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Silent Somatotroph Adenoma, Detected by Catalyzed Signal Amplification and Non-radioisotopic *In situ* Hybridization

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RECENTLY the existence of silent corticotroph or somatotroph adenomas that were formerly included in clinically non-functioning adenomas has been elucidated. In this study a silent somatotroph adenoma in a patient with clinical symptoms of amenorrhea and galactorrhea was investigated by both immunohistochemistry with catalyzed signal amplification (CSA) and non-radioisotopic *in situ* hybridization (ISH), and its endocrinological and histopathological aspects were analyzed.

Case Report

A 25-year-old woman visited the outpatient clinic of Teikyo University Ichihara Hospital with a one-year history of amenorrhea and galactorrhea. Serum PRL was elevated, being 59 ng/ml (normal range for women: 1.4–14.6 ng/ml). The basal levels of the other pituitary hormones including GH were within normal limits. T1-weighted magnetic resonance imaging (MRI) revealed a small low signal intense mass within the sella turcica. The patient was diagnosed as having amenorrhea and galactorrhea caused by a PRL-producing pituitary microadenoma. Because of the intolerable side effect of bromocriptine, i.e., nausea and vomiting, the patient was followed up without any medical treatment. In the next year, MRI revealed enlargement of the tumor, and the patient was hospitalized for further endocrinological examination. Physical examination revealed no acromegalic features in the face, hands or feet.

Endocrinological findings

The basal level of serum GH varied from 2.1 to 15.1 ng/ml (normal range for women: 0.66–3.68 ng/ml). The serum IGF-1 was within normal limits, being from 180 to 220 ng/ml (100–315 ng/ml). Serum PRL was slightly high, ranging from 17 to 24 ng/ml, and showed normal response to TRH loading. Levels of ACTH, cortisol, TSH, FSH and LH were within normal limits, with normal response to the stimulation tests. An oral glucose tolerance test (OGTT) showed slight suppression of serum GH. TRH and GnRH provocation tests showed a paradoxical rise in serum GH (Fig. 1). The serum GH was not suppressed by 2.5 mg of bromocriptine, but showing irregular secretion. These results of endocrinological studies suggested the presence of a somatotroph adenoma in a patient with amenorrhea and galactorrhea, but no acromegaly. Transsphenoidal surgery was carried out, and the pituitary tumor was extensively removed. Postoperative endocrinological studies revealed a normalized basal level of serum GH, ranging from 0.83 to 2.62 ng/ml, and a normal level of IGF-1, being 160–210 ng/ml. The paradoxical response of

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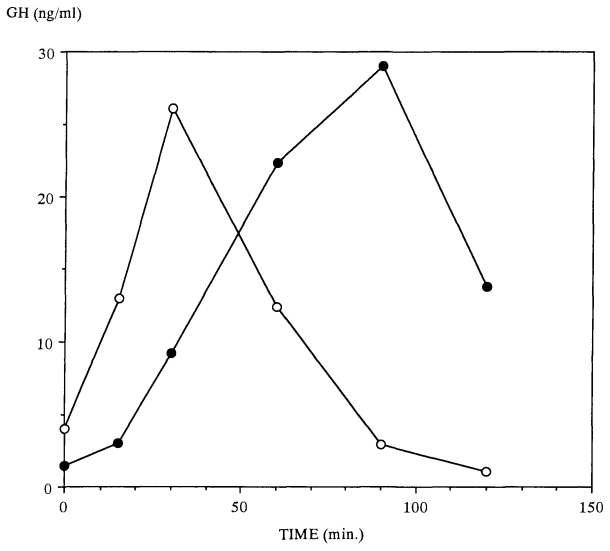

Fig. 1. After TRH stimulation, serum GH showed a paradoxical increase. GnRH provocation test also showed a paradoxical increase in serum GH. ○, TRH provocation test; ●, GnRH provocation test.

GH in the GnRH provocation test disappeared and the suppression of serum GH by 2.5 mg of bromocriptine was obtained.

Histopathological findings

Hematoxylin-eosin staining confirmed the tumor as a chromophobe pituitary adenoma (Fig. 2-a). Tumor specimens were immunostained by the indirect peroxidase method with antibodies to human pituitary hormones, supplied by the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Md., USA. In the whole sections, PRL-immunopositive cells were common (Fig. 2-b), whereas GH-immunopositive cells were not detected by this conventional indirect peroxidase method (Fig. 2-c). A novel amplified immunohistochemical method with CSA (DAKO, Carpinteria, Ca., USA) disclosed the diffuse immunopositivity for GH in the adenoma cells (Fig. 2-d). Non-radioisotopic ISH studies with biotinylated antisense oligonucleotide probes revealed diffuse expression of GH mRNA (Fig. 2-e). The expression of PRL mRNA and Pit-1 mRNA was also observed. These endocrinological and histopathological findings confirmed the adenoma as a silent somatotroph adenoma, presenting no clinical features of acromegaly.

Discussion

Only a few silent somatotroph adenomas have been reported in the literature [1–6]. Kovacs *et al.* reported three patients with galactorrhea and amenorrhea or irregular menstruation [3]. They speculated on the silence of the adenomas that the adenomas do not secrete GH in amounts needed to raise serum GH levels substantially and cause acromegaly. Yamada *et al.* also reported a patient with a large pituitary macroadenoma, slightly high serum GH levels, high serum IGF-1 levels, and abnormal GH dynamics, but no acromegaly [6]. They proposed the most likely explanation of the silence of the adenoma with high GH and IGF-1 levels to be that the duration of GH hypersecretion was too short for the development of acromegaly, which may take several years to become clinically manifest.

As shown in the present study, ISH can recognize the expression of the corresponding hormone mRNA, and can serve to make a precise diagnosis of the immunonegative adenomas or clinically silent adenomas. A novel amplified immunohistochemical method with CSA has recently been reported [7–9]. Sanno *et al.* stated that this system was 1,000 times as sensitive as the conventional indirect peroxidase method [10]. In the present adenoma, although GH was not immunostained by the conventional indirect peroxidase method, histopathological studies including immunohistochemistry with CSA and ISH studies, disclosed the immunoreactivity for GH and the expression of GH mRNA, and thus confirmed the adenoma as a silent somatotroph adenoma. Based on these histopathological findings, we speculate that a small amount of GH production in spite of frequent expression of GH mRNA could be a cause of silence, which may be attributed to some unknown translational abnormalities.

In our patient paradoxical increases in serum GH in TRH and GnRH provocation tests were observed. These abnormal endocrinological responses of GH in TRH and GnRH provocation tests, and in some cases OGTT, may be an important clue suggesting the presence of a silent somatotroph adenoma. Amenorrhea and galactorrhea are generally considered to be caused by PRL-producing adenomas. In the outpatients' clinics, endocrinological studies on GH dynamics are not

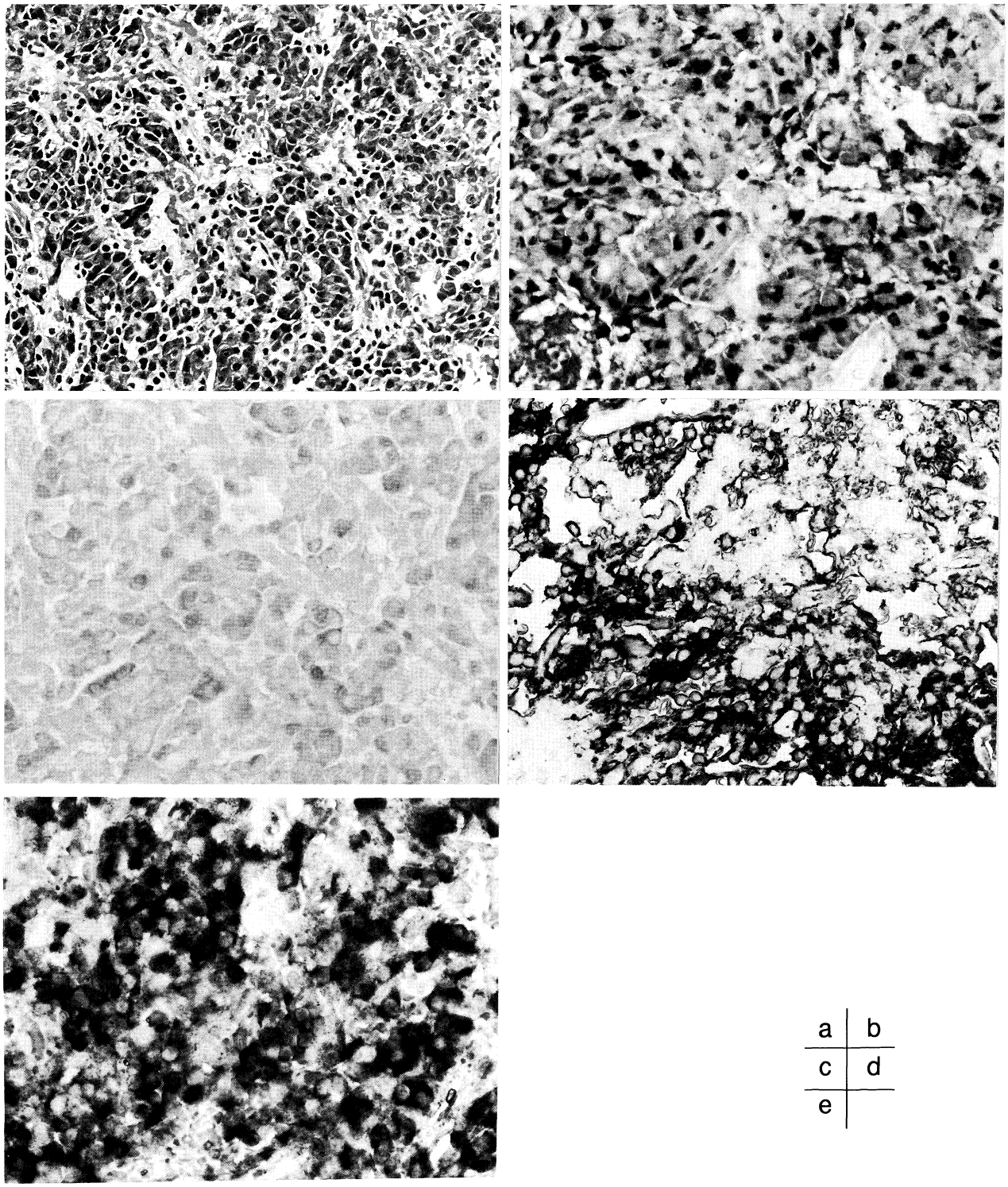

Fig. 2. Hematoxylin-eosin staining confirmed that the tumor was a chromophobe pituitary adenoma (a). In the whole sections, PRL-immunopositive cells were common (b), whereas GH-immunopositive cells were not observed by the conventional indirect peroxidase method (c). A novel amplified immunohistochemical method with CSA revealed diffuse immunopositivity for GH in the adenoma cells (d). Non-radioisotopic ISH studies revealed diffuse expression of GH mRNA (e) (Original magnification × 200).

examined for patients with amenorrhea and galactorrhea, and they are usually treated with bromocriptine. The present case study suggests that among the patients with amenorrhea and galactorrhea, those with a silent somatotroph adenoma are included, and that meticulous hormonal studies, especially endocrinological studies on GH dynamics, including TRH, GnRH provocation test or OGTT, should be recommended. If the above-mentioned endocrinological studies on GH dynamics indicate that the patient has a somatotroph adenoma, the tumor should be removed surgically and investigated histopathologically by the methods including immunohistochemistry with CSA and non-radioisotopic ISH.

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