Ectopic ACTH syndrome caused by desmopressin-responsive thymic neuroendocrine tumor

Yoshihiro Sekiguchi¹¹, Yuki Miyamoto¹¹, Ichiro Kasahara², Yoshihito Hara¹¹, Yuji Tan³, Masaru Doi⁴ and Yukio Hirata⁵

¹¹Division of Endocrinology and Diabetology, Ohme Municipal General Hospital, Ohme 198-0042, Japan
²Division of Pathology, Ohme Municipal General Hospital, Ohme 198-0042, Japan
³Division of Endocrinology and Metabolism, Kitasato University School of Medicine, Sagamihara 252-0374, Japan
⁴Doi Medical Clinic, Kamamoto 861-5255, Japan
⁵Institute of Biomedical Research and Innovation Hospital, Kobe 650-0047, Japan

Abstract. A 32-year-old Chinese woman with rapid weight gain and progressive edema was found to have typical Cushingoid features. Her endocrine data were consistent with a diagnosis of ACTH-dependent Cushing’s syndrome. To differentiate ectopic ACTH syndrome (EAS) from Cushing’s disease (CD), various dynamic endocrine and imaging tests were performed. Her ACTH response was negative to corticotropin-releasing hormone (CRH) and positive to desmopressin. Magnetic resonance imaging of the pituitary showed no mass lesion. Computed tomography scan of the chest revealed a large mass (21 × 15 mm) in the anterior mediastinum, where positron emission tomography showed accumulation of [¹⁸F] fluorodeoxyglucose. Selective venous sampling showed marked step-up in ACTH level in the internal thoracic vein but not in the cavernous sinus after CRH stimulation. These data are compatible with the diagnosis of EAS. The resected tumor was pathologically consistent with thymic neuroendocrine tumor (NET) positive for ACTH by immunohistochemistry and abundant V1b receptor gene expression by RT-PCR. Postoperatively, her circulating ACTH/cortisol levels became normalized, and responded to stimulation with CRH but not with desmopressin. Her Cushingoid appearance gradually disappeared, and she was free from recurrence 5 years after surgery. This is a rare case of desmopressin-responsive EAS caused by thymic NET with predominant V1b gene expression, which was successfully localized by imaging modalities combined with selective venous sampling.

Key words: Ectopic ACTH syndrome, Thymic neuroendocrine tumor, Selective venous sampling, Desmopressin, V1b gene expression

CUSHING’S SYNDROME (CS) resulting from chronic exposure to excessive endogenous glucocorticoids consists of adrenocorticotropin (ACTH)-dependent and ACTH-independent CS. Among ACTH-dependent CS, the majority (80-90%) is due to ACTH-producing pituitary adenoma (Cushing’s disease: CD) [1], while 10-20% is nonpituitary tumors, termed ectopic ACTH syndrome (EAS) [2, 3]. The most common cause of EAS is small cell lung carcinoma (SCLC) (45%), followed by thymic (15%), bronchial (15%) and pancreatic (10%) carcinoid tumors, currently termed neuroendocrine tumors (NET) [4]. In contrast to EAS caused by SCLC with its rapid onset and progression, slowly-growing, small NET causing EAS is often difficult to distinguish from CD based on their clinical, endocrine and biochemical features [2, 5].

Here, we report a rare case of desmopressin-responsive EAS caused by thymic NET as localized by computed tomography (CT) and [¹⁸F] fluorodeoxyglucose-positron emission tomography (FDG-PET) scans as well as selective venous sampling (SVS). Postoperatively, desmopressin failed to elicit ACTH/cortisol increase, and the resected tumor tissue was found to have abundant gene expression of V1b receptor comparable to that of ACTH-producing pituitary adenomas.
Case Report

A 32-year-old Chinese woman with a rapid weight gain (18kg/month) and progressive facial and leg edema was admitted to our hospital for endocrine evaluation. She came from Taiwan, and her family and past history was noncontributory. She was 158cm tall, weighed 68kg; body mass index 27.3. Blood pressure was 154/93mmHg. Physical examination revealed typical Cushionoid features, such as moon face, central obesity, buffalo hump, purpura and skin atrophy and pitting edema of lower limbs, but no striae or skin pigmentation noted. Laboratory examination showed a profound hypokalemia (2.7mEq/L), but plasma glucose level (122mg/dL) and glycated hemoglobin (HbA1c: 4.8%) were normal. Endocrine study at baseline revealed an increased urinary free cortisol excretion (1181µg/day) and elevated levels of plasma ACTH (68.7pg/mL) and cortisol (39.2µg/dL), both of which lacked circadian rhythm (Table 1). Dynamic endocrine tests showed no suppression to low-dose (0.5mg) dexamethasone suppression test (DST) (Table 1). There were no responses of ACTH and cortisol after CRH stimulation, but exaggerated responses of ACTH and cortisol were observed after desmopressin (DDAVP)
stimulation (Fig. 1). These physical and endocrine data were compatible with the diagnosis of ACTH-dependent CS. To differentiate between CD and EAS, various diagnostic modalities were implemented.

No mass lesion in the pituitary was detected by magnetic resonance imaging (MRI) of the brain. While CT scan of the chest showed an enlarged mass (21 × 15

Table 1 Dynamic endocrine data

<table>
<thead>
<tr>
<th>Circadian rhythm and DST*</th>
<th>ACTH (pg/mL)</th>
<th>Cortisol (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td>68.7</td>
<td>39.2</td>
</tr>
<tr>
<td>16:00</td>
<td>91.5</td>
<td>34.7</td>
</tr>
<tr>
<td>23:00</td>
<td>159.8</td>
<td>43.3</td>
</tr>
<tr>
<td>8:00(clock)</td>
<td>118.7</td>
<td>35.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Selective venous sampling before and after (→) CRH stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (pg/mL)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*DST: dexamethasone suppression test, dexamethasone (0.5mg) was given orally at 23:00 the day before (C/P ratio): central to peripheral ratio

Fig. 1  CRH and DDAVP stimulation before and after surgery
(Left) Human CRH (100µg) and (right) DDAVP (4µg) were intravenously injected before and after surgery; plasma ACTH levels before (●) and after (○) surgery, and serum cortisol levels before (▲) and after (△) surgery, are shown.
mm) in anterior mediastinum, FDG-PET scan showed a marked uptake in the same anterior mediastinal mass (Fig. 2). To localize the source of ACTH secretion, selective venous sampling was performed (Table 1). Bilateral cavernous sinus sampling showed no ACTH gradient after CRH stimulation; central to peripheral (C/P) ratios of the right and the left cavernous sinus were 1.49 and 1.38, respectively. However, selective sampling of the right internal thoracic vein showed a marked step-up of ACTH (C/P ratio: 8.16), while that of the left internal thoracic vein showed no step-up of ACTH (C/P ratio: 1.42) (Table 1). Collectively, these data were compatible with the diagnosis of EAS caused by an anterior mediastinal tumor, possibly thymic tumor. She underwent complete resection of the mediastinal tumor.

Grossly, the resected tumor (2.5 × 1.5 cm size) was covered with a smooth thin fibrous capsule. Histopathologically, the tumor cells were composed of epithelioid cells with round nuclei containing irregular chromatin and mild eosinophilic cytoplasm, which were arranged in ribbon-like or follicle-like patterns (Fig. 3A), consistent with the diagnosis of thymic neuroendocrine tumor (NET):G1 (Ki67/MIB-1 index <1.0 %). Immunohistochemically, the tumor cells showed positive immunostainings for ACTH (Fig. 3B) and neuroendocrine markers, such as chromogranin A (Fig. 3C) and synaptophysin (Fig. 3D).

Total RNAs from the surgical tumor specimens were reverse transcribed for quantification of corticotrophin releasing hormone receptor 1 gene (CRHR1) and vasopressin receptor 1b gene (V1bR) with their respective synthetic PCR primers by real-time RT-PCR as described [6]; the relative mRNA expressions of CRHR1 and V1bR to that of glyceraldehyde-3-phosphate dehydrogenase, a house-keeping gene, in the thymic tumor and the pituitary tumors from patients with CD were calculated and compared. The thymic tumor tissue showed more abundant (about 1.7-fold) V1bR gene expression and far less (<0.001-fold) CRHR1 gene expression than those of pituitary tumors, respectively. These data are compatible with the diagnosis of ectopic ACTH-producing thymic NET with predominant expression of V1b receptor.

Postoperatively, plasma levels of both ACTH (<1.0pg/mL) and cortisol (0.9μg/dL) fell dramatically, which necessitated her for glucocorticoid replacement. Postoperative dynamic endocrine tests revealed low responses of ACTH/cortisol to CRH stimulation, but no responses to desmopressin stimulation (Fig. 1). Her Cushingoid features gradually disappeared, and she has been free from recurrence for 5 years after surgery.

Discussion

Thymic NET, formerly termed thymic carcinoid tumors, account for approximately 0.4% of all NETs [7]. Thymic NET may rarely be associated with CS due
Endocrine tests have been established and widely used [20, 21]. For example, CRH stimulation test had sensitivity (86%) and specificity (90%) and high-dose DST had sensitivity (65-100%) and specificity (60-100%), respectively [4]. Lack of ACTH and cortisol responses to CRH stimulation in the present case is not consistent with CD. Unfortunately, high-dose DST was not performed in the present case because of the potential risk of severe infection under the extremely high circulating cortisol level. Desmopressin, a long-acting vasopressin analog with selective V2 agonist, has been shown to cause ACTH secretion in patients with ACTH-dependent CS [4, 21], and desmopressin test is currently recommended as one of the screening tests for ACTH-dependent CS in Japan [20]. Previously, it was suggested that desmopressin test might be useful to aid the differential diagnosis of ACTH-dependent CS because desmopressin potently induced ACTH secretion in most patients with CD, but none or very few patients with EAS [21, 22]. However, recent accumulating lines of evidence [6, 20, 23, 24] reported that 15 out of 30 EAS patients (50%) showed positive ACTH response to desmopressin, suggesting ectopic ACTH production, currently coined as EAS. There have been only 11 cases of EAS caused by thymic NET thus far reported in Japan published in the English literature [8 - 17]. Thymic NET causing EAS occurs at any age (4-64 years, mostly between 20-40 year of age). The most common manifestations include Cushingoid features indistinguishable from CD, but hypertension, severe hypokalemia, and edema are more frequent than CD [4]. Thymic NET causing EAS usually grows very slowly with the average 3 year-interval from the onset of symptoms to clinical diagnosis [18], but it may take many years or even decades in some cases [19]. In contrast, our case rapidly (within 6 months) developed an overt hyperadrenocorticism as represented by typical Cushingoid features, progressive generalized edema, and severe hypokalemia, suggesting active ACTH production by the tumor.

Basal endocrine data with elevated plasma ACTH and increased serum and urinary cortisol levels, lack of their circadian rhythm, and resistance to low-dose DST in the present case are all consistent with ACTH-dependent CS. For differential diagnosis of ACTH-dependent CS between CD and EAS, various dynamic endocrine tests have been established and widely used [20, 21]. For example, CRH stimulation test had sensitivity (86%) and specificity (90%) and high-dose DST had sensitivity (65-100%) and specificity (60-100%), respectively [4]. Lack of ACTH and cortisol responses to CRH stimulation in the present case is not consistent with CD. Unfortunately, high-dose DST was not performed in the present case because of the potential risk of severe infection under the extremely high circulating cortisol level. Desmopressin, a long-acting vasopressin analog with selective V2 agonist, has been shown to cause ACTH secretion in patients with ACTH-dependent CS [4, 21], and desmopressin test is currently recommended as one of the screening tests for ACTH-dependent CS in Japan [20]. Previously, it was suggested that desmopressin test might be useful to aid the differential diagnosis of ACTH-dependent CS because desmopressin potently induced ACTH secretion in most patients with CD, but none or very few patients with EAS [21, 22]. However, recent accumulating lines of evidence [6, 20, 23, 24] reported that 15 out of 30 EAS patients (50%) showed positive ACTH response to desmopressin, suggesting ectopic ACTH production, currently coined as EAS. There have been only 11 cases of EAS caused by thymic NET thus far reported in Japan published in the English literature [8 - 17]. Thymic NET causing EAS occurs at any age (4-64 years, mostly between 20-40 year of age).
that ACTH response by desmopressin in EAS is not so infrequent as previously thought. The underlying mechanism of ACTH response by desmopressin could be accounted for by the amount of V1bR expressed by ACTH-producing tumors irrespective of pituitary or non-pituitary origin [6, 23, 25]. There have been only two cases of desmopressin-responsive EAS associated with thymic NET thus far reported in the English literature [26, 27].

It should be noted that Newell-Price et al. [28] first reported that combination of desmopressin and CRH may improve over CRH alone due to the greater response in CD patients than in EAS patients after combination test, suggesting its potential diagnostic utility. In fact, Tsagarakis et al. [23] have later shown that the area under the ROC curve (AUC) for the percent ACTH increase (218%) after combined CRH-desmopressin test was significantly greater than that after CRH test alone, suggesting that combined test may be useful, although limited with sensitivity (88%) and specificity (80%), for the differential diagnosis of ACTH-dependent CS. Thus further study using the combination test in larger population of ACTH-dependent CS, especially EAS patients, is needed to prove its diagnostic utility.

Our study using RT-PCR clearly showed that the thymic tumor abundantly expressed V1b receptor mRNA levels comparable to those of pituitary tumors causing CD, whereas the thymic tumor expressed CRH receptor mRNA levels far less than the pituitary tumors [6]. These data lend a strong support to the preoperative endocrine data in our case showing positive ACTH response to desmopressin and negative ACTH response to CRH. Furthermore, the postoperative endocrine data showing the disappearance of ACTH response to desmopressin along with the slow recovery of ACTH response to CRH also support to the contention. Such distinct ACTH responses to desmopressin and CRH observed in vivo casually related to the in vitro gene expression profiles of their receptors in the tumor as demonstrated in this study could deserve discussion even in a single case such as ours.

We have previously reported that only one case of SCLC out of 9 cases with EAS (11%) examined by FDG-PET scan had positive result [29]. The sensitivity of PET for detecting EAS has recently been reported as high as 64% [3]. Since FDG-PET is known to identify tumors with highly proliferative nature like SCLC [30], our case of thymic NET with low Ki67 (<1%) does not agree with such assumption. However, given the larger tumor volume associated with the rapid and excessive hormone production, it is reasonable to speculate that the thymic NET could possess highly metabolic activity to accumulate and utilize glucose within the tumor.

Selective venous sampling from cavernous sinus and/or inferior petrosal sinus have been recognized as the most accurate and reliable method to differentiate CD from EAS with high sensitivities (88-100%) and specificities (90-100%) [31, 32]; a distinct step up of ACTH with C/P ratio of ≥2 before and ≥3 after CRH stimulation makes the definite diagnosis of CD. The present case with C/P ratios in CS (right: 1.49, left: 1.38) after CRH stimulation excluded the source of ACTH secretion from the pituitary.

Selective venous sampling from right internal thoracic vein where thymic vein drains showed a distinct step-up (C/P ratio: 8.16), indicating the ectopic ACTH source from the anterior mediastinal tumor. It has been reported that selective venous sampling may be valuable in localizing the ectopic source of ACTH secretion in some cases, such as in thymic tumors [33]. Thus, the present case with a distinct step-up of ACTH in the effluent vein by selective venous sampling combined with the presence of an anterior mediastinal tumor detected by CT and FDG-PET scans made the definite localization of ectopic ACTH-producing tumor in the anterior mediastinum, possibly thymic NET.

Complete surgical resection is the best choice of therapeutic options for thymic NETs. However, chemotherapy and/or radiotherapy for thymic NET has been variable response rate (30-60%) [34], and local invasion and distant metastasis have been reported 50% and 20-30%, respectively [35]. The clinical behavior of thymic NET is often correlated with degree of histopathological differentiation: disease-free survivals for well, moderately, and poorly differentiated thymic NET have been 50, 20, and 0% at 5 years, respectively [36]. In a series of 74 cases studied by Wick et al., the thymic NETs associated with CS had higher 10-year mortality rate (65%) than those without endocrinopathy (29%) or those with multiple endocrine neoplasia (50%) [35]. Recently, Neary et al. have reported 12 EAS patients with thymic NET experienced at NIH over 25 years and compared meta-analysis [37]. The median age (21 years) and the median tumor size (5cm) at NIH cohort seem similar to those of the meta-analysis. All 12 patients underwent thymectomy; six recur
20-28 months after surgery and died at median of 57 months, and six were alive at median of 49 months. Thus, some patients of thymic NET with EAS were aggressive, especially in younger age, but others were more indolent. While the present case with well-differentiated thymic NET is free from the recurrence 5 years after removal of the tumor, a long-term and careful follow-up is needed.

In summary, we had a rare case of desmopressin-responsive EAS caused by thymic NET with the abundant V1b gene expression, whose localization was successfully made by CT and FDG-PET scans in combination with a selective venous sampling.

Acknowledgments

We thank Dr. Toshizumi Shirai, Division of Cardiovascular Surgery, Ohme Municipal General Hospital, for the surgery, and Dr. Shozo Yamada, Hypothalamic Pituitary Surgery Center, Toranomon Hospital, for the analysis of pituitary tumors.

Disclosure

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

References


