CASE REPORT

Feminizing Adrenocortical Carcinoma with Selective Suppression of Follicle-Stimulating Hormone Secretion and Disorganized Steroidogenesis: A Case Report and Literature Review

Takatoshi Saito, Katsuyoshi Tojo, Nozomu Furuta, Katsuhiko Ono, Hironobu Sasano and Kazunori Utsunomiya

Abstract

We report a 61-year-old male with gynecomastia, poor libido and erectile dysfunction. Endocrinological studies showed high levels of estradiol and dehydroepiandrosterone sulfate. Although luteinizing hormone (LH) level was within the normal limit, the concentration of follicle-stimulating hormone (FSH) was under the normal limit. Delayed response of LH and poor response of FSH to gonadotropin-releasing hormone administration were detected. Magnetic resonance imaging of the abdomen revealed a left adrenal tumor. Although the surgically-resected tumor was diagnosed as a high grade ACC based on Weiss’s criteria of adrenocortical malignancy, no metastasis was detected. Since estrogen levels normalized after resection, feminizing ACC was confirmed. While LH concentration increased slightly after operation, FSH level became transiently elevated over the normal limit, and finally reached the normal range. These data may suggest that FSH was suppressed selectively by hormone produced by ACC different from estrogen.

Key words: estrogen-producing tumor, hyperestrogenemia, feminizing adenocarcinoma


Introduction

Adrenocortical carcinoma (ACC) is a rare disease with a poor diagnosis (1-3). Most ACC patients are diagnosed at an advanced stage of disease (2). Although the best treatment for ACC is clearly surgical resection, approximately 5% of patients are judged to have unresectable tumors (3). Therefore, early diagnosis is very important and signs and symptoms of ACC should not be overlooked.

Endocrinological disorders such as hypersecretion due to excess cortisol or hyperandrogenemia are present in approximately 75% of patients with ACC (4). Several reports have described ACCs identified by clinical symptoms and/or laboratory abnormalities (1-4). However, not all ACC cases have apparent symptoms stemming from endocrinological disorders. Feminization due to hyperestrogenemia is very rare. While sex steroid hormones suppress secretion of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH), inhibin B is known to selectively suppress FSH. Some reports revealed that inhibin B was produced by ACC and suppressed FSH secretion (5, 6).

Gynecomastia is defined as benign proliferation of male breast glandular tissue and is usually caused by hyperestrogenemia, hypotestosteronemia or the use of medications that hamper hormone metabolism (7). In ACC patients, it is one of the feminizing signs induced by hyperestrogenemia. We recently encountered a case complaining of gynecomastia, poor libido and erectile dysfunction. He had been aware of these symptoms for more than two years and he had already been treated with methenolone-depot 100 mg once a week for two months at another hospital. We detected high levels of estradiol and dehydroepiandrosterone sulfate (DHEA-S), low testosterone and an adrenal tumor. Although luteinizing
hormone (LH) level was within the normal limit, the concentration of follicle-stimulating hormone (FSH) was under the normal limit. Delayed response of LH and poor response of FSH to gonadotropin-releasing hormone administration were detected. Pathological examination disclosed the high grade malignancy of this tumor. Both his symptoms and the abnormal laboratory data were promptly resolved after resection, indicating that the ACC had caused feminizing symptoms and signs via elevated estrogen. Since no metastasis was detected, this carcinoma was diagnosed as stage I. While LH concentration increased slightly after operation, FSH level was transiently elevated over the normal range, and finally decreased to gradually within the normal limit. These data may suggest that FSH was suppressed selectively by inhibin B.

### Table 1a. Endocrinological Data before and after Operation (Serum or Plasma)

<table>
<thead>
<tr>
<th></th>
<th>Pre 1.0, Post 5.1 (0.79-5.72)</th>
<th>1.7 (&lt;0.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/mL)</td>
<td>0.3, Post 6.2 (2.00-8.30)</td>
<td>&lt; 1.0 (5.4-16.7)</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>10.3 (7.2-63.3)</td>
<td>0.47 (2.01-7.5)</td>
</tr>
<tr>
<td>ACTH (pg/mL)</td>
<td>7.9 (3.58-12.78)</td>
<td>360 (24-244)</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>Estradiol (pg/mL)</td>
<td>7.04 (0.04-1.16)</td>
</tr>
<tr>
<td></td>
<td>Free testosterone (pg/mL)</td>
<td>19.3 (14.3-35.1)</td>
</tr>
<tr>
<td></td>
<td>&lt; 5.0 (&lt; 5.0)</td>
<td>7.9 (4.0-19.3)</td>
</tr>
<tr>
<td></td>
<td>Cortisol (μg/mL)</td>
<td>120 (29.9-159)</td>
</tr>
<tr>
<td>17-OH pregnenolone (ng/mL)</td>
<td>11.0 (0.1-4.0)</td>
<td>11-deoxy cortisol (ng/mL)</td>
</tr>
<tr>
<td>17-OH progesterone (ng/mL)</td>
<td>11.2 (0.5-2.9)</td>
<td>7.89 (1.54-3.91)</td>
</tr>
<tr>
<td>11-deoxycortisol (ng/mL)</td>
<td>11.8 (0.1-4.0)</td>
<td>11Oxy-17Ketogenic steroids (mg/day)</td>
</tr>
<tr>
<td>11Oxy-17Ketogenic steroids (mg/day)</td>
<td>6.63 (1.54-3.91)</td>
<td>11Oxy-17Ketogenic steroids (mg/day)</td>
</tr>
<tr>
<td>Estriol (μg/day)</td>
<td>476 (&lt; 15.0)</td>
<td>3.63 (0.2-2.1)</td>
</tr>
<tr>
<td>Estradiol (μg/day)</td>
<td>73.6 (&lt; 15.0)</td>
<td>11Deoxy-17Ketogenic steroids (mg/day)</td>
</tr>
</tbody>
</table>

### Table 1b. Endocrinological Data on Admission (Urine)

<table>
<thead>
<tr>
<th></th>
<th>528 (1.8-11.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnandiol (mg/day)</td>
<td>2.15 (0.16-0.79)</td>
</tr>
<tr>
<td>Pregnantriol (mg/day)</td>
<td>5.33 (0.13-1.60)</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (mg/day)</td>
<td>7.89 (0.12-5.20)</td>
</tr>
<tr>
<td>Estrone (μg/day)</td>
<td>118 (&lt; 15.0)</td>
</tr>
<tr>
<td>Estradiol (μg/day)</td>
<td>7.89 (1.54-3.91)</td>
</tr>
<tr>
<td>Estriol (μg/day)</td>
<td>11.8 (0.1-4.0)</td>
</tr>
</tbody>
</table>

Case Report

A 61-year-old male complaining of gynecomastia, poor libido and gradually worsening erectile dysfunction was referred to our unit. He had been aware of these symptoms for more than two years and had already been treated with methenolone-depot 100 mg once weekly for two months without endocrinological evaluation at another hospital. Although the gynecomastia transiently improved with this therapy, poor libido and erectile dysfunction were unchanged. Furthermore, the gynecomastia recurred after discontinuation of treatment. Laboratory data obtained in our hospital included a high level of estradiol and low levels of testosterone, free testosterone and follicle-stimulating hormone (FSH) (Table 1). He was admitted to our hospital for a thorough endocrinological evaluation.

His height was 166 cm and weight 71 kg. His blood pressure was 128/80 mmHg, pulse 70/min and regular. Painless gynecomastia was remarkable. Peripheral lymph nodes were not palpable. Neither neurological disorders nor weight loss was observed. Urinalysis and hematological tests showed no abnormalities. Routine blood chemistry data were all within normal limits. Basal endocrinological data are presented in Tables 1a, 1b and 1c. While basal ACTH level was slightly low, basal cortisol level was normal. Delayed response of luteinizing hormone (LH) and a poor response of FSH to gonadotropin-releasing hormone (GnRH) administration were detected (Table 2). Magnetic resonance imaging of the abdomen disclosed a left adrenal mass. Although it was difficult to definitely diagnose this mass as a feminizing adrenal tumor, we determined that it should be removed because malignancy was suspected based on its size. The patient underwent a complete adrenalectomy without complications. The excised adrenal tumor measured 50×40×40 mm and weighed 67 g. It was a well-encapsulated solid tumor. The adjacent normal adrenal tissue showed thinning, although atrophic change was not detected. The cut surface appeared yellow-brown with neither hemorrhage nor necrosis. Histologically, the tumor tissue consisted of round or elliptical tumor cells, as well as bizarre cells in some places. Mitotic figures were identified throughout the tumor. Ki67/MIB1 labeling indexes ranged from 6% to 7%, on average (data not shown). The histological findings met several of Weiss’s criteria for the histopathological diagnosis of ACC; the presence of capsular and sinusoidal invasion, abundant eosinophilic cytoplasm, nuclear atypia, atypical mitosis and diffuse
Table 1c. Endocrinological Data on Admission (Metabolic Pathway)

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Reference Range</th>
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<tbody>
<tr>
<td>Pregnenolone (ng/mL)</td>
<td>0.38 (0.1-1.0)</td>
</tr>
<tr>
<td>17-OH pregnenolone</td>
<td>11.8 (0.1-4.0)</td>
</tr>
<tr>
<td>DHEA (mg/day)</td>
<td>7.89 (0.12-5.20)</td>
</tr>
<tr>
<td>DHEA-S (ng/mL)</td>
<td>360 (24-244)</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>1.7 (&lt;0.7)</td>
</tr>
<tr>
<td>17-OH progesterone</td>
<td>11.2 (0.5-2.9)</td>
</tr>
<tr>
<td>Androstenedione (ng/mL)</td>
<td>0.91 (0.41-1.57)</td>
</tr>
<tr>
<td>Estrone (μg/day)</td>
<td>476 (&lt;15.0)</td>
</tr>
<tr>
<td>Estradiol (μg/day)</td>
<td>528 (1.8-11.0)</td>
</tr>
<tr>
<td>Deoxycorticosterone (ng/mL)</td>
<td>0.20 (0.08-0.28)</td>
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<tr>
<td>Testosterone (ng/mL)</td>
<td>7.04 (0.04-1.16)</td>
</tr>
<tr>
<td>Corticosterone (ng/mL)</td>
<td>2.06 (0.38-8.42)</td>
</tr>
<tr>
<td>Cortisol (μg/dL)</td>
<td>7.9 (4.0-19.3)</td>
</tr>
<tr>
<td>Aldosterone (pg/mL)</td>
<td>120 (29.9-159)</td>
</tr>
<tr>
<td>Cortisone (ng/mL)</td>
<td>2.06 (0.38-8.42)</td>
</tr>
</tbody>
</table>

Table 2. Gonadotropin-releasing Hormone Test (GnRH 100 μg)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/mL)</td>
<td>2.0</td>
<td>6.9</td>
<td>9.0</td>
<td>13.3</td>
<td>17.6</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

architecture pattern (Weiss’s score 6). Immunohistochemical analysis of steroidogenic enzymes showed the expressions of 3BHSD, CYP21, CYP17 and dehydroepiandrosterone sulfotransferase (DHEA-ST) in tumor cells, indicating disorganized steroidogenesis in this tumor (data not shown). Based on these data, ACC was diagnosed. In addition, as shown in Fig. 1, immunohistochemical study with #677 monoclonal antibody, specific monoclonal antibody against aromatase, showed clear staining, indicating feminization in the present case to be attributable to hypersecretion of estradiol from the ACC (8). To confirm CYP19A1 expression in the tumor, RT-PCR was performed. Total RNA was extracted using TRIzol Reagent (Invitrogen Corporation, San Diego, CA, USA) from the adrenal tumor. A QuantiTect Reverse Transcription Kit (QIAGEN GmbH, Hilden, Germany) was used for the synthesis of cDNA. PCR was carried out using the LightCycler System (Roche Diagnostics GmbH, Mannheim, Germany) and the FastStart DNA Master SYBR Green I (Roche Diagnostics GmbH), with ribosomal protein L 13a (RPL13A) as the internal standard. The primer sequences of CYP19A1 and RPL13A were as described previously (9). DNA bands were visualized after staining with ethidium bromide (Fig. 2). While CYP19A1 is barely detectable in the normal adrenal gland (lane 3), its expression was clearly visualized in the adrenal tumor from this case (lane 1).

Postoperatively, the estradiol concentration decreased rapidly and all symptoms gradually improved (Table 1a). While LH concentration increased slightly after operation, FSH level was transiently elevated over the normal limit (8.8 mIU/mL reference interval: 2.0-8.3), and finally reached the normal range. Although we recommended that he undergo adjuvant therapy with mitotane, he refused this treatment. Computed tomography (CT) findings of the chest and abdomen, physical symptoms and estradiol and testosterone levels were evaluated at 3 months after the operation. Eleven months later there is no evidence of recurrence. He now regularly comes to our outpatient unit every three months and has remained recurrence free, to date.

Discussion

Early diagnosis of ACC is very important because this disease generally has a poor prognosis. Clinical stage of the tumor at the time of diagnosis is a significant prognostic factor. For stages I through IV, the approximate 5-year survival has been reported at 15-45%, 12-57%, 5-18%, and 0%, respectively (10). Another report described that 10 of 11 patients with stage IV died within 2 years despite a better prognosis in cases with stage I and stage II disease (11). These findings suggest that the present case has a good prognosis.

In the present case, however, ACC had not been suspected at a previous hospital despite symptomatic endocrinological
disorders. Because ACC is a very rare entity and gynecomastia is generally due to factors other than feminizing ACC, the final diagnosis of ACC was delayed in this case. Overproduction of estradiol persisted for more than 2 years, indicating that a highly malignant ACC had proliferated for a long time. A previous study revealed that 12 of 52 cases with feminizing adrenal tumor were diagnosed more than two years after the onset (12). Medical histories of nine cases among them were described and all nine cases died within seven years. The observation that this case was diagnosed as ACC more than two years after the emergence of symptoms may indicate a poor prognosis.

A previous report described that the average size and weight of ACC that passed for more than two years from the onset was 145 mm (80-210 mm) and 900 g (200-2,100 g), respectively (12). Most ACCs in those cases had already metastasized at diagnosis. In comparison with those cases, the tumor of the present case was small and light and no metastatic tumor has been found. There may be something that affects the influence on growth and metastasis. Further investigation concerning growth and metastasis is necessary.

Morimoto et al described that patients with Ki67/MIB labeling index of 7% or more were associated with significantly shortened disease-free survival (13). McNicol et al also reported that patients with a Ki67/MIB labeling index of over 3% had significantly shortened disease-free survival (14). On the other hand, Stojadinovic et al showed no significant differences in disease-specific survival when 31 patients were stratified according to the cut-off value of Ki67/MIB labeling index 7% (15). It is difficult to precisely predict his prognosis from the value of Ki67/MIB labeling index 6-7%. However, we think that it is difficult to expect long-term disease-free survival in the present case.

Whether the functional status of the ACC influences the prognosis is controversial. Abiven et al showed a poor outcome of cortisol-secreting ACC (4). On the other hand, Luton et al described that 79% of 105 patients with ACC showed adrenal steroid hormone excess and the functional status of the ACC had no effect on prognosis (16). Further investigation regarding the prognosis is necessary.

Luton et al also showed that the steroids most commonly oversecreted were glucocorticoid: 34 patients (41% of patients) had a tumor secreting only glucocorticoid, 35 patients had tumors secreting both glucocorticoid and androgens, 8 patients had a tumor secreting only androgens, 1 patient had a tumor secreting only aldosterone, and 1 patient had tumors secreting glucocorticoid, estradiol and androgens. Since there was no case with only estrogen-secreting ACC, they described that estrogen-secreting ACC was rare. As shown in Table 3, only five cases have been reported in Japan to date (17).

Cortisol overproduction is often detected in cases with estrogen-producing tumors (17). No hypercortisolemia was observed, although the present case had high levels of 17-OH progesterone, 17-OH pregnenolone and 11-deoxy cortisol, all precursors of cortisol. As shown in Tables 1a and 1b, not only estrogen but also other steroid hormones were elevated, indicating disorganized steroid hormone production in this tumor (18, 19). Due to an incidentally low expression of CYPB11, we considered cortisol elevation to be absent.

Terzolo et al reported that adjuvant mitotane might prolong recurrence-free survival in patients with radically resected ACC (20). Although we strongly recommended the current patient to receive adjuvant therapy, he refused it because he was feared the side effects of mitotane. Each time he comes to our outpatient unit, we recommend adjuvant mitotane to him.

Estrogen-producing tumors produce two types of symptoms. One involves feminization such as gynecomastia, erectile dysfunction and/or poor libido, the other is focal signs such as lumbago, mass effect or a palpable tumor (4). While gynecomastia was an important clue to identifying ACC in the present case, he had no local signs. Because his tumor was not particularly large, it was thought not to have produced local signs.

Two cases with hypersecretion of not only estrogen but also inhibin B by ACC were reported (5, 6). These cases showed poor responses of FSH to GnRH and high serum concentrations of inhibin B. In addition, immunohistochemical study with anti-inhibin B antibody showed intense staining in the adrenocortical tumor cells. Although no investigation concerning inhibin B was carried out in the present case, immunohistochemical analysis of aromatase using #677 monoclonal antibody. Immunoreactivity of aromatase is detectable in tumor cells.
The authors state that they have no Conflict of Interest (COI).

**Table 3. Case Reports of Estrogen-secreting ACC in Japan**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Gynecomastia</th>
<th>Virilization</th>
<th>Cushing's Syndrome</th>
<th>Androstenedione/DHEA-S</th>
<th>Cortisol</th>
<th>Aromatase</th>
<th>Tumor Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37yr/M</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>ND/ND</td>
<td>ND</td>
<td>(+)</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>19yr/M</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>ND/ND</td>
<td>ND</td>
<td>(+)</td>
<td>1600</td>
</tr>
<tr>
<td>3</td>
<td>18mo/M</td>
<td>(+)</td>
<td>(+)</td>
<td>ND</td>
<td>ND/ND</td>
<td>ND</td>
<td>(+)</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>2yr10mo/F</td>
<td>(+)</td>
<td>(+)</td>
<td>ND</td>
<td>ND/ND</td>
<td>ND</td>
<td>(+)</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>26yr/M</td>
<td>(+)</td>
<td>(-)</td>
<td>ND</td>
<td>ND/ND</td>
<td>ND</td>
<td>(+)</td>
<td>330</td>
</tr>
<tr>
<td>6</td>
<td>61yr/M</td>
<td>(+)</td>
<td>(-)</td>
<td>ND</td>
<td>ND/ND</td>
<td>ND</td>
<td>(+)</td>
<td>67</td>
</tr>
</tbody>
</table>

Abbreviations: E1 (estrone), E2 (estradiol), DHEA-S (dehydroepiandrosterone sulfate), yr (years), mo (months), M (male), F (female), ND (not determined), ↑ (increase), → (normal)

The present case was included as case 6.

### References

22. Bouraima H, Lireux B, Mitre H, et al. Major hyperestrogenism in...

