], we classify Cushing's disease or corticotroph adenoma according to the grade of clinical features, cortisol production levels, and autonomous secretion of ACTH (Table 1).

Pituitary ACTH-secreting tumor presents shows a variety of clinical features, from overt to subtle ones. Cushing's disease is defined by autonomous/dysregulated secretion of ACTH and excess cortisol production, with their obvious manifestation of the clinical features of Cushing's disease. In this review, we outlined the

Table 1 Spectrum of clinical symptoms and ACTH expression by corticotroph adenomas

Classification	Cushingoid features	Hyper-cortisolism	Autonomous secretion*	ACTH staining
Cushing's disease	+	+	+	+
Subclinical Cushing's disease	-	+ (mild)	+	+
Silent corticotroph adenoma	-	-	-	+

*Dysregulation of ACTH-dependent cortisol secretion; ACTH, adrenocorticotropic hormone

features of ACTH release and characteristics of corticotroph adenoma cells. We especially focused on the corticotroph adenomas in patients with no clinical features of Cushing's disease. Subclinical Cushing's disease is defined by ACTH-induced mild hypercortisolism without typical features of Cushing's disease. Silent corticotroph adenomas (SCAs) are defined by normal cortisol secretion and ACTH-immunopositive staining without autonomous ACTH secretion. Clinicians who are not well-informed about the disease may sometimes confuse SCAs (because of their clinically silent nature) with "subclinical Cushing's disease".

Subclinical and preclinical Cushing's syndrome has been reported especially in the adrenal tumors [6, 7]. Subclinical and preclinical conditions are generally distinguished by their long-term prognosis. Subclinical Cushing's syndrome would refer to a biochemical abnormality that never becomes clinically manifest, whereas preclinical Cushing's syndrome would refer to a stage in the development of the clinical syndrome [8]. Subclinical Cushing's disease was previously named as preclinical in Japan. However, it has not been determined whether such condition develops to overt ones. Therefore, it would be appropriate at presence that the term "subclinical" has been used in the pituitary. Subclinical Cushing's disease is defined by ACTH-induced mild hypercortisolism without typical features of Cushing's disease [9, 10]. However, clinicians often may confuse the manifestation of the clinical features. Therefore, we propose that the disorders that found by chance, incidentally or as a screening, and show ACTH-dependent mild hypercortisolism, are considered as subclinical Cushing's disease. The disease is often accompanied with some metabolic diseases such as diabetes and hypertension.

SCAs are defined by normal cortisol secretion and ACTH-immunopositive staining without autonomous ACTH secretion. Patients with SCAs exhibit none of the clinical features of Cushing's disease, have normal plasma cortisol levels, and do not have autonomous ACTH secretion, but tumor cells are immunopositive for ACTH [11, 12]. Immunopositivity for ACTH varies ranging from low to high in SCAs [11-13]. Preoperative SCA presentation was similar to that observed in nonfunctioning adenomas. However, SCAs completely differentiate from ACTH-negative nonfunctioning adenomas. SCAs have a notably higher rate of recurrence and new-onset postoperative hypopituitarism than nonfunctioning adenomas [12]. SCAs show characteristics of poorly differentiated corticotroph tumors [13].

Epidemiology of subclinical Cushing's disease

Previous studies have shown a prevalence of unsuspected Cushing's syndrome in patients with type 2 diabetes ranging from 0 to 9.4% [14, 15]. On the other hand, a prevalence of subclinical Cushing's disease has not been reported. However, it might be considered to be approximately one-tenth, compared with the patients with Cushing's disease [16]. In screening for secondary hypertension in 80 younger patients, one patient with overt Cushing's disease was found [17]. One patient with corticotroph adenoma was pointed out among newly-diagnosed 100 diabetic patients without Cushingoid features, suggested subclinical Cushing's disease [18]. A frequency of previously unsuspected Cushing's syndrome of 0.7% in a cohort of 813 patients with type 2 diabetes [15]. Among them, only one patient was pointed out subclinical Cushing's disease [15]. The data do not support widespread screening of patients with type 2 diabetes for ACTH-dependent and -independent Cushing's syndrome without Cushingoid features. Terzolo *et al.* recommend a case-finding approach in patients with uncontrolled diabetes and hypertension despite appropriate treatment [15]. In such patients, subclinical Cushing's disease may be efficiently detected.

Diagnostic approach for subclinical Cushing's disease

The existence of subclinical or preclinical Cushing's diseases has raised intriguing questions [8]. The first case of preclinical Cushing's disease was reported by Sakai *et al.* in Japan [19], and we have since reported another case of the disease [10]. Sakai *et al.*'s case showed extremely elevated plasma ACTH levels in response to corticotropin-releasing factor and desmopressin, whereas in our case both were within the normal range. A low-dose overnight dexamethasone suppression test (DST) failed to sufficiently reduce plasma ACTH and cortisol levels in both cases. Furthermore, the circadian rhythms of plasma ACTH and cortisol were disturbed in both cases. In Sakai *et al.*'s case, transsphenoidal resection of pituitary adenoma was performed, and immunohistochemistry revealed an ACTH-producing tumor. Further cases of subclinical or preclinical Cushing's disease have been reported in the English literature [20, 21].

The following diagnostic criteria, based on endocrinological findings, for subclinical Cushing's disease were established in Japan in 2010 [4]: (1) presence of a pituitary adenoma on magnetic resonance imaging (MRI); (2) normal to high plasma ACTH levels and normal cortisol levels in the morning; and (3) absence of a typical Cushingoid appearance. When laboratory data suggest ACTH-dependent dysregulation of cortisol secretion, screening tests are recommended to determine autonomic or abnormal secretion of ACTH. In terms of subclinical Cushing's disease, the following endocrinological findings are considered diagnostic criteria: (1) incomplete suppression of plasma cortisol levels (>3 µg/dL) after a low-dose (0.5 mg) DST; (2) high plasma cortisol levels (>5 µg/dL) during nighttime sleep; (3) response of plasma ACTH levels to the desmopressin test; and (4) high salivary cortisol levels (>1.5, compared with mean level) during nighttime sleep.

The Endocrine Society reported a clinical practice guideline for diagnosing Cushing's syndrome recommending 1 mg DST to be used as one of the primary screening tests [22]. Their guidelines outlined the plasma cortisol cutoff concentration for the 1 mg DST to be 1.8 µg/dL. However, cortisol values, especially at low levels, vary between cortisol assay kits [23]. Our previous studies revealed that low cortisol levels are associated with poor measurement accuracy. For example, a measurement of 1.8 µg/dL ± 2SD had a variation of ± 1.36 µg/dL [24-26]. Recently, standardization of cortisol measurements with seven assay kits using standard plasma material containing synthetic d4-hydrocortisone (NMIJ CRM 6007-a) and the ID-LC/MS/MS method was carried out in Japan [24, 25]. The resulting relative standard deviation was set within 10% in Japan, but not in other countries. Therefore, plasma cortisol cutoff level should be reconsidered for the diagnosis. In addition, people from East and Southeast Asia are leaner than those in Western countries, thus it has been suggested that the 1 mg DST may be too strong in suppressing plasma cortisol concentration in Japanese patients [16]. Additionally, in patients with subclinical Cushing's disease plasma cortisol concentration is easily suppressed by DST as their levels of excess cortisol are only mild [10, 16]. Therefore, a 0.5 mg DST has been developed and validated to provide a more reliable and sensitive screening test in Japan [16].

The screening and confirmatory tests for subclinical Cushing's disease (except for the 3.0 µg/dL cutoff of plasma cortisol level by 0.5 mg overnight DST) are the same as those for clinical Cushing's disease. The difference between Cushing's disease and subclinical Cushing's disease is due to be with or without typical Cushingoid appearance, respectively. Degree of excess cortisol production may be associated with the typical Cushingoid appearance. However, there are some reports that subclinical Cushing's disease may be associated with metabolic change [21, 27]. It is possible that mild hypercortisolism due to autonomous secretion of ACTH contributes to the metabolic change in subclinical Cushing's disease. Such evidence needs to be accumulated. An alternative pathology is, in rare cases, plurihormonal pituitary adenoma with growth hormone (GH) and ACTH production [28]. GH and ACTH function antagonistically with regard to protein and fat metabolism. The potent anabolic effects of GH may, therefore, conceal the effects of cortisol in the metabolism. Tomlinson *et al.* reported a case of Cushing's disease that failed to present with a classical phenotype [29] due to partially defective systemic 11β-hydroxysteroid dehydrogenase (HSD) type 1 (11β-HSD1) activity, which resulted in defective cortisone to cortisol conversion, thus increasing cortisol clearance and protecting the patient from the effects of excess cortisol.

Pathology of subclinical Cushing's disease

The differences of molecular pathogenesis between

subclinical Cushing's disease and Cushing's disease have not clarified. Ebisawa *et al.* suggest two potential differences [30]. One possibility is that plasma cortisol levels in subclinical Cushing's disease are within normal limits due to inactive ACTH precursor secretion. A discrepancy between plasma ACTH and cortisol levels would be found in such cases. Another possibility is that glucocorticoid resistance at the level of transcription is more prominent in subclinical Cushing's disease than in Cushing's disease. Glucocorticoid resistance may be caused by 11β-HSD type 2 (11β-HSD2) that converts cortisol to cortisone, expression levels or mutation of glucocorticoid receptors, and coactivation or corepression of glucocorticoid receptors. However, they failed to show the differences of 11β-HSD2 expression levels between subclinical Cushing's disease and Cushing's disease, and suggested that a mechanism other than 11β-HSD2 may be involved in the impaired action of glucocorticoid [30].

Management and follow up of subclinical Cushing's disease

Pituitary surgery is the first-line treatment for subclinical Cushing's disease. In a patient with subclinical Cushing's disease specifically, resection of a pituitary tumor improved all metabolic comorbidities, including hypertension, obesity, and impaired glucose tolerance [27]. In addition, surgery should be considered when pituitary adenomas are associated with hypopituitarism, visual impairment, or progressive enlargement during radiologic review [31]. It is better to treat the disease when there is evidence that subclinical hypercortisolism can reverse metabolic change [32].

In fact, there are some reports that subclinical Cushing's syndrome may be associated with metabolic change. Di Dalmazi *et al.* reported that patients with mild hypercortisolism have an increased risk of cardiovascular events and mortality, even when clinical signs of overt hypercortisolism are not present [33]. Improvement in hypertension and/or glycemic control, and the normalization of markers of bone turnover were reported after unilateral adrenalectomy in patients with subclinical Cushing's syndrome [34]. Although no long-term prospective data are available for guidance in the choice between medical and surgical therapy for subclinical Cushing's syndrome [35], Young has proposed a commonsense strategy to operate on younger patients (<40 years old) with a recent onset or worsening of dia-

betes, hypertension, or osteoporosis [34].

Some reports also show that treatment of subclinical Cushing's disease improved metabolic change. Nagai *et al.* [21] reported improved blood pressure and glucose levels after resection of pituitary adenoma, even in cases of subclinical Cushing's disease. For instance, before the operation, blood pressure was uncontrolled under treatment with furosemide, nifedipine, doxazosin mesilate, and cilazapril, and the fasting blood glucose level was 241 mg/dL, and HbA1c level was 11.0%. After the operation, her blood pressure was controlled enough even when cilazapril alone was decreased to 0.5 mg/day, and HbA1c level was improved to around 6.0% with diet therapy only. The data suggest that malignant hypertension and deteriorated diabetes mellitus may have been due to subclinical Cushing's disease in this case. However, at presence, evidence may be insufficient to conclude that treatment of all the cases of subclinical hypercortisolism can reverse metabolic change. Therefore, purposing the indications of treatments may be an important target of research in future studies.

Epidemiology of pituitary silent corticotroph adenomas

SCAs are considered preoperatively to be nonfunctioning pituitary adenomas [11]. Approximately 6% of all nonfunctioning pituitary adenomas have been reported to be SCAs [36], while Yamada *et al.* found a 19% incidence of clinically nonsecreting pituitary adenomas removed at surgery [37]. SCAs are macroadenomas, and tend to have a more aggressive presentation with a higher chance of hemorrhage and invasion of anatomical structures [38]. SCA patients are younger than the patients with ACTH-negative nonfunctioning pituitary adenomas [39].

Pathology of pituitary silent corticotroph adenomas

The precise mechanisms behind the "silence" of clinical SCAs have not been clarified [13]. Some pituitary adenomas may have impaired secretion of ACTH or may autonomously secrete ACTH at levels insufficient for producing cortisol to show Cushingoid features [40]. Translational or post-translational abnormalities of ACTH have been suggested in SCAs [41]. Despite the absence of clinical hypercortisolism in patients with SCAs, elevated plasma ACTH levels

are observed [13]. Therefore, some SCAs have been reported to secrete mostly biologically inactive, high-molecular-weight ACTH [42], which can be detected on gel chromatography [42]. Defective prohormone convertase 1/3 expression may lead to preferential production of unprocessed, biologically inactive ACTH variants in SCAs [43]. Corticotroph adenomas have glucocorticoid resistance, which may be involved in their tumorigenesis. 11β-HSD2, which converts cortisol to cortisone, might play important roles in regulating glucocorticoid hormone action. It has been reported that ACTH-secreting pituitary tumors show enhanced 11β-HSD2 gene expression but suppressed 11β-HSD1 gene expression [44, 45]. Expression levels of 11β-HSD2 mRNA in the ACTH-secreting pituitary tumors that cause Cushing's disease were greater than those in SCAs [46], suggesting a partial or weak resistance by glucocorticoids in SCAs.

Furthermore, SCAs have been characterized two morphologic variants of SCAs by electron microscopy studies [11, 47]. Type 1 adenomas were similar to functional corticotroph adenomas in that they are densely granulated basophilic tumors with abundant cytokeratin filaments. Subtype 2 adenomas were chromophobic and lack cytoplasmic intermediate filaments.

SCAs might be less differentiated tumors. T pit is an essential transcription factor for proopiomelanocortin transcription and corticotroph differentiation [13], and both T pit and proopiomelanocortin expression levels are lower in SCAs [13]. SCAs differentiate from overt or subclinical Cushing's disease in terms of hormonal and molecular behaviors [13]. SCAs exhibit different levels of corticotroph differentiation with some being poorly differentiated corticotroph tumors [13]. In fact, in addition to corticotroph features, SCAs were shown to incorporate gonadotroph elements as evidenced by the presence of honeycomb Golgi or transcriptional factors [12].

Management and follow up of silent corticotroph adenomas

The majority of SCAs are macroadenomas upon presentation. SCAs are recognized preoperatively as a distinct clinical entity of nonfunctioning adenomas and generally have an aggressive postoperative course. However, a rare case was reported in which a silent corticotroph macroadenoma developed into Cushing's disease [48]. The development of overt Cushing's disease due to a previously resected SCA has been suggested to be a potential indicator for future malignant behavior [49, 50]. Alahmadi *et al.* reported that two recurrent cases had a relatively high MIB-1 score, and recommended reserving adjuvant radiotherapy (radiation) for the subset of SCAs that demonstrate more aggressive clinical behavior on close surveillance MRI or exhibit potential indications for high recurrence, such as a higher MIB-1 labeling index, in the operated tumor [38]. Other predictors should be established in the future.

A subset of aggressive pituitary adenomas and carcinomas has been shown to respond to the second-generation alkylating agent temozolomide [50, 51]. Because low levels of O-6-methylguanine DNA methyltransferase are indicative of a favorable response to temozolomide, low expression levels in nonfunctioning pituitary adenomas are indicative of a potential candidate for temozolomide treatment. These findings provide a rationale for the use of temozolomide as an alternative treatment for SCAs when conventional therapy such as reoperation and radiotherapy is ineffective [52, 53]. Because SCA patients are typically young with a high incidence of postoperative tumor regrowth [39], long-term follow up is necessary.

Conclusion

The recent criteria for diagnosing subclinical Cushing's disease in Japan are presented and benefits for treatment of subclinical Cushing's disease are also provided. Some reports show that removal of pituitary tumors in cases of subclinical Cushing's disease improved metabolic change. However, there is too limited evidence to conclude at presence that treatment of all the cases of subclinical hypercortisolism can reverse this change. Such evidence needs to be accumulated. SCAs generally demonstrate an aggressive postoperative course. Due to the typically aggressive postoperative course of SCAs, long-term follow up on surveillance MRI is necessary.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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