

TSH ratio as a novel diagnostic method for Cushing's syndrome

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Abstract. Circadian variations impact thyrotropin (TSH) secretion; in Cushing's syndrome (CS) patients, the nocturnal serum TSH surge is abolished. The aim of this prospective study is to examine whether serum TSH surge may be a useful diagnostic method for CS. This prospective study recruited 136 inpatients for differential diagnosis of CS or subclinical CS (SCS), and 21 inpatients with depression at Osaka University Hospital. Serum TSH surge was assessed by the midnight-to-morning serum TSH ratio (2300–2400 h to 0800–0900 h). The diagnostic accuracy (sensitivity and specificity) between TSH ratio and ordinary screening tests [low-dose dexamethasone suppression test (LDDST), late-night serum cortisol and urine free cortisol (UFC)] were compared. Twenty-two patients were diagnosed as CS (12 overt CS and 10 SCS) and the remaining 120 patients were excluded for CS. The diagnostic accuracy of TSH ratio (cutoff value 1.0) yielded sensitivity 90.9% [95% confidence interval (CI) 70.8–98.9], specificity 95.0% (95% CI 89.4–98.1), and a high positive and low negative likelihood ratio [18.2 (95% CI 8.2–40.1) and 0.096 (95% CI 0.026–0.359), respectively]. The specificity of TSH ratio was significantly higher than LDDST and midnight serum cortisol test. The sensitivity of TSH ratio was significantly higher than UFC. TSH ratio showed more than 1.0 in all patients with depression and CYP3A4 inducer users. TSH ratio is a novel supportive diagnostic method with higher specificity than the current diagnostic methods for CS.

Key words: Cushing's syndrome, Thyrotropin, TSH surge, TSH ratio, Dexamethasone suppression test

CUSHING'S SYNDROME (CS) is a clinical state caused by chronic excess of glucocorticoid (GC) [1]. It can be divided into adrenocorticotrophic hormone (ACTH)-dependent CS, such as ACTH-producing pituitary adenoma [Cushing's disease (CD)], and ACTH-independent CS, such as cortisol-producing adrenal tumors [2, 3]. The clinical features of CS include central obesity, moon face, hirsutism, and plethora [4, 5]. GC excess in CS results in hypertension, impaired glucose tolerance, dyslipidemia, osteoporosis, depression and cognitive impairment [5, 6]. In addition to the classical overt CS, a mild form of ACTH-independent CS (sub-

clinical Cushing's syndrome; SCS) [7] and that of ACTH-dependent CS (subclinical Cushing's disease; SCD) [8] have been identified in recent years. These mild forms of CS are defined as those lacking typical physical features of CS, while harboring autonomy in cortisol and/or ACTH secretion. SCS was reported to show an increased prevalence of risk factors for cardiovascular diseases [9], and these metabolic disorders were partially or totally reversed by surgical treatment of SCS [10, 11]. CS-associated comorbidities contribute to a decreased quality of life [12], and the estimated 5-year survival rate of patients with untreated CS is 50%, which is mainly due to cardiovascular complications [13]. Therefore, the practical physician must diagnose CS and SCS certainly.

The evidence of the excessive secretion of cortisol in the endocrinological examination is indispensable in the diagnosis of CS and SCS. Currently, the overnight low-

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dose dexamethasone suppression test (LDDST), late-night salivary and blood cortisol and urine free cortisol (UFC) are recommended as initial examinations for cortisol excess [14]. However, cortisol as a stress hormone, is easily elevated by mental and physical stress; this is particularly true of depression, alcoholism, morbid obesity and poorly controlled diabetes, and false positive results are often found in these tests [14]. In addition, because dexamethasone is metabolized by CYP3A4, use of this enzyme-related drug may render difficulty to interpret the LDDST in the diagnosis of CS [15]. Thus, to date, the diagnosis of CS by cortisol levels has remained a problem, especially in terms of specificity.

On the other hand, it is well established that thyrotropin (TSH) secretion has circadian variations, and that the highest serum concentrations of the hormone are found in the late evening or early morning [16, 17]. This nocturnal serum TSH surge is abolished in CS patients regardless of dependence on ACTH [18, 19]. These findings suggest a possibility of new diagnostic method for CS other than by cortisol level analysis.

The aim of this prospective study was to examine whether serum TSH surge may be a useful diagnostic method for CS.

Subjects and Methods

Research subjects

This prospective study was approved by the Ethical Committee of the Osaka University (reference no. 12354), and according to the requirements of the Declaration of Helsinki, written informed consent was obtained from all participants before participation in the study.

We recruited 136 consecutive patients (49 patients with pituitary tumor, 68 patients with adrenal tumor and 18 other patients, such as obesity and diabetes) who were hospitalized for the purpose of differential diagnosis of CS and SCS in the Department of Metabolic Medicine in Osaka University Hospital between January 2013 and March 2015. Furthermore, we recruited another 21 patients who were hospitalized for the purpose of treatment for major depression in the Department of Psychiatry in Osaka University Hospital. Major depressive disorder was diagnosed according to DSM-V criteria. Exclusion criteria included withdrawal of the research consent, age of <20 years, pregnancy, the presence of malignant neoplasm, the presence of primary thyroid diseases, or therapy with GCs or thyroid hormone. According to the

exclusion criteria, 8 subjects were excluded because they withdrew an agreement, and 2 subjects with malignant neoplasm, 3 subjects with chronic thyroiditis and 2 subjects with GCs or thyroid hormone replacement were excluded. A total of 142 patients [83 females, median age, 56 years (44–66 years); median body mass index, 24.1 kg/m² (21.2–27.4 kg/m²)] were enrolled in the present study.

Diagnostic criteria of CS and SCS

The diagnosis of overt CS (CD and adrenal CS) and SCS (SCD and adrenal SCS) were based on clinical and biochemical data according to the guideline proposed by the working group of the Japanese Ministry of Health, Welfare and Labor.

The diagnosis of CD and SCD were based on the following criteria: 1) The presence of typical Cushingoid appearance (moon face, central obesity, dorsocervical fat pad (buffalo hump), purple striae, thin skin and easy bruising, proximal myopathy) is a prerequisite for CD, while that for SCD is the absence of typical Cushingoid features; 2) Evidence of autonomic or abnormal secretion of ACTH and cortisol, such as (a) normal-to-high plasma ACTH and serum cortisol levels, (b) incomplete suppression of cortisol (>5.0 µg/dL in overt CD and >3.0 µg/dL in SCD) by low-dose overnight dexamethasone suppression test (LDDST), (c) high cortisol level (>5.0 µg/dL) during night time sleeping, and (d) response of plasma ACTH level to the desmopressin (DDAVP) test; 3) The differential diagnosis between CD and ectopic ACTH syndrome shows (a) normal or exaggerated response of plasma ACTH level to the corticotropin-releasing hormone (CRH) test, (b) suppression of cortisol (less than half, compared with the basal level) by high-dose (8 mg) overnight DST, (c) the presence of a pituitary adenoma by magnetic resonance imaging (MRI), (d) positive results by a selective venous sampling test, and finally CD and SCD are diagnosed by histopathological examination of the surgically excised lesions and confirmation by immunohistochemical staining for ACTH.

The diagnosis of adrenal CS and SCS were based on the following criteria: 1) The presence of typical Cushingoid appearance is a prerequisite for adrenal CS, while that for SCS is the absence of typical Cushingoid features; 2) Evidence of autonomic or abnormal secretion of cortisol, such as (a) normal-to-high serum cortisol level and suppression of ACTH, (b) incomplete suppression of cortisol (>5.0 µg/dL in CS and >3.0 µg/dL in SCS) by LDDST, (c) incomplete suppression of cortisol

(>1.0 µg/dL) by high-dose (8 mg) overnight DST, (d) high cortisol level (>5.0 µg/dL) during night time sleeping, (e) low DHEA-S level, and (f) adrenocortical scintigraphy, show increased uptake at the affected adrenal side and decreased uptake at the unaffected side. Furthermore, the presence of transient adrenal insufficiency symptoms and/or the atrophy of adhesion cortical tissue are evidence of adrenal CS diagnosis following adrenalectomy.

TSH surge and other screening tests of CS

Serum TSH and cortisol circadian rhythm were evaluated by measurement at morning (0800–0900 h) and at sleeping midnight (2300–2400 h). TSH ratio (midnight serum TSH to morning serum TSH) ≤ 1.0 means abolition of nocturnal serum TSH surge. In LDDST, 0.5 mg (ACTH dependent) or 1 mg (ACTH independent) dexamethasone was given between 2300 and 2400 h, and cortisol was measured between 0800 and 0900 h the following morning. UFC was measured at 24 h urinary sample, and a UFC level greater than the normal range (100 µg/24 h) was considered to be high.

Laboratory assays

Serum TSH level was assessed by immunoenzymometric assay (TOSOH-II ST AIA-PACK TSH, Tosoh Bioscience Inc.), with an intra-assay CV and inter-assay CV of <5.0%. Serum cortisol was measured by chemiluminescent enzyme immunoassay (Access cortisol kit, Beckman Coulter, Tokyo). This kit has a sensitivity of 0.4 µg/dL, with an intra- and inter-assay CV less than 4.3% and 5.9%, respectively. UFC was measured by radioimmunoassay (cortisol kit TFB, TFB Inc., Tokyo), with an intra-assay CV and inter-assay CV of <10.0%.

Statistical analysis

Continuous variables are expressed as median values (1st and 3rd quartiles). Wilcoxon rank sum test was used to examine group differences, and Wilcoxon signed-rank test was used to compare morning and midnight TSH levels. Differences in diagnostic accuracy between TSH ratio and other screening tests were examined by comparing the sensitivity and specificity using the McNemar test. A 95% confidence interval (CI) was constructed for each sensitivity and specificity. The 95% CIs were estimated by the Clopper-Pearson exact method. Two-sided $p < 0.05$ denoted a statistically significant difference. All statistical analyses were performed with IBM SPSS Statistics Version 22 (International Business Machines

Table 1 Clinical diagnosis of all subjects ($n = 142$)

Disease (Number of subject)	
Non-CS group (120)	
Pituitary tumor (43)	Nonfunctional adenoma (24), GH-secreting adenoma (7), PRL-secreting adenoma (6), Rathke's cleft cyst (5), Chordoma (1)
Adrenal tumor (48)	Nonfunctional adenoma (18), primary aldosteronism (25), Pheochromocytoma (4), Adrenal cortical carcinoma (1)
Others (16)	Obesity (13), Diabetes mellitus (3)
Depression (13)	Depression (13)
CS group (22)	
Overt CS (12)	CD (1), Adrenal CS (11)
SCS (10)	SCD (3), Adrenal SCS (7)

CD, Cushing's disease; SCD, subclinical Cushing's disease; CS, Cushing's syndrome; SCS, subclinical Cushing's syndrome

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Results

The final diagnosis in all 142 subjects is shown in Table 1. Twenty-two patients were diagnosed as CS (12 overt CS and 10 SCS). The remaining 120 patients (43 pituitary tumor, 48 adrenal tumor, 13 obesity, 3 diabetes mellitus and 13 depression patients) were excluded for CS according to the diagnostic criteria of CS and SCS, as described in Subjects and Methods.

Morning and midnight serum TSH and cortisol levels were measured in 142 subjects (100%). LDDST was performed in 139 subjects (98%), and UFC was measured in 132 subjects (93%).

Effects of glucocorticoid autonomy on TSH ratio

Morning and midnight serum TSH levels are shown in Fig. 1. Midnight serum TSH level in CS group was significantly lower than that of non-CS group ($p < 0.001$), and morning serum TSH levels did not differ between the two groups ($p = 0.980$). Furthermore, midnight TSH level was significantly increased compared with the morning level in non-CS group ($p < 0.001$). In contrast, midnight TSH level was significantly decreased compared with the morning level in CS group ($p < 0.001$).

TSH ratio is shown in Fig. 2. TSH ratio was more than 1.0 in all non-CS patients except for six patients (2 non-

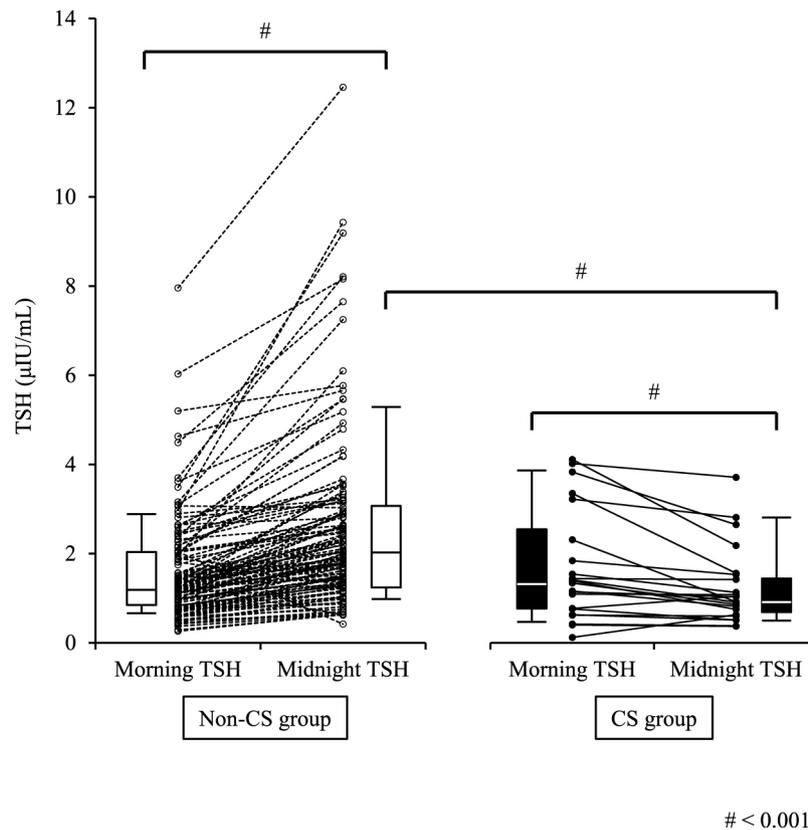


Fig. 1 Morning and midnight serum TSH levels in non-CS and CS groups. Midnight serum TSH levels in CS group were significantly lower than those of non-CS group ($p < 0.001$). Midnight TSH levels were significantly increased compared to the morning levels in non-CS group ($p < 0.001$). In contrast, midnight TSH levels were significantly decreased compared to the morning levels in CS group ($p < 0.001$).

CS, Cushing's syndrome; TSH, thyrotropin

functional pituitary adenoma, 2 PRL-secreting adenoma, 1 chordoma and 1 nonfunctional adrenal adenoma). In 91% of CS patients (20 patients) TSH ratio was less than 1.0. Surprisingly, TSH ratio was over 1.0 in all 13 patients with depression, which indicated that nocturnal serum TSH surge was maintained.

Compared with diagnostic criteria of CS, TSH ratio had a negative relation to cortisol level after LDSDT ($r = -0.284$, $p < 0.001$) as well as its level during night time sleeping ($r = -0.323$, $p < 0.001$). TSH level during night time sleeping also had a negative relation to cortisol level after LDSDT ($r = -0.262$, $p = 0.002$) as well as its level during night time sleeping ($r = -0.240$, $p = 0.004$).

Comparison of differential performance between typical CS screening tests and TSH ratio

The diagnostic performance of typical CS screening tests and TSH ratio is shown in Table 2. Although the

optimum cutoff value of the TSH ratio for CS diagnosis by the ROC curve was 0.99 (sensitivity 90.9%, specificity 95.0%, AUC 0.91), we decided to use 1.0 for clinical convenience. The diagnostic accuracy of TSH ratio (cutoff value 1.0) was sensitivity 90.9% (95% CI 70.8–98.9) and specificity 95.0% (95% CI 89.4–98.1). The diagnostic accuracy of the post-LDDST cortisol (cutoff value 5.0 $\mu\text{g}/\text{dL}$) was sensitivity 95.5% (95% CI 77.2–99.9) and specificity 87.2% (95% CI 79.7–92.6). With a cutoff value of 3.0 $\mu\text{g}/\text{dL}$, sensitivity was 100.0% (95% CI 84.6–100.0) and specificity 80.3% (95% CI 72.0–87.1), and with a cutoff value of 1.8 $\mu\text{g}/\text{dL}$, sensitivity was 100.0% (95% CI 84.6–100.0) and specificity 65.0% (95% CI 55.6–73.5). The midnight serum cortisol test (cutoff 5.0 $\mu\text{g}/\text{dL}$) was sensitivity 90.9% (95% CI 70.8–98.9) and specificity 86.7% (95% CI 79.3–92.2). The UFC test (cutoff 100 $\mu\text{g}/24 \text{ h}$) was sensitivity 34.6% (95% CI 20.7–63.6) and specificity 94.6% (95% CI

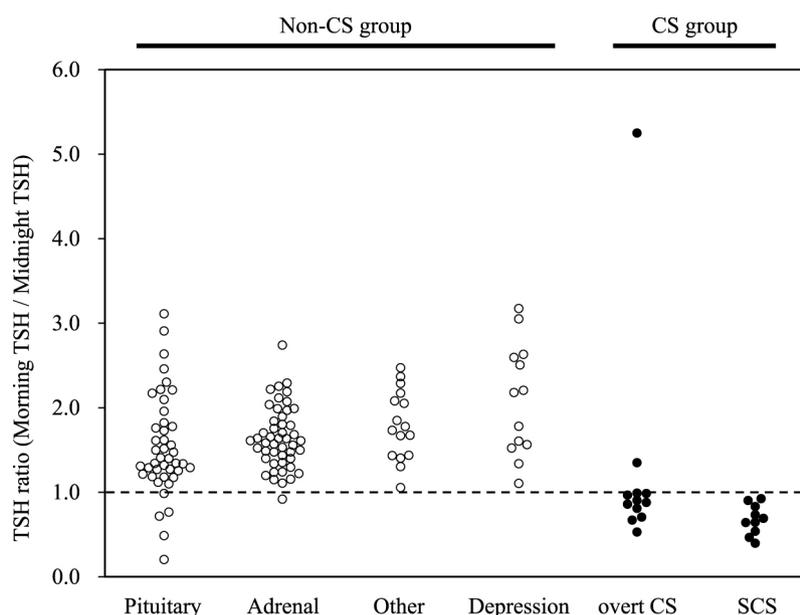


Fig. 2 TSH ratio in each disease group. CS, Cushing's syndrome; SCS, subclinical Cushing's syndrome; TSH, thyrotropin

Table 2 Comparison of differential performance between ordinary CS screening tests and TSH ratio

Test	cut off	Sensitivity (95% CI)	<i>p</i> vs. TSH ratio	Specificity (95% CI)	<i>p</i> vs. TSH ratio	LR ^{pos} (95% CI)	LR ^{neg} (95% CI)
TSH ratio	1.0	90.9 (70.8 to 98.9)	—	95.0 (89.4 to 98.1)	—	18.2 (8.2 to 40.1)	0.096 (0.026 to 0.359)
post-LDDST cortisol	5.0 µg/dL	95.5 (77.2 to 99.9)	1.000	87.2 (79.7 to 92.6)	0.078	7.5 (4.6 to 12.1)	0.052 (0.008 to 0.354)
post-LDDST cortisol	3.0 µg/dL	100.0 (84.6 to 100.0)	0.500	80.3 (72.0 to 87.1)	0.003	5.1 (3.5 to 7.3)	—
post-LDDST cortisol	1.8 µg/dL	100.0 (84.6 to 100.0)	0.500	65.0 (55.6 to 73.5)	<0.001	2.9 (2.2 to 3.7)	—
Midnight serum cortisol	5.0 µg/dL	90.9 (70.8 to 98.9)	1.000	86.7 (79.3 to 92.2)	0.041	6.8 (4.2 to 11.0)	0.105 (0.028 to 0.394)
Urinary free cortisol	100 µg/24 h	40.9 (20.7 to 63.6)	0.007	93.6 (87.3 to 97.4)	1.000	6.4 (2.7 to 15.4)	0.631 (0.444 to 0.896)

CS, Cushing's syndrome; LDDST, low-dose dexamethasone suppression test; LR^{pos}, positive likelihood ratio; LR^{neg}, negative likelihood ratio; CI, confidence interval

LR^{neg} not computable if sensitivity = 100%

87.3–97.4). The specificity of TSH ratio was significantly higher than that of post-LDDST cortisol (cutoff values 3.0 µg/dL and 1.8 µg/dL, $p = 0.003$ and $p < 0.001$, respectively) and midnight serum cortisol test ($p = 0.041$). The sensitivity of TSH ratio was significantly higher than that of UFC ($p = 0.007$).

Among 13 patients with depression, 2 patients showed false positive in LDDST cortisol and 1 patient showed

false positive in midnight serum cortisol test. However, all of TSH ratios yielded normal responses.

Effect of CYP3A4 inducer on TSH ratio

In this study, 22 patients (5 CS and 17 non-CS) were receiving CYP3A4 inducer medications (12 atorvastatin, 2 simvastatin, 2 pioglitazone, 3 valproate, 2 clonazepam, 1 terbinafine and 1 estrogen) interfering with dexametha-

sone metabolism [20]. In non-CS patients, the false positive rate of post-LDDST cortisol in patients with CYP3A4 inducer medications tended to be higher than that in patients without CYP3A4 inducer medications (35.3% vs. 17.0%). However, the TSH ratio yielded a normal response in all 17 non-CS patients with CYP3A4 inducer medications.

Discussion

In this prospective study, we investigated whether serum TSH surge was a useful diagnostic method for CS and SCS. The specificity of TSH ratio was significantly higher than LDDST and midnight serum cortisol, and the diagnostic sensitivity of TSH ratio was significantly higher than UFC. In addition, we found that TSH ratio also had a high diagnostic accuracy, even in patients with depression and in CYP3A4 inducer users who did not use cortisol level for differential diagnosis of CS. TSH ratio is a novel supportive diagnostic method with higher specificity than the current diagnostic methods for CS.

LDDST, late-night salivary and blood cortisol and UFC are currently used as the initial examination of CS [14]. However, these typical screening tests have several problems related to diagnostic specificity. The diagnosis of CS and SCS is often determined by the cortisol kit used, but the cortisol levels differ among the kits [21]. It is suggested that the lack of the standardization of cortisol measurements is one of the causes of confusion in the diagnosis of CS and SCS. In addition, the cutoff levels of cortisol in these tests are close to the functional limit of detection of the assays. Therefore, the physician must be mindful of the possibility of false-positive test results when they assess cortisol levels after typical screening tests for CS. On the other hand, with the development of modern TSH assays, there is a 10-fold improvement in functional sensitivity between each new generation of assays [22]. Therefore, the modern TSH assays yield reliable data even within the low concentration range. Additionally, because the TSH ratio is a relative value not an absolute value, which is the ratio of midnight TSH to morning TSH, this ratio is not affected by the use of any type of TSH assay kit. In particular, this is true of pseudo-Cushing's syndrome (such as depression, alcoholism, morbid obesity, poorly controlled diabetes and extreme physical stress), which often shows a false-positive result when analyzed by typical screening tests using cortisol levels. TSH ratio is simpler to perform than the desmopressin test [23] and

dexamethasone-CRH test [24], which were previously recommended to confirm or exclude the CD diagnosis.

Moreover, the drug-affected CYP3A4 might confound the interpretation of the low dose DST in the diagnosis of CS [15], because CYP3A4 inducer enhances the metabolism of dexamethasone. TSH ratio was useful even in the patients taking such drugs.

The serum concentration of TSH is reported to show significant circadian periodicity as nocturnal surge [16, 17]. The nocturnal surge of TSH starts after the first month of life [25], and is observed regardless of age or gender [26]. The onset of the nocturnal TSH surge is delayed by increasing BMI and advanced by increasing age, however, almost all subjects show nocturnal surge at midnight (2300 h to 2400 h) [27].

Previous studies have reported that the nocturnal TSH surge is absent in patients with CS regardless of dependence on ACTH [18, 19], and that high-dose GC infusion (hydrocortisone 100 mg/24 h) completely suppress the nocturnal TSH surge in healthy controls [28]. Interestingly, we confirmed the lack of nocturnal TSH surge not only in overt CS patients, but also in SCS patients as mild hypercortisolism in present study. It is reported that some conditions affect the circadian TSH rhythm. The nocturnal serum TSH surge is absent in primary hyperthyroidism [29] and in severe primary hypothyroidism [30]. The nocturnal TSH surge is decreased in subclinical hypothyroidism with high serum TSH and normal thyroid hormone levels [30] and in patients with non-thyroidal illness [31]. We did not evaluate the TSH ratio in patients with primary thyroid disease and GCs users in this study. Previous studies reported the nocturnal serum TSH surge is decreased in poorly controlled diabetes [32, 33]. The interpretation of the TSH ratio for the diagnosis of CS in these patients might require special care, described above.

In pituitary tumor patients, TSH deficiency might influence a nocturnal serum TSH surge. We evaluated whether TRH stimulates the TSH secretion in 37 of 43 pituitary patients (TRH stimulation test). The TSH ratio was independent of basal TSH levels in TRH stimulation test ($p = 0.070$) but it was correlated with the TSH hold response ($r = 0.394$, $p = 0.016$). Yamakita *et al.* reported a lack of nocturnal surge of serum TSH in idiopathic isolated TSH deficiency patients in whom the serum TSH levels are less than 0.20 mIU/L [34]. In this study, 5 of 6 non-CS patients that TSH ratio was less than 1.0, had pituitary diseases (2 nonfunctional adenoma, 2 PRL-secreting adenoma and a Chordoma). In 3 of 5 these

patients with pituitary diseases, TSH deficiency was diagnosed by TRH stimulation test. Therefore, the use of the TSH ratio must be carefully considered in patients with severe TSH deficiency.

The evaluation of nocturnal TSH in patients with depression has been controversial in previous studies [35-37]. We evaluated the TSH ratio in 13 depression patients who were hospitalized in the Department of Psychiatry. Their TSH ratios differed from the CS group and were more than 1.0. Therefore, the TSH ratio is useful for the differential diagnosis between CS and pseudo-Cushing's syndrome, such as patients with depression.

The strength of our current survey is its prospective

design and the evaluation of not only overt CS but also SCS. The limitation is the relatively small number of CS group patients, especially overt CD. However, the complete lack of nocturnal serum TSH surge in overt CD patients has been established in previous studies [18, 19]. A prospective study with a large number of CS patients will be needed to clarify the optimal TSH ratio in the diagnosis of CS.

Disclosure

The authors have nothing to disclose.

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