Ectopic Luteinizing Hormone Secretion and Anovulation

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In patients with amenorrhea and infertility, increased levels of serum luteinizing hormone and follicle-stimulating hormone are usually due to primary gonadal failure, whereas a selective increase in luteinizing hormone can be seen with polycystic ovary syndrome. By contrast, an isolated elevation in luteinizing hormone above the postmenopausal range is uncommon and prompts evaluation of the luteinizing hormone assay. The luteinizing hormone value may be spuriously elevated if human chorionic gonadotropin levels are increased and assay antibodies recognize both hormones. In addition, circulating heterophilic antibodies may interact in an assay, causing an apparent elevation of luteinizing hormone.[1]

A verified abnormal result raises the possibility of tumoral hormone production. Dysgerminomas and bronchogenic, testicular, hepatic, and renal-cell carcinomas may secrete human chorionic gonadotropin; patients with these cancers present with precocious puberty, gynecomastia, or hirsutism and amenorrhea.[2-8] Pituitary[9] or, rarely, adrenal[10,11] tumors may secrete luteinizing hormone. We describe a patient who had anovulation with elevated plasma luteinizing hormone levels caused by luteinizing hormone secretion from a benign neuroendocrine tumor of the pancreas.

CASE REPORT

A 40-year-old woman was referred for evaluation of elevated levels of luteinizing hormone. After she had discontinued the use of oral contraceptives, her menses were irregular and she remained infertile for 10 years. She had intermenstrual intervals of six to eight weeks, a monophasic oral temperature, and plasma luteinizing hormone levels of 475 to 707 IU per liter (normal midcycle peak, 17.5 to 49.0) according to three two-site immunometric assays. The plasma levels of follicle-stimulating hormone were 2.7 to 5.9 IU per liter (normal, 5.1 to 34.2). The serum progesterone level was consistently less than 2 ng per milliliter (6.36 nmol per liter). After 35 days of administration of a gonadotropin-releasing hormone analogue (leuprolide, up to 1.5 mg daily), the serum luteinizing hormone level was 707 IU per liter.

When she was seen at the National Institutes of Health, the patient reported no headaches, hirsutism, visual disturbances, or vasomotor symptoms but did report gradual weight gain. Her height was 163 cm, and her weight was 109 kg. The physical examination was otherwise unremarkable; there was no hirsutism, acne, or acanthosis nigricans.

Because of the nonsuppressible plasma luteinizing hormone values, we investigated the possibility of tumoral secretion of luteinizing hormone.

METHODS

Clinical Protocol

After obtaining written informed consent from the patient, we measured hormones (follicle-stimulating hormone, prolactin, thyrotropin, progesterone, estradiol, dehydroepiandrosterone, testosterone, 17-hydroxyprogesterone, and androstenedione) and tumor markers (chromogranin A, the alpha subunit

of luteinizing hormone, and the beta subunit of human chorionic gonadotropin) using commercial assays. The cross-reactivity of the assay for the human chorionic gonadotropin beta subunit with luteinizing hormone was 0.05 percent. The cross-reactivity of the luteinizing hormone alpha subunit assay with luteinizing hormone was 0.8 percent, and its cross-reactivity with the human chorionic gonadotropin beta subunit was less than 0.1 percent. Covance Laboratories measured the luteinizing hormone beta subunit[12]using reference preparations from the National Hormone and Pituitary Program; a cross-reactivity evaluation was not performed.

To reduce the chance of spurious results caused by heterophilic antibodies or cross-reactivity with other hormones, luteinizing hormone was measured by a two-site microparticle enzyme immunoassay (Abbott Diagnostics) and a two-site immunoenzymometric assay (Tosoh Medics); the cross-reactivity of human chorionic gonadotropin was 0.01 percent with each method. We also pretreated plasma with a reagent that binds to heterophilic antibodies (Heterophilic Blocking Tube, Scantibodies Laboratory) or serially diluted plasma to 1:50 before performing the assay for luteinizing hormone. Luteinizing hormone bioactivity was measured by the mouse Leydig-cell assay[13] with the luteinizing hormone standard from the Abbott kit. For comparison, luteinizing hormone in serum from postmenopausal and premenopausal women was assayed with the use of the Abbott kit and bioassay.

To rule out a luteinizing hormone–secreting pituitary tumor, we performed pituitary magnetic resonance imaging (MRI) with gadolinium and measured thyrotropin, prolactin, luteinizing hormone, follicle-stimulating hormone, the luteinizing hormone beta subunit, and the luteinizing hormone alpha subunit after administering thyrotropin-releasing hormone.[9] To rule out a nonpituitary tumor, we performed computed tomography (CT) and MRI of the chest, abdomen, and pelvis. On the basis of these results, selective venous sampling of the hepatic, azygous, petrosal, and peripheral veins was performed for research purposes, with measurement of luteinizing hormone, the luteinizing hormone beta subunit, and the luteinizing hormone alpha subunit. Somatostatin scintigraphy was not performed.

Immunohistochemical Evaluation of Tumor

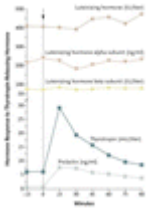
Immunohistochemical evaluation of an abdominal mass was performed by an automated immunostainer with 5-μm-thick paraffin sections of tumor and appropriate controls and colorimetric detection. Monoclonal antibodies were diluted as follows: from Boehringer Mannheim, chromogranin A (1:3000) and cytokeratin AE1/AE3 (AE1 1:500, and AE3 1:200); from the National Institute of Diabetes and Digestive and Kidney Diseases–National Hormone and Pituitary Program, luteinizing hormone (1:1600), follicle-stimulating hormone (1:400), and alpha subunit (1:300); from Dako, glucagon (1:1000), somatostatin (1:1000), gastrin (1:2000), and human chorionic gonadotropin (1:60,000); and from Zymed, cytokeratin MAK-6 (1:2).

RESULTS

Laboratory Tests and Imaging

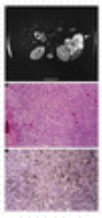
Laboratory measurements showed elevated plasma levels of luteinizing hormone, luteinizing hormone alpha subunit, human chorionic gonadotropin, chromogranin A, and 17-hydroxyprogesterone and normal levels of follicle-stimulating hormone, dehydroepiandrosterone

sulfate, testosterone, estradiol, insulin, and androstenedione (Table 1**TABLE 1** Circulating Hormone Levels in a 40-Year-Old Woman with Anovulation and Infertility.). The luteinizing hormone levels were 408 to 644 IU per liter and 388 to 551 IU per liter in the two immunoassays and were not affected by blocking of heterophilic antibodies or dilution of plasma. The values of bioactive luteinizing hormone were 575 and 602 IU per liter, with corresponding immunoassay results of 407 and 408 IU per liter, producing biologic:immunologic ratios of 1.4 and 1.5. These ratios were between those for premenopausal women (0.8 and 1.0) and postmenopausal women (1.5 to 2.9). After the administration of thyrotropin-releasing hormone, the levels of prolactin and thyrotropin increased and



other hormones were unchanged (Figure 1**FIGURE 1** Hormone Levels before and after Intravenous Administration of Thyrotropin-Releasing Hormone in a Woman with a Luteinizing Hormone–Secreting Pancreatic Tumor.).

Imaging demonstrated a 2-to-3-mm pituitary abnormality suspected of being a microadenoma, normal-sized ovaries with a 2-cm left ovarian cyst, and a mass in the left upper abdomen (Figure



2A**FIGURE 2** Magnetic Resonance Imaging (MRI) and Pathological Evaluation of Tumor.). There was no hormonal gradient on venous sampling, and the levels of luteinizing hormone did not vary by more than 10 percent during one hour of sampling. We proceeded with surgical exploration of the abdomen. At surgery, a polypoid mass 5 by 6 by 11.5 cm was removed by a distal pancreatectomy.

Pathological Evaluation

Hematoxylin-and-eosin staining of the tumor revealed cells with a lightly eosinophilic cytoplasm and uniform round, purple nuclei with infrequent mitoses (Figure 2B).

On immunohistochemical testing, 40 percent of the tumor cells showed a cytoplasmic distribution of intact luteinizing hormone (Figure 2C) and luteinizing hormone alpha subunit and membranous staining for chromogranin A (data not shown). Approximately 25 percent of the cells in the same nodule were positive on staining for human chorionic gonadotropin. The cells did not stain for follicle-stimulating hormone, glucagon, somatostatin, or gastrin but were positive for the cytokeratins MAK-6 and AE1/AE3. The pathological diagnosis was a neuroendocrine tumor.

Recovery of the Hypothalamic–Pituitary–Ovarian Axis

Luteinizing hormone decreased from a preoperative level of 667 IU per liter to 15, 5, 3, and 2 IU per liter on postoperative days 1, 2, 3, and 4, respectively. Plasma luteinizing hormone and follicle-stimulating hormone were undetectable on day 5 and detectable on day 13, when they were next measured. On postoperative day 7, pelvic ultrasound examination showed a right ovary 2.2 by 1.6 by 2.4 cm, with several follicles up to 8 mm in diameter, and a left ovary 3.0 by 1.4 by 2.1 cm, without follicles. The endometrial thickness was 3 mm. Luteinizing hormone was detected in the urine on postoperative day 31. A luteal-phase progesterone level (4.6 ng per milliliter [15 nmol per liter]) was measured on day 38, and menstrual bleeding occurred on day 43. During ovarian stimulation one month later, normal suppression of luteinizing hormone occurred in response to luteinizing hormone–releasing hormone analogue, and normal ovarian stimulation occurred in response to human chorionic gonadotropin, with successful retrieval of 10 oocytes.

DISCUSSION

During an evaluation for infertility, extremely high plasma levels of luteinizing hormone were found in this 40-year-old woman. Excision of a benign pancreatic tumor that contained luteinizing hormone resulted in a decrease in luteinizing hormone to undetectable levels, followed by an ovulatory menstrual cycle within two months. This is an unusual case of ectopic secretion of luteinizing hormone from the neuroendocrine cells of the pancreas.

Patients with ectopic secretion of gonadotropin may present with reproductive abnormalities. In males, precocious puberty may herald hepatic carcinoma or a germ-cell tumor,[4] and gynecomastia has led to the detection of liver,[4] bronchogenic,[5] testicular,[6] and renal-cell[7]carcinomas. In females, secondary amenorrhea has been associated with an androgen- and gonadotropin-secreting ovarian dysgerminoma[8] and bronchogenic carcinoma.[2]

Because luteinizing hormone and human chorionic gonadotropin both stimulate the same receptor, either hormone might cause these clinical effects. When assays that distinguish the beta subunits of these hormones are used,[14] it is clear that human chorionic gonadotropin is the gonadotropin associated with these cancers.[4,15-18] In our patient, two-site immunoassays for luteinizing hormone demonstrated elevated levels. Cross-reactivity of luteinizing hormone in the human chorionic gonadotropin assay could account for most, but not all, of the mild increase in human chorionic gonadotropin. This observation and the fact that there was immunohistochemical staining for human chorionic gonadotropin in the tumor suggest that it secreted both gonadotropins.

Plasma antibodies to nonhuman immunoglobulins may interact in immunoassays and produce falsely elevated hormone results.[1,19] The correct normal result may be achieved by using two-site assays,[19] serial dilutions of the sample, or a blocking reagent. In our patient, these strategies did not reduce measured plasma luteinizing hormone levels.

The differential diagnosis of a verified elevated luteinizing hormone value includes inactivating mutations of the luteinizing hormone receptor,[20] polycystic ovary syndrome, and secretion of luteinizing hormone from a eutopic (pituitary) tumor[9] or ectopic tumor. In our patient, the degree of luteinizing hormone elevation and the presence of chronic oligomenorrhea made the possibility of an inactivating mutation of the luteinizing hormone–receptor gene less likely. The absence of hirsutism, acne, and hyperandrogenism argued against the diagnosis of polycystic ovary syndrome. We ruled out a gonadotrope tumor by petrosal-sinus sampling and thyrotropin-releasing hormone stimulation;

the pituitary imaging abnormality might have represented a false positive result or a nonsecreting tumor.

Because of the unique characteristics of this case, we proceeded with whole-body imaging that led to the discovery of a large luteinizing hormone–containing pancreatic tumor. Two previous cases of ectopic secretion of luteinizing hormone have been well substantiated, both in young boys with precocious puberty, mild elevations in luteinizing hormone (to 15 IU per liter), and adrenal tumors that contained luteinizing hormone.[10,11]

The case of our patient also presents a physiological question. Since luteinizing hormone is critical for folliculogenesis, ovulation, and androgen production, how could this patient have disordered folliculogenesis and anovulation without hyperandrogenism? Reduced bioactivity of tumoral luteinizing hormone might account for this situation, but it was ruled out. We speculate that the ovaries became desensitized to luteinizing hormone after invariant exposure to elevated levels.

A loss-of-function mutation in the mannose–*N*-acetylgalactosamine-4-sulfate receptor, which normally clears luteinizing hormone from the circulation, reduces steroidogenesis, thus providing evidence of the importance of normal pulsatile secretion of luteinizing hormone.[21]Since our patient did not have normal levels of luteinizing hormone, we speculate that tumoral luteinizing hormone secretion was continuous, that abnormal glycosylation of the molecule decreased its clearance, or that both of these processes occurred. Although all measured values were consistently elevated (in the range of 440 to 640 IU per liter), frequent sampling would be necessary to rule out normal pulsatile secretion. We were unable to perform isoform analysis to evaluate glycosylation.

Excess human chorionic gonadotropin is a model for tonic excess of luteinizing hormone because of its long half-life and its ability to activate the luteinizing hormone receptor. When injected or secreted by a persistent mole or bronchogenic adenocarcinoma, human chorionic gonadotropin causes dose-dependent anovulation.[22-24] In one study examining the effects of exogenous human chorionic gonadotropin before oophorectomy, ovaries showed increased atresia of antral follicles and luteinization of follicles 6 to 8 mm in diameter.[22] The finding of a serum progesterone level of 2 ng per milliliter (6.36 nmol per liter) in our patient provides evidence of the preservation of some luteinizing activity.

We believe that receptor desensitization was the underlying pathophysiological mechanism of the tonic excess of luteinizing hormone. Chronic exposure to elevated levels of human chorionic gonadotropin or luteinizing hormone induces both uncoupling and down-regulation of the luteinizing hormone receptor.[25] The mild elevation of 17-hydroxyprogesterone, without corresponding increases in androgens, suggests reduced responsiveness to luteinizing hormone with a relative mismatch between the 17-hydroxylase and the 17,20-lyase functions of the ovarian P450c17 enzyme, as described in women with polycystic ovary syndrome.[26]

It is also possible that the levels of follicle-stimulating hormone in our patient were insufficient to promote normal folliculogenesis and induce luteinizing hormone receptors on maturing follicles. This would prevent recruitment of a dominant follicle and reduce the cohort of follicles capable of steroidogenesis. The findings in our patient are similar to those in a patient who had a deficiency of follicle-stimulating hormone and elevated luteinizing hormone levels but no hyperandrogenism.[27]

The possibility of an ectopic luteinizing hormone–producing tumor in the adrenal gland or pancreas should be considered in women with extremely high levels of luteinizing hormone. The case we describe also underscores the importance of normal luteinizing hormone pulsatility for folliculogenesis