Clinically silent hypersecretion of growth hormone in patients with pituitary tumors

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Hypersecretion of growth hormone (GH) was found in three women aged 25 to 35 years old, with somatotroph adenomas without clinical stigmata of acromegaly. The patients had previously been diagnosed as having nonfunctioning pituitary macroadenomas, with extrasellar extension. Concentrations of GH were elevated preoperatively in all subjects and could not be suppressed during oral glucose tolerance testing. Somatomedin-C concentrations were elevated in two patients. Immunocytochemical studies of surgically obtained tumor tissue demonstrated sparse positive staining for GH in all subjects. Gel-chromatographic analysis of serum and tumor tissue samples demonstrated that the immunoactive GH was authentic GH. On pathological examination, the tumor was cellular in all cases, consisting of partly acidophilic and partly chromophobic cells. Electron microscopic analysis of one tumor showed a cell composition not previously described. These studies further characterize GH hypersecretion in a subset of patients with clinically nonfunctioning pituitary macroadenomas.

KEY WORDS • pituitary adenoma • growth hormone • chromophobe adenoma

GROWTH hormone (GH)-secreting pituitary adenomas characteristically cause clinical acromegaly. It is important to diagnose hypersecretion of GH since sustained excess of GH has long-term deleterious cardiovascular and metabolic effects including hypertension, median neuropathy, glucose intolerance, and hyperlipemia. The endocrine evaluation of patients with pituitary tumors is usually dependent upon their clinical presentation; approximately 25% of patients with pituitary adenomas are diagnosed as having nonfunctioning tumors. Although an immunocytochemical study noted GH staining in pituitary tumor tissue obtained from patients without acromegaly, the significance of this was unknown, since GH hypersecretion, abnormal GH dynamics, and somatomedin-C levels were not investigated. Tourniaire, et al., recently reported two women who presented with galactorrhea and were found to have pituitary tumors with pathological features typical of somatotroph adenomas. We report the cases of three young women with somatotroph macroadenomas without clinical acromegaly, who initially presented with visual loss, galactorrhea, or fatigue. They were found to have GH hypersecretion, elevated somatomedin-C concentrations and, in one patient, a response to bromocriptine.

Clinical Material and Methods

Subjects

Case 1. This 25-year-old woman first presented in 1979 with bitemporal visual field loss and was found to have a pituitary macroadenoma with suprasellar extension. She had a history of normal menstrual periods and denied galactorrhea. Her physical examination was otherwise normal and her only endocrine abnormality was a minimally elevated serum prolactin (PRL) level of 23 ng/ml. She underwent transphenoidal resection of a presumed nonfunctioning adenoma. She was readmitted in 1983 with a right sinus mass which was biopsied and diagnosed as a pituitary tumor. Tumor immunocytochemical tests were positive for GH and PRL. Although there were no clinical stigmata of acromegaly, tumor immunocytochemical tests demonstrated adenoma cells staining positive for GH. Studies for GH were therefore performed as outlined in Table 1. The patient underwent subtotal transphenoidal resection of the recurrent intra- and suprasellar pituitary.
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**TABLE 1**

<table>
<thead>
<tr>
<th>Case No. &amp; Time of Test</th>
<th>Fasting GH (ng/ml)</th>
<th>Somatomedin-C (U/ml)</th>
<th>GH (ng/ml) on OGTT</th>
<th>GH (ng/ml) on ITT</th>
<th>GH (ng/ml) on TRH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 Min</td>
<td>30 Min</td>
<td>60 Min</td>
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<tr>
<td>Case 1 preop</td>
<td>36</td>
<td>7.9</td>
<td>18</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Case 1 postop (1 mo)</td>
<td>18</td>
<td>3.7</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Case 2 preop</td>
<td>24</td>
<td>2.6</td>
<td>19</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Case 2 postop (1 mo)</td>
<td>7</td>
<td>1.2</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Case 3 preop</td>
<td>8</td>
<td>—</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Case 3 postop (1 mo)</td>
<td>7</td>
<td>0.53</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

* GH = growth hormone; OGTT = oral glucose tolerance testing; ITT = insulin tolerance testing; TRH = thyrotropin-releasing hormone study; — = study not performed.

adenoma. She received a postoperative course of 4500 rads of conventional radiation directed to the pituitary.

**Case 2.** This 35-year-old woman presented in 1983 with a 6-month history of fatigue. She had a normal reproductive history with four pregnancies; her last delivery was in 1981. Physical examination was remarkable for bilateral galactorrhea, and she was found to have a minimally elevated serum PRL level of 19 ng/ml. A computerized tomography (CT) scan revealed a pituitary macroadenoma with suprasellar extension, and a "nonfunctioning pituitary adenoma" was diagnosed. She underwent transsphenoidal resection of a pituitary adenoma with positive tumor immunocytochemical testing for GH and PRL. The endocrine test results are shown in Table 1. She received a postoperative course of 4500 rads of conventional radiation directed to the pituitary.

**Case 3.** This 29-year-old woman presented in 1983 with a 1-year history of galactorrhea, irregular menstrual periods, and fatigue. Physical examination was remarkable only for galactorrhea. The serum PRL level was somewhat elevated at 43 ng/ml, and a CT scan documented a macroadenoma with suprasellar extension. She underwent transsphenoidal resection of a diagnosed "nonfunctioning adenoma," and immunocytochemical testing of the tumor was positive for GH and PRL. The results of the endocrine evaluation are shown in Table 1.

**Study Methods**

**Assays.** Growth hormone levels in serum and tumor lysate were measured by radioimmunoassay with H5840-A as the standard. Serum and tumor lysate concentrations of PRL were measured using previously described methods. Somatomedin-C concentrations were determined by radioimmunoassay.

**Tumor Tissue Analysis.** Pituitary tumor tissue obtained at transsphenoidal adenomectomy was immediately placed in normal saline at 4°C, minced into approximately 1-cu mm fragments, and washed repeatedly. After the final wash and centrifugation, the tissue pellet was resuspended in distilled water and sequentially frozen and thawed five times. Cellular debris was then pelleted by centrifugation at 800 G for 10 minutes. The solubilized cellular contents in the supernatant were decanted and stored at -20°C before radioimmunoassay and determination of the protein concentration using the method of Lowry, et al.15

**Gel-Chromatography.** Two-milliliter aliquots of serum or samples of approximately 40 ng GH from the pituitary tumor lysate from Case 1 were analyzed by chromatography on a Sephadex G-100 column.

**Morphological Techniques.** Formalin-fixed paraffin-embedded sections were stained with hematoxylin and eosin (H & E) and periodic acid-Schiff. Tests for the presence of adenohypophysial hormones were performed by previously described immunoperoxidase techniques on tumor tissue obtained during transsphenoidal surgery. Tumor tissue was analyzed using specific antibodies to PRL, GH, alpha subunit, thyroid-stimulating hormone (TSH-α), luteinizing hormone (LH-β), follicle-stimulating hormone (FSH-β), adrenocorticotropic hormone (ACTH), and β-endorphin. Glutaraldehyde-fixed osmicated epoxy resin embedded tissue was used for ultrastructural study. Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a Philips 300 electron microscope.

**Results**

**Endocrine Studies**

None of the patients had clinical signs of acromegaly. There was no history of acral changes as assessed by serial photographs and shoe or ring size. All three patients were normotensive without acromegalic stigmata. Heel pad thickness in Cases 1, 2, and 3 was 19, 22, and 12 mm, respectively. Cases 2 and 3 had evidence of preoperative impaired oral glucose tolerance
testing (OGTT) results, with mean glucose levels of 223 and 206 mg/dl at 90 and 30 minutes, respectively. Postoperative OGTT was normal in Cases 1 and 2 but remained abnormal in Case 3 (glucose levels of 236 and 243 mg/dl at 60 and 90 minutes, respectively). Fasting calcium and phosphorus concentrations were normal.

All three patients were clinically euthyroid, and their serum thyroxine concentrations were normal. Thyrotropin-releasing hormone (TRH) was administered (200 μg intravenously) to assess the TSH and PRL reserve. The TSH reserve was normal in Cases 1 and 3 (peak TSH concentrations of 7.9 and 6.9 μU/ml, respectively) and slightly blunted in Case 2 with a peak TSH concentration of 4.5 μU/ml (normal peak TSH response 5.5 to 18 μU/ml). All of the patients had normal ACTH reserve studies as determined by insulin tolerance testing (0.15 U crystalline zinc insulin/kg).

Serum PRL concentrations were increased preoperatively in all three patients at 23, 19, and 43 ng/ml, respectively (normal < 15 ng/ml), and were unresponsive (less than twofold rise) to TRH stimulation (200 μg intravenously). Serum PRL levels were normal (3 ng/ml) postoperatively in Case 1 after the initial operation in 1979, but remained slightly elevated at 24 ng/ml following the second operation. The postoperative serum PRL concentrations were normal at 10 ng/ml in both Cases 2 and 3.

**Growth Hormone Studies**

The results of the basal, stimulatory, and suppressive tests for GH secretion are shown in Table 1. Fasting preoperative serum GH concentrations in Cases 1, 2, and 3 were 36, 24, and 8 ng/ml, respectively, and failed to show normal suppression during OGTT. Postoperatively, Cases 1 and 2 had persistently abnormal GH suppressibility.

Cases 1 and 3 demonstrated a rise in GH during preoperative insulin tolerance testing from 18 to 40 ng/ml and 6 to 41 ng/ml, respectively. The preoperative GH of 24 ng/ml in Case 2 was unresponsive to hypoglycemia. All three patients had adequate hypoglycemia with glucose concentrations of less than 30 mg%. Postoperatively, GH concentrations in Case 2 decreased from 24 to 4 ng/ml and exhibited a rise to 19 ng/ml during insulin tolerance testing.

None of the patients demonstrated a rise in serum GH level following TRH (200 μg) administration. Serum samples obtained at a TRH study in Case 1 in 1979 and frozen at -4°C revealed a basal GH level of 17 ng/ml which was unresponsive to TRH stimulation (18 and 16 ng/ml at 30 and 60 minutes, respectively). Fasting serum GH determinations were obtained at hourly intervals for 8 hours postoperatively in Case 1 prior to and during administration of bromocriptine (10 mg/day). Mean GH concentrations were 14.3 ± 2.5 ng/ml (± standard deviation) prior to bromocriptine administration. During bromocriptine therapy, mean GH concentrations decreased to 4.9 ± 2.3 ng/ml.

**Plasma Somatomedin-C Concentrations**

Plasma somatomedin-C concentrations were determined in all subjects as shown in Table 1. In Case 1, the somatomedin-C level was elevated at 7.9 U/ml (normal 0.45 to 2.2 U/ml) and decreased to 3.7 U/ml postoperatively. During treatment with bromocriptine (15 mg/day) for 3 months, the somatomedin-C level became normal at 1.8 U/ml. In Case 2, the somatomedin-C level was elevated at 2.6 U/ml preoperatively and was normal postoperatively at 1.2 U/ml. A somatomedin-C concentration obtained 6 months after transsphenoidal adenomectomy in Case 3 was normal at 0.53 U/ml.

**Tumor Tissue Analysis**

A tumor specimen taken from Case 1 at transsphenoidal surgery was analyzed for GH and PRL content. Growth hormone was found in quantities of 10,909 ng/mg of cellular protein, and the PRL determination was 15 ng/mg of cellular protein.

**Gel-Chromatographic Analysis of Serum and Tumor Tissue**

Gel-chromatographic analysis of 2 ml of serum from Case 1 showed that immunoactive GH (Kav = 0.47) comigrated with labeled GH (Kav = 0.49). Tumor tissue from Case 1 was homogenized, and an aliquot containing approximately 40 ng of GH was also analyzed. Immunoactive GH (Kav = 0.45) coeluted with labeled GH (Kav = 0.45).

**Morphological Findings**

In all three cases, the tumor was cellular, consisting of partly acidophilic and partly chromophobic cells as
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observed by light microscopy with H & E staining. The majority of cells in all three cases failed to react with any hormone tested using the immunoperoxidase technique; however, positive staining for GH in scattered adenoma cells of all three patients was detected. There was no correlation between the intensity of GH staining and serum GH concentrations. Immunochemical staining for GH of tumor tissue from Case 1 is shown in Fig. 2. Prolactin immunopositivity was noted in occasional adenoma cells in all three cases. Comparison of overlapping microscopic sections indicated that the PRL and GH cells were separate populations. In Case 1, ACTH immunopositivity was also noted in occasional adenoma cells. Immunostaining of adenoma cells for TSH-β, FSH-β, LH-β, and alpha subunit was negative in all patients.

Electron Microscopic Analysis

Electron microscopic analysis was performed on the tumor tissue from Case 1, and representative electron micrographs are shown in Fig. 3. Cells of a monomorphic pituitary adenoma were identified. They were large and irregular, possessing long cytoplasmic processes. The nuclei were large, often pleomorphic, and contained conspicuous nucleoli. The cytoplasm was abundant and well differentiated. The rough endoplasmic reticulum membranes and the Golgi apparatus were prominent. Large aggregates of smooth-surfaced endoplasmic reticulum membranes were noticeable in the cytoplasm of some cells. The mitochondria showed regular features. The secretory granules were spherical and evenly electron-dense with distinct limiting membranes; they were sparse and measured 50 to 150 nm. Some adenoma cells contained larger secretory granules but had the same ultrastructure as those with smaller secretory granules. Many secretory granules were present under the plasmalemma but exocytoses were not observed. Multiple interdigitations were noted between adjacent adenoma cells.

The cellular composition and derivation of this tumor are uncertain. Although several adenoma cells contained immunoreactive GH, the ultrastructural features of these adenoma cells did not resemble those of nonadenomatous or adenomatous GH cells.9,12

Discussion

We have studied three young women with pituitary macroadenomas and documented GH hypersecretion without clinical acromegaly. All three patients had large tumors with suprasellar extension and presented with visual loss, galactorrhea, and/or menstrual disturbances. Detailed laboratory analysis revealed elevated serum GH concentrations, abnormal GH dynamics including glucose nonsuppressibility, and elevated somatomedin-C concentrations. In one patient treated with bromocriptine, GH decreased and the elevated somatomedin-C concentrations normalized. Investigation of the tumor tissue obtained at transsphenoidal surgery by gel-chromatography revealed the presence of authentic GH in the tumor. Morphological analysis of tumor tissue demonstrated partly acidophilic and partly chromophobic cells, with scattered GH positivity seen on immunocytochemical studies, and unique ultrastructural features.

The identification of GH excess is important not only as a means of following tumor activity, but also because GH hypersecretion may have deleterious metabolic and systemic consequences. Several investigators have found that untreated acromegalic patients have an increased incidence of fatal cardiovascular, cerebrovascular, and respiratory disease.1,7,24 Other effects of GH excess include peripheral neuropathies, sleep apnea, and an increased risk of colon carcinoma.1,11,19 Acromegalic acral changes do not correlate with the metabolic consequences of GH excess.5,17 Therefore, patients with unrecognized GH hypersecretion may be at risk for the development of adverse end-organ GH effects.

Despite documented GH excess and somatomedin-C elevations, the patients in the present report did not appear acromegalic. The most likely explanation is that they presented prior to developing the chronic changes of GH excess, which may take years to become apparent. Although these patients may represent cases of early biochemical recognition of acromegaly, the large tumor size, clinical presentation, and aggressive clinical course (particularly in Case 1) are atypical for somatotroph adenomas. Morphological studies demonstrated that the tumors are partly acidophilic and partly chromophobic, and immunocytochemical analysis demon-
We have further characterized a subset of young patients with large pituitary tumors, clinically asymptomatic GH excess, and unique pathological features. Although acromegaly is typically a clinically insidious disease reflecting chronic GH excess, a subset of patients with large somatotroph adenomas may present prior to the development of acral changes. This study confirms that the presence of asymptomatic GH hypersecretion should be considered, particularly in young women with pituitary macroadenomas.

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References

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