Obstructive sleep apnea syndrome causes a pseudo-Cushing’s state in Japanese obese patients with type 2 diabetes mellitus

Daisuke Tamada1), Michio Otsuki1), Susumu Kashine2), Ayumu Hirata1), Toshiharu Onodera1), Tetsuhiro Kitamura1) and Iichiro Shimomura1)

1) Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
2) Department of Internal Medicine, Suita Municipal Hospital, Osaka, Japan

Abstract. Activation of the hypothalamic-pituitary-adrenal axis has been reported in some patients with the obstructive sleep apnea syndrome (OSAS). In current study, we investigated whether OSAS affect the screening test for subclinical Cushing’s disease using 0.5 mg overnight dexamethasone suppression test (DST) in Japanese obese diabetic patients with OSAS. Among Japanese obese patients with type 2 diabetes mellitus who had been hospitalized in our department, we selected 20 patients with moderate to severe untreated OSAS (apnea-hypoxia index, AHI, of ≥15 events/hour). All patients underwent 0.5 mg DST. The same test was repeated in patients with positive response of it within a few days after continuous positive airway pressure (CPAP) therapy. We found that five patients showed positive response of DST (25%). Three of these patients continued to use CPAP, and they showed normal response of DST after CPAP therapy. Serum cortisol after 0.5 mg DST measured before CPAP therapy correlated significantly with fasting serum cortisol level (r=0.764, p<0.0001), but not with various clinical parameters, including AHI (p=0.784), body mass index (p=0.984), waist circumference (p=0.957), HbA1c (p=0.261), fasting plasma glucose (p=0.420) and HOMA-IR (p=0.500). Our study show that OSAS causes a pseudo-Cushing’s syndrome in obese patients with type 2 diabetes mellitus, which phenomena can be reversed by CPAP therapy.

Key words: Obstructive sleep apnea syndrome, Pseudo-Cushing’s syndrome, Cushing’s disease, Dexamethasone suppression test

OBSTRUCTIVE SLEEP APNEA SYNDROME (OSAS) is associated with increased risk of hypertension, diabetes mellitus, myocardial infarction, congestive heart failure, and stroke [1, 2]. Continuous positive airway pressure (CPAP) therapy improves not only upper airway patency but also the quality of life and reduces the frequency of OSAS-related cardiovascular complications [2-4].

The underlying mechanisms of the multi-organ complications associated with OSAS are considered to include repetitive upper airway obstruction, hypoxemia and sleep fragmentation. These abnormalities likely result in various neural and metabolic changes, including activation of peripheral sympathetic activity, inflammatory pathways, hypothalamic-pituitary-adrenal (HPA) axis, impairment of insulin sensitivity and generation of reactive oxygen species, which could predispose to vascular damage [5, 6]. Previous clinical studies suggested that activation of the sympathetic nervous system in OSAS results in hypertension and tachycardia, which were both improved by CPAP therapy [7, 8]. OSAS is also reported to activate the HPA axis. Bratel, et al. indicated that OSAS was associated with a significant increase of fasting serum cortisol levels [9]. However, several other studies did not find any OSAS-related changes in cortisol levels, compared with healthy subjects [10-12]. Thus, the effect of OSAS on the HPA axis remains to be defined.

Pseudo-Cushing’s syndrome should be differentiated from Cushing’s syndrome and Cushing’s disease. Alcoholism, depression and severe obesity are known to cause pseudo-Cushing’s syndrome [13-16].
Subclinical Cushing’s syndrome (subCS) and subclinical Cushing’s disease (subCD) have been identified [17, 18]. Subclinical Cushing’s state is defined as the lack of clinical features of Cushing’s syndrome and Cushing’s disease, such as moon face, central obesity, buffalo hump, red-purple striae, bruising and muscle weakness, with the presence of autonomous ACTH or cortisol secretion. The diagnostic criteria for subCS and subCD have been proposed by the Japan Endocrine Society [19, 20]. In order to exclude subCS or subCD, obese patients should be evaluated for autonomous ACTH or cortisol secretion.

In the present study, we performed screening tests to distinguish subCD from simple obesity, including overnight dexamethasone suppression test (DST) in Japanese obese diabetic patients with OSAS, and investigated the effect of CPAP therapy on ACTH and cortisol secretion.

**Materials and Methods**

**Subjects**

Among Japanese obese patients with type 2 diabetic mellitus who had been hospitalized at our department between April 2011 and March 2013, we selected 20 patients (14 males and 6 females) with moderate to severe untreated OSAS, which was defined as apnea-hypopnea index (AHI) of ≥15 events/hour. Severe OSAS patients (AHI of ≥30 events/hour) were started CPAP therapy. The median age of these patients was 56.5 years (range, 44-64) and median body mass index (BMI) was 32.6 kg/m$^2$ (28.1-38.9) (Table 1). Each patient underwent an overnight 0.5 mg DST to distinguish subCD from simple obesity. Patients with alcoholism, depression or glucocorticoid treatment were excluded from the study. The study protocol was reviewed and approved by the ethics committee of Osaka University.

**Endocrinological evaluation**

The circadian rhythms of plasma ACTH and serum cortisol levels were evaluated at 0600 h and 2300-2400 h. The DST was performed using the following protocol: 0.5 mg dexamethasone was taken at 2300 h, and blood samples were collected at 0600 h for measurement of plasma ACTH and serum cortisol levels. According to the diagnostic criteria of subCD proposed by the Japan Endocrine Society, the normal serum cortisol level at midnight is less than 5.0 μg/dL and a normal response of 0.5 mg DST is suppression of serum cortisol to less than 3.0 μg/dL. Pseudo-Cushing’s syndrome is defined as the presence of some or all of clinical features of Cushing’s syndrome with some evidence for hypercortisolism [13]. The data in Table 2 describe the circadian rhythm of plasma ACTH and serum cortisol, plasma ACTH and serum cortisol after 0.5 mg DST and urinary cortisol levels.

To differentiate CD from pseudo-Cushing’s syndrome, we used the desmopressin test in patients with a positive response of DST. In this test, injection of desmopressin, a vasopressin analog, stimulates ACTH release in patients with CD but not in the majority of obese subjects. The test involves intravenous injection of 4 μg of desmopressin (DDAVP™, Kyowa Hakko, Tokyo, Japan) followed by collection of blood at 0 (pre-desmopressin), 15, 30, 60 and 90 minutes. A peak ACTH value of >1.5 times the basal value was defined as a positive desmopressin test.

The screening criteria of subCD are the following: 1) positive response of serum cortisol after 0.5 mg DST, 2) increased serum cortisol at midnight, and 3) positive response of serum cortisol in desmopressin test.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of the patients</th>
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</thead>
<tbody>
<tr>
<td>Males/Females</td>
<td>14/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.5 (44-64)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>32.6 (28.1-38.9)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>112.5 (98.3-123.9)</td>
</tr>
<tr>
<td>AHI (events/hour)</td>
<td>41.1 (28.6-66.7)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 (6.6-9.1)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126 (111-134)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 (64-86)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>76 (67-86)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>90</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>75</td>
</tr>
</tbody>
</table>

Data are median (1st and 3rd quartiles) values. AHI, Apnea Hypopnea Index; HbA1c, glycated hemoglobin; SBP, Systolic blood pressure; DBP, Diastolic blood pressure.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Endocrinological data of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Values</td>
</tr>
<tr>
<td>Morning serum cortisol (μg/dL)</td>
<td>11.5 (8.8-14.8)</td>
</tr>
<tr>
<td>Morning plasma ACTH (pg/mL)</td>
<td>35.5 (22.0-60.0)</td>
</tr>
<tr>
<td>Midnight serum cortisol (μg/dL)</td>
<td>2.7 (2.3-3.9)</td>
</tr>
<tr>
<td>Midnight plasma ACTH (pg/mL)</td>
<td>10.0 (5.5-13.5)</td>
</tr>
<tr>
<td>Post DST serum cortisol (μg/dL)</td>
<td>1.3 (0.4-3.3)</td>
</tr>
<tr>
<td>Post DST plasma ACTH (pg/mL)</td>
<td>1.0 (1.0-6.8)</td>
</tr>
<tr>
<td>Urinary free cortisol (μg/day)</td>
<td>45.2 (32.7-55.4)</td>
</tr>
</tbody>
</table>

Data are median (1st and 3rd quartiles) values.
mg DST is a prerequisite for this screening.

Patients with positive response of serum cortisol after 0.5 mg DST were treated with CPAP. The same test was repeated in patients with positive response of it within a few days after CPAP therapy.

**Hormonal assays**

Plasma ACTH was measured by electrochemiluminescence Immunoassay (ECLusys ACTH kit, Roche Diagnostics, Tokyo, Japan). Serum cortisol was measured by chemiluminescent enzyme immunoassay (Access cortisol kit, Beckman Coulter, Tokyo, Japan). This kit possess a sensitivity of 0.4 µg/dL, with an intra-assay CV less than 4.3 % and an inter-assay CV less than 5.9 %.

**Statistical analysis**

The measured variables are expressed as median values (1st and 3rd quartiles). The relationship between serum cortisol in 0.5 mg DST and each of the following parameters, including AHI, BMI, waist circumference, glycated hemoglobin (HbA1c), fasting plasma glucose, homeostasis model assessment for insulin resistance (HOMA-IR) and fasting serum cortisol was determined by calculating the Pearson correlation coefficient. Statistical analyses were performed using JMP 9.0.2 (SAS Institute Inc. Cary, NC). A \( p \) value <0.05 denoted the presence of statistically significant difference.

**Results**

**Screening of subCD in obese diabetic patients with OSAS**

Five (25%) patients showed positive response of serum cortisol after 0.5 mg DST (Fig. 1). These patients also underwent the desmopressin test and measurement of serum cortisol at midnight. None of the patients satisfied the diagnostic criteria of subCD; serum cortisol level at midnight was normal and the desmopressin test was normal.

Following these tests, CPAP therapy was started in these 5 patients. All five patients were tested again after 5 days (range, 2-8) of the commencement of CPAP therapy. Specifically, they underwent the 0.5 mg DST again. Three patients continued to use CPAP (CPAP group). The CPAP group showed improvement in the AHI (less than 5 events/hour) at the time of the second 0.5 mg DST. The other two patients have felt the difficulty in wearing the mask of CPAP since the start of this therapy and we could not evaluate the effect of CPAP therapy for about a week. Thus they were performed the second DST after 7-8 days of it. There was not any improvement of AHI for them at the second time of 0.5 mg DST (non CPAP group). Finally they stopped using CPAP. CPAP group but not non CPAP group showed normal response of cortisol after the second time of 0.5 mg DST (Fig. 2). CPAP group demonstrated a tendency to decrease in ACTH after the second time of 0.5 mg DST. Non CPAP group did not show such a response of ACTH after the second time of 0.5 mg DST (Fig. 2).

Non CPAP group were perfomed magnetic resonance imaging of the brain because of positive response of cortisol after the second time of 0.5 mg DST. We did not find any pituitary lesion in non CPAP group.

**Relationship between serum cortisol after DST and clinical parameters in obese diabetic patients with OSAS**

We investigated the relationship between various clinical parameters and serum cortisol after 0.5 mg DST in obese diabetic patients with OSAS.

Among the various clinical parameters measured before CPAP therapy, serum cortisol after 0.5 mg DST correlated only with fasting serum cortisol (Fig. 3),
Obese patients should be performed the screening tests to exclude subCD and subCS. In the present study, we selected the screening tests of subCD because there was no patient with abnormally low plasma ACTH. Twenty five percent of the patients showed positive response of serum cortisol after 0.5 mg DST, which should be the prerequisite for screening of subCD. Remarkably, only three, not all, of the patients who had positive response to first DST showed normal response to the second DST after CPAP therapy. These results suggest that activation of the HPA axis in obese diabetic patients with moderate to severe OSAS seems to induce a pseudo-Cushing’s state.

In agreement with our results, Carneiro, et al. showed that a blunted response of cortisol suppression after 0.25 mg DST in obese men with OSAS compared with obese male controls, which were recovered by CPAP therapy [21]. The previous reports including Carneiro’s study were that the effect of CPAP therapy for the HPA axis in OSAS patients was examined three months after CPAP therapy [11, 21-23]. On the other hand we performed 0.5 mg DST for them within a few

**Discussions**

Obese patients should be performed the screening tests to exclude subCD and subCS. In the present study, we selected the screening tests of subCD because there was no patient with abnormally low plasma ACTH. Twenty five percent of the patients showed positive response of serum cortisol after 0.5 mg DST, which should be the prerequisite for screening of subCD. Remarkably, only three, not all, of the patients who had positive response to first DST showed normal response to the second DST after CPAP therapy. These results suggest that activation of the HPA axis in obese diabetic patients with moderate to severe OSAS seems to induce a pseudo-Cushing’s state.

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days after improvement of AHI (less than 5 events/hour) in response to CPAP therapy. We also demonstrated that BMI, waist circumference, HbA1c, fasting plasma glucose and HOMA-IR were not found any relation with serum cortisol after DST. Our results indicated that CPAP therapy was more directly involved in the normalization of cortisol response after DST.

Serum cortisol after DST had strongly positive relation with fasting serum cortisol, but not urinary free cortisol and serum cortisol at midnight in the patients. This positive relationship may reflect the activation of HPA axis at night in OSAS patients. Only at night, stress stimuli such as upper airway constriction, progressive hypoxemia and sleep fragmentation may activate the HPA axis in OSAS patients.

Pseudo-Cushing’s syndrome should be differentiated from subCD and CD. Our study showed false positive response of serum cortisol after 0.5 mg DST in obese diabetic patients with untreated OSAS. Thus, OSAS, similar to alcoholism, depression, and massive obesity, may cause pseudo-Cushing’s syndrome. The results also showed that the desmopressin test and serum cortisol at midnight could be used to discriminate between pseudo-Cushing’s syndrome and subCD.

As a limitation, our study was a small sample size and lack of control group. Although further prospective studies in large number of patients are needed to confirm our findings, the results presented here indicate that OSAS should be considered in the differential diagnosis of pseudo-Cushing’s syndrome.

In conclusion, our findings demonstrate that OSAS cause pseudo-Cushing’s syndrome in obese patients with type 2 diabetes mellitus and that this syndrome can be reversed by CPAP therapy.

Disclosures

The authors have no personal financial or institutional interest in this article.

References