Thyrotropin-Producing Pituitary Adenoma Discovered as a Pituitary Incidentaloma

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A 46-year-old woman with incidentally discovered thyrotropin (TSH)-producing pituitary adenoma showed endocrine data which was consistent with TSH-producing pituitary tumor. However, she showed only slight hyperthyroidism and the oversecretion and autonomous secretion of TSH from the tumor seemed to be limited from the results of several endocrine examinations. Immunohistochemical examination revealed that not only TSH- β and TSH- α but also prolactin and growth hormone synthesizing cells were present in the tumor tissue. Pituitary-transcription activator 1 (Pit-1) immunoreactivity was also detected in the adenoma cell nuclei. It was conceivable that the presented TSH-producing adenoma clinically located close to the non-functioning adenoma and Pit-1 may have played an important role in the multidirectional differentiation or development of this tumor. (Internal Medicine 34: 1055–1060, 1995)

Key words: Pit-1, immunohistochemistry

Introduction

Thyrotropin (TSH)-producing pituitary adenoma is relatively rare and constitutes about 1% of pituitary adenoma (1). The patients usually show symptoms of hyperthyroidism (sweating, palpitation, weight loss, and diffuse goiter), and are often misdiagnosed and treated as Graves' disease (2–4). The tumor is usually huge when discovered. However, since the method of measuring TSH has recently become very sensitive, pituitary tumor can be suspected when the serum TSH level is inappropriately high for the serum thyroid hormone levels even if it is not absolutely high (4). On the other hand, magnetic resonance imaging (MRI) examination has become popular and now the possibility of incidentally discovering pituitary tumor has increased. In adult autopsies the incidence of pituitary microadenoma has varied between 6 and 23% (1). In one series, the incidence of pituitary tumor with state-of-the-art MRI and gadolinium enhancement has been reported to be 10% (5). Thus, it is likely that pituitary incidentaloma is not rare. In the present paper we report a patient with incidentally discovered TSH-producing pituitary adenoma who showed only slight symptoms of hyperthyroidism and present results of the immunohistochemistry for several pituitary hormones and pituitary-transcription activator 1 (Pit-1) (6) in the resected tumor.

Materials and Methods

Hormone measurement

All hormone levels in serum or plasma were measured by commercially available radioimmunoassay kits except for the measurement of serum TSH- α subunit (TSH- α) level. Serum free triiodothyronine (fT3) and free thyroxine (fT4) levels were measured by AMERLEX-M free T3 kitTM and AMERLEX-M free T4 kitTM (Amersham International plc, Bucks, UK). Serum TSH level was measured by TSH•RIA BEADS IITM (Dainabot, Tokyo, Japan). Plasma growth hormone (GH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were measured with GH kit DaiichiTM, SPAK-S LH kitTM and SPAK-S FSH kitTM (Daiichi Radioisotope, Tokyo, Japan), respectively. Plasma prolactin (PRL) and adrenocorticotropin (ACTH) levels were measured using SPAK-S PRLTM (Daiichi Radioisotope) and ALLEGRO HS-ACTHTM (Nichols Institute, Sari Juan Capistrano, CA, USA), respectively, which are immunoradiometric assay systems. Reference ranges of serum or plasma hormone levels were as follows; fT3, 4.65–9.00 pmol/l; fT4, 10.9–27.7 pmol/l; TSH, 0.6–5.1 mU/l; TSH- α , 29.3–353.1 pmol/l; PRL, 1.4–14.6 μ g/l; GH, 0.66–3.68 μ g/l, LH in post menopausal phase, 8.7–38.0 IU/l; FSH in post menopausal phase, 26.2–113.3 IU/l; ACTH, 1.98–11.5 pmol/l.

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Case Report

The molar ratio of serum levels of TSH- α to TSH was calculated on the basis of the following molecular weight values: TSH, 28,000; and TSH- α , 14,700 (1 ng TSH corresponding to 3.226 μ U).

TSH receptor antibodies (TRAb) in serum were measured by radioreceptor assay using TSH Receptor Antibody KitTM (Incstar Co., Stillwater, MN, USA). Measuring of anti-T3 and anti-T4 antibodies in serum was previously described (7). Serum sex hormone binding globulin (SHBG) level was measured by DELFIA SHBG kitTM (Wallac, Turku, Finland), with a reference range of 18.6–117 nmol/l.

All studies were performed in accordance with the Helsinki Declaration.

Histological examination

The pituitary tumor specimen was examined light-microscopically and immunohistochemically. Hematoxyline-eosin and Azan stain were used for the light microscopic examination. The indirect immunoperoxidase method was applied for immunohistochemistry, using antisera specific to TSH (Histofine Anti-TSH AntibodyTM, Nichirei, Tokyo, Japan), TSH- α (provided by Dr. T. Yamaji), TSH- β subunit (TSH- β) (provided by National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD, USA), PRL (Histofine Anti-PRL AntibodyTM, Nichirei), GH (mouse anti-human growth hormoneTM, Zymed Laboratories Inc., San Francisco, CA, USA), LH (Histofine Anti-LH AntibodyTM, Nichirei), and FSH (Histofine Anti-FSH AntibodyTM, Nichirei). The cross-reactivity of antisera to TSH with LH and FSH was 0.1% and 0.2%, respectively. The production of the anti-human Pit-1 antibody and the details of the antibody were described elsewhere (8). Two-micron thick sections of paraffin embedded sections were stained for anti-TSH, anti-PRL and anti-GH antibodies. Four-micron thick ones were stained for anti-TSH- α , anti-TSH- β , anti-LH, anti-FSH, anti-ACTH and anti-Pit-1 antibodies.

Clinical and endocrine findings

A 46-year-old woman consulted Matsunami General Hospital complaining vertigo on February 20, 1994. She underwent hysterectomy and left ovariectomy six months earlier due to myoma uteri and the left ovarian cyst in a hospital. She showed no remarkable neurological findings on physical examination except for slight nystagmus on the right side in both eyes. The vertigo and nystagmus spontaneously disappeared without any medications in a week. On MRI of the head, however, a pituitary tumor with suprasellar extension and invasion to the left cavernous sinus was revealed (Fig. 1). No visual disturbance was seen on ophthalmological examination. On admission, the skin was moist and Achilles' tendon reflex was slightly accelerated, but no goiter, finger tremor or weight loss was found. Pulse rate was 84/min and blood pressure was 132/84 mmHg. On laboratory examination, blood count and biochemical data were unremarkable. The plasma glucose levels after oral administration of 75g glucose showed oxyhyperglycemic pattern, increasing from 5.16 mmol/l to a peak of 11.21 mmol/l at 30 minutes and returning to 6.72 mmol/l at 120 minutes. Serum fT3 and fT4 levels were slightly high or in the upper limit of the reference range, being 10.1–10.7 pmol/l and 27.4–27.7 pmol/l, respectively. Serum TSH level was, however, within the normal range (2.3–3.7 mU/l), which was an inappropriately high level for serum fT3 and fT4 levels. Serum SHBG level was slightly high, at 120 nmol/l. No diurnal rhythm was shown in serum TSH levels, being 2.2 mU/l at 0600, 2.0 at 0900, 2.0 at 2100, and 2.1 at 2300. Serum TSH- α level was high, at 642 to 925 pmol/l. The molar ratio of TSH- α to TSH in serum was high, being 24.5. Basal metabolic rate was normal, being 8% (reference range, –10 –10%) and ¹²³I uptake of thyroid gland for 24 hours was 20%. TRAb and TSBAb were negative, being 4.2% and 7.5%, respectively. Both of anti-T3 and anti-T4

Figure 1. Magnetic resonance imaging of the pituitary tumor. a) Pituitary tumor (large arrowheads) showing suprasellar extension was found in the sagittal image. However, the optic nerve (small arrowheads) was still intact. b) In the coronal image, invasion of the pituitary tumor to the left cavernous sinus (large arrowheads) was found.

antibodies were negative. Antithyroglobulin antibodies and antimicrosomal antibodies were also negative. Serum TSH level did not respond to intravenous administration of 500 μ g thyrotropin-releasing hormone (TRH test); basal level was 2.2 mU/l and peak one was 2.3. No paradoxical response of serum TSH level was shown after intravenous administration of 100 μ g gonadotropin-releasing hormone (GnRH test), 100 μ g GH-releasing hormone (GRH test) and 100 μ g corticotropin-releasing hormone (CRH test), respectively. By the oral consecutive administration of 75 μ g of triiodothyronine daily for 15 days (T3 suppression test), the serum TSH and TSH- α levels were incompletely suppressed (0.75 mU/l and 520 pmol/l in the final day, respectively) (Fig. 2). However, the molar ratio of serum level of TSH- α to TSH gradually increased during T3 suppression test. At the final day of the administration of T3, 123 I uptake of thyroid gland for 24 hours was suppressed to 6%. By a single oral administration of 2.5 mg bromocriptine, serum TSH and TSH- α levels slightly decreased from 2.9 mU/l to 1.8 at 12 hours and from 782 pmol/l to 566 at 12 hours, respectively. After a subcutaneous administration of 100 μ g octreotide acetate, serum TSH and TSH- α levels slightly decreased from 2.1 mU/l and 642 pmol/l to 1.7 at 3 hours and 524 at 12 hours, respectively.

Basal plasma levels of other pituitary hormones and their responses to hypothalamic hormones were within normal limits; PRL was 5.6 μ g/l and increased to 13 by TRH test; LH and FSH were 23 IU/l and 89 IU/l and increased to 83 and 200 in GnRH test, respectively; GH was 0.75 μ g/l and increased to 45 in GRH test; ACTH was 3.74 pmol/l and increased to 15.2 in CRH test. The increase of the plasma PRL level in GnRH, GRH

and CRH tests was equivocal. However, the plasma GH level paradoxically increased from 0.19 and 0.20 μ g/l to 3.0 and 4.4 in GnRH and CRH tests, respectively.

From these results, a TSH-producing pituitary tumor was strongly suspected and transsphenoidal hypophysectomy was performed on May 15, 1994. However, complete removal of the tumor was impossible due to tumor invasion to the cavernous sinus. A few days after the operation serum TSH, TSH- α , fT3 and fT4 levels transiently decreased to 0.76–0.87 mU/l, 471 pmol/l, 2.33 pmol/l, and 14.9 pmol/l, respectively. However, serum TSH and TSH- α levels returned to the pre-operative levels two weeks later. Serum fT3 and fT4 levels were 7.75 pmol/l and 17.0 pmol/l, respectively. The responses of plasma PRL, LH, FSH, GH and ACTH levels to the simultaneous venous administration of TRH, GnRH, GRH and CRH were normal as well as their basal levels. No response of serum TSH level was shown in TRH test. Serum level of SHBG was 68 nmol/l. The 123 I uptake of thyroid gland for 24 hours was 27% and basal metabolic rate was 1%. Moist skin ameliorated and the patient was discharged on July 15, 1994, and is being followed in the out patient clinic now.

Light microscopic and immunohistochemical findings

The resected pituitary tumor was pituitary adenoma constituted with chromophobe cells and a few acidophilic cells. Immunohistochemically, anti-TSH antibodies stained the majority of the tumor cells strongly (Fig. 3a). Anti-TSH- α antibodies (Fig. 4a) and anti-TSH- β antibodies (Fig. 4b) also stained these cells. Furthermore, tumor cells were stained with anti-PRL antibodies (Fig. 3a). Anti-GH antibodies stained the same

Figure 2. Suppressibility of thyrotropin (TSH) and α -subunit of TSH (TSH- α) by triiodothyronine (T3) administration (T3 suppression test). Serum TSH and TSH- α levels were incompletely suppressed by consecutive oral administration of 75 μ g of T3 daily for 15 days. The molar ratio of serum levels of TSH- α to TSH gradually increased. During this period serum free T3 levels increased to more than 23.3 pmol/l.

Figure 3. Immunohistochemical staining of 2 μ sections; a, b and c are serial sections of tumor tissue ($\times 400$). a) TSH immunostaining; b) PRL immunostaining; c) GH immunostaining. Cells have a tendency to have gland formation and each arrow indicates the same cell which was stained with anti-TSH, anti-PRL and anti-GH antibodies, respectively.

Figure 4. Immunohistochemical staining of 4 μ sections of tumor tissue ($\times 400$). a) Cytoplasm was stained with anti-TSH- α and b) anti-TSH- β antibodies, and c) nuclei were stained with anti-Pit-1 antibody.

cells faintly (Fig. 3b). Serial semithin sections revealed that the PRL-positive cells were consistently positive for TSH. A few cells were stained with anti-TSH, anti-PRL and anti-GH antibodies. However, there were no cells stained with anti-LH, anti-FSH and anti-ACTH antibodies. Anti-Pit-1 antibodies stained

cell nuclei of the adenoma cells (Fig. 4c).

Discussion

Ambiguous symptoms of hyperthyroidism in this patient

were supported by normal findings of ^{123}I uptake of thyroid gland and the basal metabolic rate. The oxyhyperglycemic pattern of plasma glucose level in glucose tolerance test and the slightly increased serum SHBG level were compatible with hyperthyroidism. The basal serum levels of fT_3 , fT_4 , and TSH were consistent with inappropriate secretion of TSH, which includes TSH-producing tumor and selective pituitary resistance to thyroid hormone action (4). The high TSH- α levels and high TSH- α to TSH molar ratios in this patient virtually excluded the diagnosis of the latter (4, 9). These results indicate that the pituitary tumor of this patient overproduces TSH, although hyperactivity and autonomy of the tumor is likely limited. The pathogenesis of hyperthyroidism in the setting of normal TSH levels in this patient is poorly understood, but biologic activity of TSH molecules may be increased as previously postulated (10) and reported (3).

The response of serum TSH levels to TRH is variable in patients with TSH-producing pituitary adenoma; about 60% of reported patients showed no response, which is similar to the present patient (4) and the absence of TRH receptor in such tumors was reported (11). Disappearance of diurnal rhythm of serum TSH level was also consistent with the previously reported patients with TSH-producing tumor (3, 12). The decreasing effect of a single administration of bromocriptine on serum TSH and TSH- α levels in this patient may be rather a minor (13), because a majority of the previous patients with TSH-producing tumor did not show a significant fall in serum TSH level (14). However, the reduction of serum TSH level by an acute challenge of somatostatin analog (octreotide acetate) in this patient was consistent with the previous reports (12, 15).

The serum TSH level has been reported to be partially suppressed by daily administration of T_3 in 25% of 40 reviewed patients with TSH-producing tumor (4), which was also seen in this patient, and was not suppressed in the remaining patients. It has been reported that the synthesis of both mRNA of α and β subunits of TSH in pituitary cells are suppressed by thyroid hormone. The degree of suppression is greater for TSH- β than for TSH- α (16). However, the increase of the ratio of TSH- α to TSH during T_3 suppression test in this patient indicated that the degree of suppression with T_3 was greater for TSH than for TSH- α . Accordingly, TSH- β seemed to be conversely more suppressed than TSH- α in this patient. We can postulate one possibility that the gene expression only in TSH- α -producing cells might have been unsuppressed by T_3 administration. In the immunohistochemical examination, however, we could not demonstrate clearly whether tumor cells producing TSH- α alone were present. As another possibility, the α -subunit of LH and FSH may have affected this phenomenon, because they were overproduced due to the postmenopausal state of this patient and were not suppressed by T_3 administration.

Simultaneous overproduction of PRL and/or GH and/or LH and FSH in TSH-producing pituitary adenoma has been reported (3, 17–19). According to the review of 161 TSH-producing pituitary tumors by Refetoff et al (4), 28 tumors cosecreted PRL, 22 cosecreted GH, and six cosecreted LH and/

or FSH. In the pituitary adenoma in the present patient, we did not examine the secretability of pituitary hormones in vitro. In *in vivo* studies, we had no evidence of overproduction of pituitary hormones other than TSH. However, we demonstrated a paradoxical increase of plasma GH level in GnRH and CRH tests and demonstrated the synthesis of not only TSH but also PRL and GH by immunohistochemistry. Furthermore, we demonstrated the co-synthesis of TSH, PRL and GH in the same cells as previously reported (13, 20). On the other hand, the most frequently occurring variant of plurihormonal adenomas produces GH, PRL and TSH (21), as similarly seen in the adenoma of this patient.

Pit-1 has been known as a pituitary-specific transcription factor that regulates the functional differentiation toward GH, PRL and TSH (22). In a few previous reports, Pit-1 immunoreactivity (19) or its mRNA (23–25) is positive only in adenoma cells producing GH, PRL and/or TSH, but not in the cells producing ACTH, FSH or LH. The results of the present patient are consistent with these reports. Sanno et al postulated from the results of their study about the expression of Pit-1 in TSH-producing adenoma (19) that Pit-1 may have a role in multidirectional differentiation or development of TSH-producing adenoma which may arise from a common progenitor cell. The results of the present case support their report.

Clinically nonfunctioning pituitary adenoma represent 30 to 40% of pituitary adenomas, and although not resulting in distinct endocrine syndromes, they frequently secrete glycoprotein hormones and/or their free subunit (26). Clinically, the TSH-producing pituitary adenoma of this patient was located close to the nonfunctioning adenoma because of its ambiguous symptoms, although it was histologically plurihormonal pituitary adenoma.

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