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A Case of Macroprolactinoma with Subclinical Growth Hormone Production

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Abstract. We describe a rare case of macroprolactinoma with subclinically synchronous growth hormone (GH) production. A 59-year-old man with a giant adenoma in his pituitary had elevated serum prolactin (PRL) and insulin-like growth factor (IGF)-I levels, despite normal levels of basal GH. Serum GH levels were paradoxically increased in response to an intravenous administration of thyrotropin-releasing hormone (TRH). Prolonged exposure to glucose as a result of oral glucose tolerance testing (oGTT) failed to decrease GH levels. Two-week treatment with cabergoline, a dopamine D2 receptor agonist, decreased serum PRL and GH levels, and size of the tumor. Immunohistochemistry and *in situ* hybridization revealed PRL-producing cells capable of synchronous GH production. Acidophilic stem cell adenoma may be responsible for these phenomena. The nature of high proliferation and invasive tumor growth should be kept in mind when managing patients with this cell type of adenoma. IGF-I levels should be followed in PRLoma, even when basal GH levels are within the normal range, because mixed PRL- and GH-producing tumors would lie underneath. Further endocrinological examinations such as TRH test and oGTT are recommended when elevated IGF-I levels are detected.

Key words: Prolactin, Prolactinoma, Growth hormone, Acromegaly, Insulin-like growth factor I
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APPARENT pituitary adenomas are clinically multihormonal in 10–15% of cases [1]. Multihormonal adenomas may consist of one cell type secreting multiple hormones (monomorphous) or of several cell types, each secreting one hormone (plurimorphous) [2]. The most frequently occurring multihormonal adenomas co-secrete growth hormone (GH) and prolactin (PRL) [1, 2]. Thirty to 40% of individuals with acromegaly are hyperprolactinemic, but even so a case of apparent prolactinoma with GH mRNA expression is quite rare. We herewith describe the nature of a multihormonal pituitary adenoma with predominant PRL and subclinical GH production. In our case, serum PRL and insulin-like growth factor (IGF)-I levels were elevated, despite normal levels of basal GH. GH levels were paradoxically increased in response to thyrotropin-releasing hormone (TRH). Immunohistochemistry and *in situ* hybridization showed PRL-producing cells with synchronous GH production.

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Case report

Clinical summary

In October, 2000, a 59-year-old man was referred to our department for further evaluation of a giant pituitary adenoma. On admission, his height was 171 cm, and body weight 81 kg with body mass index of 28.0. Blood pressure was 108/80 mmHg. He did not complain of visual field defects, galactorrhoea, impotence, headache, or enlargement of hands and feet. He had no goiter or gynecomastia, and face was not typically acromegalic, i.e., with deep nasolabial furrows, thick lips, broad nose, or prominent supraorbital ridge. Heel pad thickness was 22 mm. Serum PRL (528 ng/ml; normal range: 3.4–16.2 ng/ml, enzyme immunoassay) and IGF-I levels (600 ng/ml; normal range: 59–215 ng/ml, immunoradiometric assay) were elevated, despite normal basal level of serum GH (1.9 ng/ml; normal range: <3.1 ng/ml, enzyme immunoassay). Serum calcium, phosphorus, and alkaline phosphatase were 8.9 mg/dl (normal range: 8.2–10.2 mg/dl), 3.7 mg/dl (normal range: 2.5–4.5 mg/dl), and 265 IU/l (normal range: 104–338 IU/l), respectively. As shown in Table 1, serum GH levels were paradoxically increased in response to intravenous administration of TRH. Both GH and PRL levels were enhanced in response to intravenous administration of GH-releasing hormone (GRH) (Table 1d). Prolonged exposure to glucose as a result of oral glucose tolerance testing (oGTT) failed to decrease GH levels (Table 1e). Both adrenocorticotropic hormone (ACTH) and cortisol levels were suppressed by dexamethasone (Table 1f). Magnetic resonance imaging (MRI) of the head revealed the presence of a pituitary macroadenoma enclosing bilateral carotid arteries (Fig. 1). No visual defects were detected by Goldmann perimetry. Cabergoline, a dopamine D2 receptor agonist, decreased both serum PRL and GH levels (Table 1g). A two-week treatment with cabergoline (1 mg/day) decreased serum PRL and GH levels (53.8 ng/ml and 0.5 ng/ml, respectively), and size of the tumor markedly. Under diagnosis of macroprolactinoma with GH production, transsphenoidal resection of the pituitary macroadenoma was performed. After operation, we continued to treat the residual tumor with cabergoline.

Pathological findings

Light microscopic findings

The resected tissue in the pituitary was fixed in 10% formalin for 8 h. Serial sections were prepared and stained with the hematoxylin-eosin (HE). HE stained sections revealed a papillary structure consisting of large cuboidal chromophobic cells with slightly pleomorphic nuclei (Fig. 2).

Immunohistochemistry

For immunohistochemistry, tissue sections were rinsed in 0.05 M phosphate-buffered saline (PBS). Sections were incubated in the following primary antibodies raised in rabbit: anti-GH, PRL (1:1,000, Dako, Carpinteria, CA) in PBS with 0.4% Triton X-100 at room temperature for 1 h, followed by 4°C for 24 h. After the incubation, the tissues were rinsed in PBS and incubated in biotinylated donkey anti-rabbit IgG (Histofine Kit, Nichirei, Tokyo, Japan) in PBS with 0.4% Triton X-100 for 20 min at room temperature. This was followed by another 20 min incubation at room temperature in avidin-biotin complex solution (Histofine Kit, Nichirei, Tokyo, Japan). The antibody-peroxidase complex was visualized with a mixture of 3,3-diaminobenzidine (0.2 mg/ml) and 3% H_2O_2 (0.83 μl/ml) in 0.05 M Tris buffer-saline solution. Immunohistochemistry on serial tissue sections showed tumor cells with both GH and PRL stained within Golgi areas (Fig. 3A and B).

In situ hybridization

GH or PRL complementary RNA (cRNA) probe was transcribed from the GH or PRL probe in the presence of ^{33}P-labeled UTP. The slides were exposed to the specific probe overnight in moist chambers at 42°C. After hybridization, the slides were washed in saline sodium citrate (SSC), and signals were detected with streptavidin-biotin-alkaline phosphatase using nitroblue tetrazolium-bromochloroindolyl phosphate (Dako). Control experiments were carried out using sense probes, which have a complementary sequence to the antisense probes (not shown). Most of tumor cells also showed GH and /or PRL mRNA by *in situ* hybridization (Fig. 4A and B).

Table 1. Endocrinological examinations

(a) TRH test

Time	0	30	60	90	120 (min)
TSH (μIU/ml)	3.2	17.9	16.6	9.8	9.5
PRL (ng/ml)	589.8	1948.1	1916.3	1558.7	1153.0
GH (ng/ml)	1.7	6.8	4.5	3.0	2.1

(b) LHRH test

Time	0	15	30	60	90	120 (min)
LH (mIU/ml)	3.8	33.0	45.4	42.7	34.6	29.4
FSH (mIU/ml)	4.7	10.0	14.4	16.0	17.4	15.6
GH (ng/ml)	2.3	2.2	2.1	2.2	1.9	2.1

(c) CRH test

Time	0	15	30	60	90	120 (min)
ACTH (pg/ml)	42	108	93	70	28	27
F (μg/dl)	14.3	18.4	19.7	19.1	14.4	11.4
GH (ng/ml)	1.6	1.5	1.2	1.2	1.2	1.3
PRL (ng/ml)	558.9	619.4	627.5	595.0	593.1	518.0

(d) GRH test

Time	0	15	30	60	90	120 (min)
GH (ng/ml)	1.7	2.3	3.4	3.8	2.5	1.8
PRL (ng/ml)	663.0	771.4	941.4	1098.4	825.9	760.5

(e) 75 g oGTT

Time	0	30	60	90	120 (min)
BG (mg/dl)	100	154	146	114	114
GH (ng/ml)	1.1	1.4	1.1	1.4	1.3

(f) Dex test

Dose (mg)	0	1	8
ACTH (pg/ml)	40	6	<5
F (μg/dl)	8.1	0.4	<0.2

(g) Cabergoline test

Time	0	1	3	6	12 (h)
PRL (ng/ml)	568.6	632.1	601.6	445.2	346.1
GH (ng/ml)	1.3	1.4	1.5	1.0	1.3

Time	1	2	2	4 (day)
PRL (ng/ml)	288.1	249.9	293.7	350.6
GH (ng/ml)	1.1	1.0	1.2	1.3

(a) TRH test (TRH 500 μg, intravenous bolus), (b) LHRH test (LHRH 100 μg, intravenous bolus), (c) CRH test (CRH 100 μg, intravenous bolus), (d) GRH test (GRH 100 μg, intravenous bolus), (e) oGTT (oral glucose tolerance test; 75 g, per os), (f) Dex test (dexamethasone per os at 11:00 pm), (g) Cabergoline test (cabergoline 1 mg, per os)
Normal basal ranges: TSH (0.38–3.64 μIU/ml); PRL (3.4–16.2 ng/ml); GH (<3.1 ng/ml); LH (0.3–7.1 mIU/ml); FSH (1.6–10.6 mIU/ml); ACTH (<60 pg/ml); F (cortisol, 4.5–21.1 μg/dl); BG (blood glucose, 70–110 mg/dl).

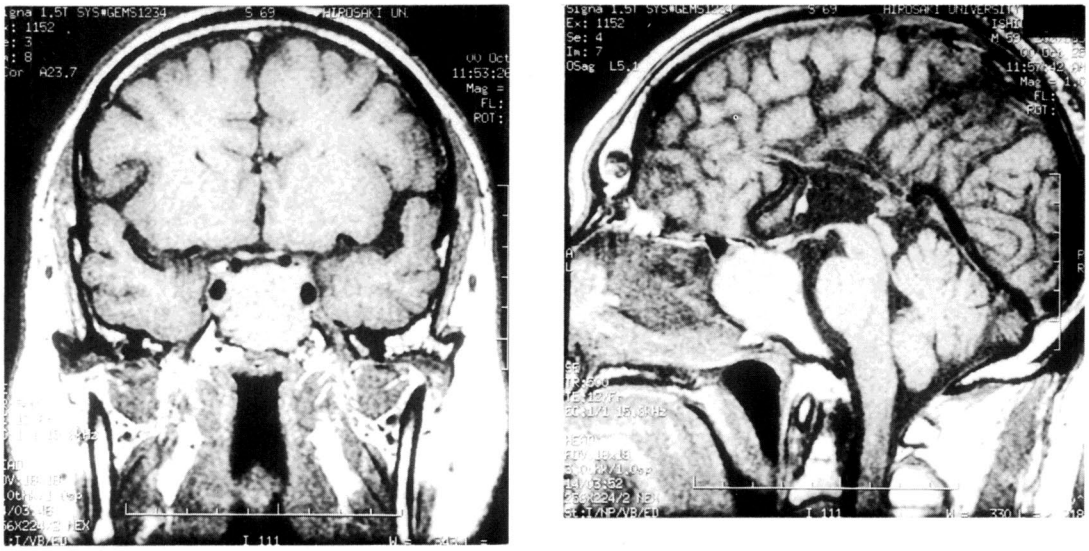


Fig. 1. Magnetic resonance imaging of the head. Coronal and sagittal T1-weighted images enhanced with gadolinium diethylenetriamine penta-acetic acid demonstrate the presence of macroadenoma in the pituitary.

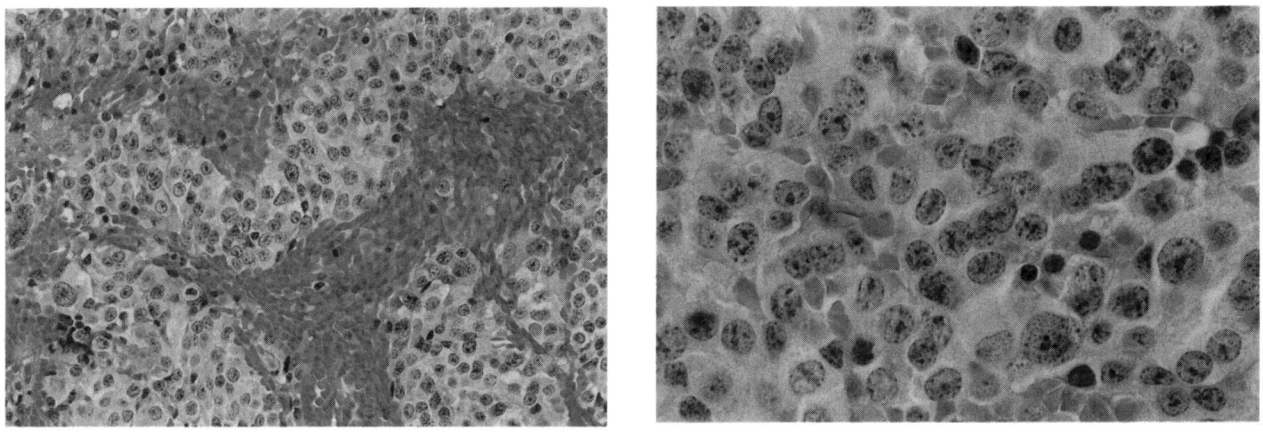
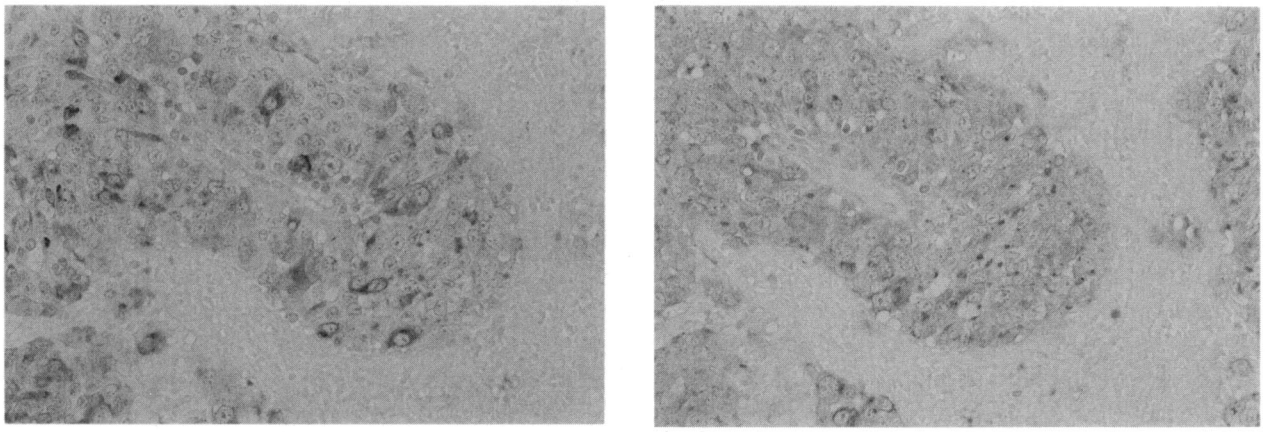


Fig. 2. Hematoxylin-eosin stain showing a large chromophobic cell type of pituitary adenoma. (Original magnification ×400 (left panel) and ×1000 (right panel).)

Discussion

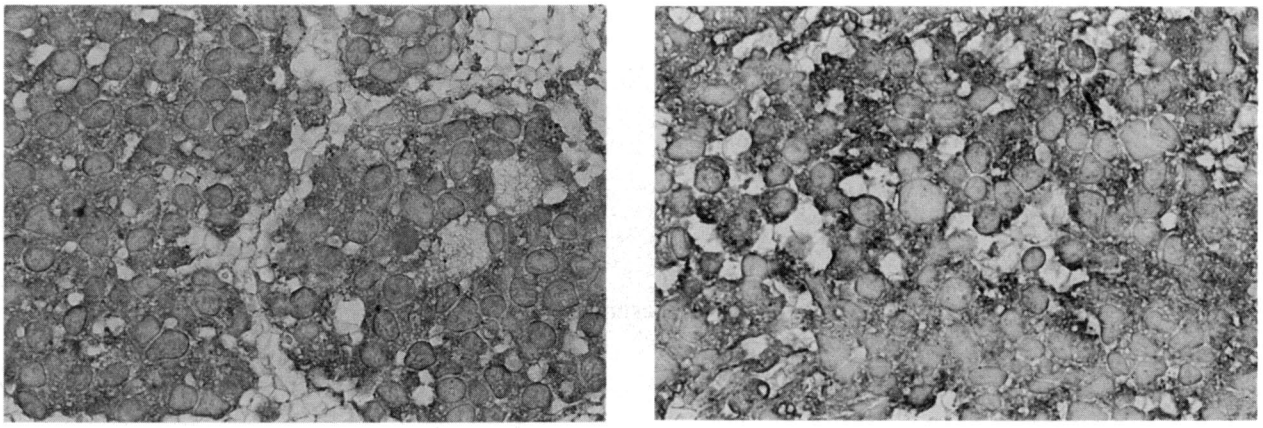
Our patient was suspected of having a GH-producing tumor co-existing with a PRL-producing one because of concomitant elevation of both serum IGF-I and PRL levels. Serum GH levels were paradoxically increased in response to TRH, and were not suppressed by prolonged exposure to glucose, further suggesting the presence of a GH-producing tumor. This hypothesis was confirmed by both immunohistochemistry and *in situ* hybridization.

A multihormonal pituitary adenoma is found when more than two pituitary hormones are elevated. In 1978, Tolis *et al.* reported a case of acromegaly and hyperprolactinemia due to both GH- and PRL-secreting pituitary adenomas [3]. In approximately 30–40% of patients with GH-producing adenomas, serum PRL levels are elevated, mostly due to two possible mechanisms: 1) hypothalamic-pituitary disconnection, resulting in attenuation of PRL inhibitory factor, or 2) either monomorphous or plurimorphous tumors capable of producing both GH and PRL [4, 5]. In fact, both GH and PRL mRNA were detected in 28 out of 59 GH- and/or PRL-



(A) (B)

Fig. 3. (A) Immunological staining for GH. GH-positive cells are visualized by the brown precipitates. The data demonstrate that 43% of tumor cells are GH-positive. (Original magnification ×400.)
(B) Immunological staining for PRL. PRL-positive cells by the brown precipitates are considered as the same cells as GH-positive on serial tissue sections. (Original magnification ×400.)



(A) (B)

Fig. 4. Localization of GH or PRL mRNA in the pituitary adenoma.
(A) A representative photomicrograph showing tumor cells probed with antisense probe for GH mRNA. GH mRNA positive signals were found in many of the tumor cells. (Original magnification ×200.)
(B) A representative photomicrograph showing tumor cells probed with antisense probe for PRL mRNA. PRL mRNA positive signals were found in many of the tumor cells close to GH mRNA positive cells. (Original magnification ×200.)

producing adenomas by *in situ* hybridization [6]. Even among GH- and PRL-coproducing adenomas, it is quite rare that PRL is predominantly produced, while GH is subclinically secreted, as found in our case.

The tumor consisted of one cell type that produces both GH and PRL, and it was assumed to have originated in the common precursor of somatotrophs and lactotrophs. The occurrence of such precursor cell adenomas that produce GH and PRL has also been described [7, 8]. Acidophilic stem cell adenomas, which are chromophobic or slightly acidophilic tumors with no periodic-acid-Schiff (PAS) positivity, share characteristics with both sparsely-granulated somatotrophs and lactotrophs, as classified by the Kovacs group [6, 9]. The presence of acidophilic stem cell adenomas is accompanied by hyperprolactinemia, and serum GH levels are elevated or within

the normal range [9]. These facts agree with our findings in this case. The nature of high proliferation and invasive tumor growth should be kept in mind in managing patients with this cell type of adenoma. GH- or TSH-secreting adenomas are often accompanied with multihormonal production of GH, PRL, and TSH. Pituitary specific transcription factor (Pit-1) is frequently co-expressed with GH, PRL, and TSH-producing tumors [10], and may contribute to the functional differentiation toward these tumor cells from the common precursor. Alternatively, it seems possible that the tumor has transformed morphologically because some fetal somatotrophs differentiate into mammosomatotrophs and then into mammototrophs [11]. This transformation could account for some cases of acromegaly without GH elevation but with increased PRL [12].

Serum IGF-I levels were elevated in our patient, although basal GH levels were not. It is probable that GH secretion during the night was enough to stimulate increased IGF-I levels, even within the normal ranges of the basal levels. It is also possible that IGF-I secretagogues other than GH, such as hypernutrition and hyperinsulinemia, would enhance IGF-I production [13].

In our case of a monomorphous adenoma, PRL production was predominant and GH production subclinical; basal GH level was within the normal range. Only a few cases of acromegaly with normal GH levels are reported [14, 15]. In such cases, IGF-I might clinically cause the acromegalic features. However, our patient did not present with acromegalic features, although IGF-I was elevated. Both GH and IGF-I act either independently or synergistically to induce skeletal and organ growth. In our patient, therefore, the magnitude or the duration of elevated serum IGF-I levels may have not been enough to cause the typical acromegalic features without apparent GH oversecretion. It is also known that both total and free IGF-I levels do not correlate with the clinical activity of acromegaly [16].

In summary, we report a rare case of a multihormonal pituitary adenoma, with predominant PRL and subclinical GH production. An acidophilic stem cell adenoma might be responsible for their production. IGF-I levels should be followed in PRLoma, even within the normal range of basal GH levels, because mixed PRL- and GH-producing tumor would lie underneath. Further endocrinological examinations such as TRH test and oGTT are recommended when elevated IGF-I levels are detected.

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