Lymphocytic Hypophysitis of Pregnancy Resulting in Hypopituitarism: A Distinct Clinicopathologic Entity

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Two patients presented with abnormalities suggestive of pituitary adenoma; one during pregnancy and one in the postpartum period. However, pathologic examination of the pituitary showed extensive destruction by a lymphoplasmacytic infiltrate; no tumor was identified. Both patients developed hypopituitarism. We know of eight additional cases of lymphocytic hypophysitis, seven of which have been reported in the literature. In only three cases, including the two reported here, the diagnosis was established by biopsy. In each of those cases, the entity mimicked a pituitary tumor. This is the first report of electron microscopy of this lesion and the ultrastructural features support the previously suggested autoimmune etiology. The lesion has been described only in women and seven of ten patients were pregnant or postpartum at the onset. This fact and previously reported experimental evidence, including the identification of anti-prolactin cell antibodies, support our suggestion that lymphocytic hypophysitis associated with pregnancy represents a distinct clinicopathologic entity.

Pregnancy induces substantial changes in the structure and function of the anterior pituitary. During pregnancy, the pituitary increases in size because of hyperplasia of prolactin cells, reflecting increased prolactin synthesizing activity (1). The pituitary gland in pregnancy seems to be especially vulnerable to circulatory disturbances; postpartum pituitary necrosis develops after complicated delivery associated with hemorrhage and shock, and is caused by ischemia (2). If pituitary destruction is severe, hypopituitarism may ensue. The effects of pregnancy on pituitary adenomas are poorly understood, although rapid deterioration of visual field defects in pregnant patients with pituitary adenomas has been documented (3). Whether these changes are the result of sudden enlargement of the pituitary tumor or of the nonhomogenous portion of the gland, or are caused by other phenomena is not known.

We describe two patients with a distinct pituitary lesion associated with pregnancy. This entity presents mass loss of the sella turcica in one pregnant and clinically mimicked pit adenoma. Detailed morphologic study, including electron microscopy, led to a diagnosis of lymphocytic hypophysitis.

Case Reports

Patient 1

A 28-year-old black woman, gravida 3, para 0, sought call attention during the sixth month of pregnancy because of 4-week history of progressive blurring of vision and scotomas across both visual fields. During the 2 weeks before vision, she had noted accelerated deterioration of visual acuity in her left eye. Her pregnancy had been uneventful until symptoms. No headaches or symptoms suggestive of endocrine deficiency were present. Family history was negative for diabetes mellitus or any other endocrinopathy.

Physical examination showed a pregnant woman with size compatible with the estimated length of gestation signs were normal. Ophthalmologic assessment on the admission showed a left temporal hemianopia and a central constriction of the right visual field. Visual acuity was to the right eye and 20/200 in the left eye. Two days later opthalmologic findings had deteriorated with bitemporal hemianopia, a left central scotoma, and visual acuities of 20, the right and 20/400 on the left.

Biochemical evaluation documented gestational diastolic systolic blood pressure of 204 mg/dL and an insulin of 38 μU/mL. The serum electrolytes, creatinine, and urine osmolalities were normal. Serum thyroxine was 5.7 μg/dL (normal, 4 to 11 μg/dL); triiodothyronine (T3), 20.8% (normal, 25% to 35%); free thyroxine (normal, 1 to 3.8); T3 by radioimmunoassay, 62.2 (normal, 70 to 210 ng/dL). Morning plasma cortisol was 6 μg/dL; 1 hour after intramuscular administration of 0.50 synthetic adrenocorticotropic hormone (ACTH) (Cortef, Organon Canada Ltd., Montreal, Quebec, Canada), plasma cortisol was 54.5 μg/dL. Follicle stimulating hormone (FSH) less than 2.0 mIU/mL; and luteinizing hormone was less than 80 mIU/mL, reflecting human chorionic gonadotropin (HCG) cross reactivity. Prolactin levels after bromocriptine 2.5 mg twice per day, were suppressed at 6.7 ng/mL before treatment were unavailable.

Skull roentgenogram showed a normal pituitary fossa...
thinning of the dorsum sellae. Computed tomographic (CT) scan showed a lobulated contrast-enhancing mass arising out of the pituitary fossa, extending into the suprasellar cistern and indenting the antero-inferior recesses of the third ventricle. There was also bilateral extension into the parasellar regions (Figure 1). A diagnosis of pituitary adenoma with suprasellar extension was made. Prolactinoma was felt to be the most likely tumor type.

The patient was treated initially with bromocriptine, 2.5 mg twice per day for 4 days, with daily visual field assessments. On the third day, improvement in visual acuity was noted with decrease in the left temporal hemianopia and central scotoma and visual acuities of 20/20 in the right eye and 20/40 in the left eye. Computed tomographic scan was not repeated before surgery.

A bifrontal craniotomy was done and a moderate sized mass was seen arising from the pituitary fossa, compressing the optic chiasm. The pituitary capsule was dense and difficult to remove, making total extirpation of the mass not possible. Postoperatively, the patient developed transient diabetes insipidus and the postoperative diabetes required temporary treatment with insulin until high dose prednisone therapy had been tapered. Repeat visual field examination showed almost complete recovery with only minimal residual left temporal hemianopia.

Two weeks after surgery and after discontinuation of all medication, serum prolactin was 86 ng/mL; serum thyroxine (T4), 7.1 μg/dL; T3 resin uptake, 20.6%; free thyroxine index, 1.5; T3 by radioimmunoassay, 148.6 ng/dL; and plasma cortisol, 28.2 μg/dL.

Two months after surgery, normal spontaneous labor led to the delivery of a healthy female infant. At term, the prolactin level was 23.1 ng/mL and the patient was unable to lactate. Over the next 2 months, thyroid and adrenal function deteriorated; T4 was 3.2 μg/dL; T3 resin uptake, 26.7%; free thyroxine index, 0.9; thyrotropic hormone (TSH), 2 μU/mL. The PM cortisol was 1.0 μg/dL, with a brisk response to synthetic ACTH, 0.25 mg intramuscularly, rising to 54.4 μg/dL at 1 hour. Dynamic pituitary function testing showed impaired cortisol and absent growth hormone responses to insulin hypoglycemia, absent TSH and prolactin responses to thyrotropin releasing hormone (TRH) and hyporesponsiveness to FSH and normal LH response to luteinizing hormone releasing hormone (LRH) (Table 1). Prednisone and thyroid hormone therapies were instituted. A repeat CT scan at this time showed a large residual pituitary mass, 1.26 cm in diameter, that enhanced with contrast and extended into the suprasellar region. Visual fields and acuity were normal.

**PATIENT 2**

A 29-year-old white woman, gravida 2, para 2, presented 6 months after normal delivery of her second child, with amenorrhea and an inability to lactate. She had had a normal pregnancy with delivery 18 months earlier that was followed by normal lactation and subsequent return of normal menses. During the seventh month of her second pregnancy, the patient noted the onset of frequent headaches, nausea and vomiting, with a weight loss in the last 2 months of gestation. After delivery, the patient noted that although pubic hair grew, there was no further growth of axillary hair. She was lethargic with decreased libido and dyspareunia. She had no history of cold intolerance or change in skin texture or pigmentation, nor did she have any symptoms of visual abnormalities. Family history revealed that a grandfather had diabetes mellitus.

Examination showed a lethargic woman with dry skin, normal pubic and scalp hair but almost no axillary hair. Vital signs were normal. The thyroid gland was palpable but not enlarged. Neurologic examination showed symmetrically hypoactive reflexes with a prolonged relaxation phase. The remainder of the examination was within normal limits.

Serum electrolytes, creatinine and blood glucose levels were normal. She had hypothyroidism with a serum thyroxine level of 1.8 μg/dL (normal 5 to 13 μg/dL), and a TSH of 3.0 μU/mL. Prolactin was elevated at 58.7 ng/mL. Her growth hormone levels were less than 1 ng/mL; FSH was 11.0 mIU/mL; LH, 8.4 mIU/mL; testosterone, less than 0.1 ng/mL; 17β-estradiol, less than 20 pg/mL; estrone, 46 pg/mL; and afternoon plasma cortisol, less than 1.6 μg/dL.

Skull roentgenogram and tomograms of the sella turcica showed slight enlargement of the sella and CT scan confirmed the presence of a pituitary fossa mass. Bilateral carotid arteriograms showed minimal suprasellar extension of the intrasellar mass, elevating the supracarotid portion of the right carotid
Table 1. Pituitary Function Tests of Patient 1 at 10 Weeks Postpartum

<table>
<thead>
<tr>
<th>Time</th>
<th>Insulin Tolerance Test*</th>
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<tbody>
<tr>
<td></td>
<td>Blood Sugar</td>
</tr>
<tr>
<td>min</td>
<td>mg/dL</td>
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<tr>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
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<tr>
<td>40</td>
<td>24</td>
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<tr>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
</tr>
</tbody>
</table>

* Regular insulin, 0.05 U/kg body weight, intravenously.
† Thyrotropin-releasing hormone, 200 µg, intravenously.
‡ Luteinizing hormone releasing hormone, 100 µg, intravenously.

siphon and A-1 segment of the anterior cerebral artery.

A diagnosis of prolactin-secreting pituitary adenoma with suprasellar extension was made. Because of clinical and biochemical evidence of hypothyroidism and adrenal insufficiency, the patient was treated with levothyroxine sodium, 0.15 mg per day orally and hydrocortisone, 20 mg orally each morning and 10 mg orally each afternoon.

A transphenoidal hypophysectomy was done. After surgery, the patient developed transient diabetes insipidus; serum sodium rose to 145 mEq/L despite weight gain and urine specific gravity of 1.003. Treatment with vasopressin tannate resulted in immediate improvement. Thyroxine and hydrocortisone replacement therapies were continued at maintenance levels and no further biochemical investigations were pursued.

**Morphologic Methods**

For light microscopy, pituitary tissue from both cases was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin, hematoxylin-phloxine-saffron and the periodic acid Schiff technique. The immunoperoxidase method (4) was done for the localization of prolactin, growth hormone, ACTH, FSH, LH, and TSH. For electron microscopy, small pieces of tissue were fixed in glutaraldehyde, postfixed in OsO₄, dehydrated and embedded in epoxy-resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Phillips 300 electron microscope (Philips Electronic, Mount Vernon, New York).

**Findings**

Light microscopic findings showed marked diffuse changes in the anterior lobe in both cases. Tien extensive cellular infiltration consisting chiefly of lymphocytes accompanied by some plasma cells; in some areas, follicles with germinal centers were noted (Fig 2a). The residual gland showed destruction (Fig 2b). Foci of uninvolved pituitary tissue were morphologically normal. Neurohypophysis was not present in the tissue. No tumor was identified in either specimen.

The immunoperoxidase technique showed the presence of immunoreactive prolactin, growth hormone, ACTH, FSH, LH and TSH within surviving adenohypophyseal cells.

Electron microscopic features were similar in both cases. Adenohypophyseal cells of all types appeared intact or exhibited varying degrees of injury. In the most dense inflammatory cell infiltration (Figure 3a), pituitary cells showed interdigitation with activated phagocytes at the common interface (Figure 3b). Some of these pituitary cells contained large lysosomal bodies with secretory granules as well as increased numbers of swollen mitochondria, indicating oncocytic transformation (Figure 4). Some pituitary cells showed vacuolated damage that they could not be classified by electron microscopy. No immune complex deposits were identified, and the vessels examined showed no pathologic change.

**Figure 2.** Case 2. a. Pituitary gland shows scattered mononuclear cell infiltrate and a large aggregate of lymphocytes. (Hematoxylin and eosin stain; original magnification, × 80.) b. Effacement of pituitary architecture by lymphocytic infiltrate sparing only a few adenohypophysial cells. (Hematoxylin and eosin stain; original magnification, × 128.)
The indirect fluorescent technique to detect organ-specific antibodies to thyroid, liver, and kidney microsomal antigens; thyroglobulin; smooth muscle; stomach parietal cells; adrenal cortex; and ovary yielded negative results in both cases. Tests for antinuclear and antiribosomal antibodies were negative. Patient 1 had no antimitochondrial antibodies; Patient 2 had 2+ to 3+ antimitochondrial activity.

In Patient 1, antibodies to pancreatic islet cells and anterior pituitary cells were not detected. HLA typing, done on Patient 1, showed a phenotype of A2A28Bw35.

Discussion

To our knowledge, seven cases of lymphocytic hypophysitis have been reported previously in association with various degrees of hypopituitarism (5-11). In addition to the two cases reported here, we are aware of one additional patient with a similar morphologic lesion (VELASCO M. Personal communication). In only three cases including our two cases was a diagnosis of lymphocytic hypophysitis made on biopsy before death (11); in all three, the lesion mimicked a pituitary tumor.

The pathogenesis of lymphocytic hypophysitis is unclear. The first case was reported by Goudie and Pinkerton in 1962 (5). These authors suggested an autoimmune etiology, and subsequent authors have concurred (6-10, 12). The morphologic features of this lesion resemble those of autoimmune thyroiditis, adrenitis, oophoritis, orchitis, and gastritis (13), which are assumed to be caused by delayed or cellular immune mechanisms. Our cases are the first to be studied by electron microscopy, and the ultrastructural features resemble those seen in autoimmune thyroiditis (14).

Similar histologic features can be produced in rats by injection of homogenates of whole pituitary glands or of anterior lobes from which most of the posterior and intermediate lobes have been removed (15). These results are consistent with the suggestion that an autoimmune reaction may be implicated in the genesis of human lymphocytic hypophysitis.

Another prominent feature of autoimmune disease is the tendency for such a lesion to affect multiple organs in the same individual. Six of the 10 cases of lymphocytic hypophysitis known to us have been associated with other endocrine autoimmune phenomena (5, 6, 8-10) (Table 2).

Further support for the existence of anterior pituitary autoimmunity is found in the demonstration of circulating antihypophysial antibodies in patients with varied endocrine autoimmune disease. Bottazzo and associates (16) were able to identify, by immunofluorescent staining, autoantibodies that reacted with anterior pituitary tissue. Of 287 patients having one or more autoimmune endocrine diseases, sera from 19 reacted with pituitary tissue. Antipituitary antibodies were tested in only two cases of lymphocytic hypophysitis; they were detected in the blood of one patient (11) but not found in our first case. Using specific antibodies to each of the six pituitary hormones, it has been shown that antibodies reacted specifically with hyperplastic pro lifer cells in pituitary tissue obtained at hypophysectomy from estrogen-treated breast cancer patients (16).
### Table 2. Clinical Features of Lymphocytic Hypophysitis in Ten Women

<table>
<thead>
<tr>
<th>Authors (Reference)</th>
<th>Patient Age</th>
<th>Menstrual and Pregnancy History</th>
<th>Associated Disorders</th>
<th>Method of Diagnosis</th>
<th>Investigative Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goudie and Pinkerton (5)</td>
<td>yrs 22</td>
<td>Onset of symptoms 2 months postpartum; only two menstrual periods from delivery to death 14 months postpartum; amenorrhea last 6 months of life</td>
<td>Hashimoto’s thyroiditis</td>
<td>Autopsy</td>
<td>Histology</td>
</tr>
<tr>
<td>Hume and Roberts (6)</td>
<td>74</td>
<td>Nulliparous; 24 years postmenopausal</td>
<td>Chronic atrophic gastritis with pernicious anemia focal lymphocytic thyroiditis</td>
<td>Autopsy</td>
<td>Histology</td>
</tr>
<tr>
<td>Egloff et al. (7)</td>
<td>29</td>
<td>Onset of symptoms 4 weeks postpartum; amenorrhea from delivery to death 1 year later</td>
<td>None</td>
<td>Autopsy</td>
<td>History</td>
</tr>
<tr>
<td>Lack (8)</td>
<td>42</td>
<td>History of miscarriages with hysterosalphingography-oophorectomy</td>
<td>Parathyroiditis, mild adrenalitis</td>
<td>Autopsy</td>
<td>History</td>
</tr>
<tr>
<td>Gleason et al. (9)</td>
<td>60</td>
<td>History of hysterosalphingography-oophorectomy</td>
<td>Severe arthralgia*</td>
<td>Autopsy</td>
<td>History</td>
</tr>
<tr>
<td>Richtsmeier et al. (10)</td>
<td>31</td>
<td>Onset of symptoms postpartum; death 3 months postpartum</td>
<td>Chronic thyroiditis, autoimmune pancreatitis*</td>
<td>Autopsy</td>
<td>Histology, immunocytoLOGY</td>
</tr>
<tr>
<td>Mayfield et al. (11)</td>
<td>23</td>
<td>Onset of symptoms 7 months postpartum; normal menses on contraception</td>
<td>None</td>
<td>Biopsy</td>
<td>Histology, immunology</td>
</tr>
<tr>
<td>Velasco†</td>
<td>22</td>
<td>Onset of symptoms 4 months postpartum; galactorrhea; suicide 14 months postpartum</td>
<td>None</td>
<td>Autopsy</td>
<td>Not known</td>
</tr>
<tr>
<td>Asa et al. (Case 1)</td>
<td>28</td>
<td>Onset of symptoms and diagnosis at 6 months gestation; amenorrhea and failure of lactation postpartum</td>
<td>Gestational diabetes</td>
<td>Biopsy</td>
<td>Histology, immunocytoLOGY, electron microscopy, immunology</td>
</tr>
<tr>
<td>Asa et al. (Case 2)</td>
<td>29</td>
<td>Onset of symptoms at 7 months gestation; amenorrhea and failure of lactation postpartum</td>
<td>None</td>
<td>Biopsy</td>
<td>Histology, immunocytoLOGY, electron microscopy, immunology</td>
</tr>
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* Not confirmed medically.
† Velasco M. Personal communication.

The importance of prolactin cell antibodies is particularly noteworthy as all 10 patients with lymphocytic hypophysitis were women. Furthermore, in five of the 10 cases (5, 7, 10, 11 and Velasco M. Personal communication), the disease was detected in the postpartum period and in the two cases reported here, onset was during pregnancy in Case 1 and probably also in Case 2. This close temporal relationship suggests that the association is not coincidental and that pregnancy and lymphocytic hypophysitis may be related. Moreover, organ-specific antibody-like activity against the pituitary has been reported in the serum of 18% of women 7 days postpartum and in one woman with clinical signs of Sheehan’s syndrome after a pregnancy 5 years earlier. Six to 12 months after delivery, 25% of those patients with elevated pituitary antibodies developed signs of decreased adenohypophysial function compared to only 4% of those with no demonstrable antibodies (17).

Experimental hypophysitis can be induced in pregnant rats and may be more severe in animals postpartum (15), lending further support to the association of lymphocytic hypophysitis with pregnancy.

The reason for this association is unclear. It may be that pregnancy initiates an autoimmune reaction. The studies of Bottazzio and associates (16) have shown that prolactin cell antibodies are not directed against the hormone itself, but rather against the cytoplasmic organelles involved in the synthesis or release of the hormone.

Whether in certain persons this antigen is modified during pregnancy, or an increased amount of antigen supplied by hyperplastic prolactin cells precipitates an autoimmune phenomenon in genetically predisposed persons remains to be determined.

Recent theories of autoimmune phenomena ascribe a major role to lack of suppressor T cell function with loss of immune surveillance and self-recognition (18). In general, pregnancy is associated with remission of activity of autoimmune disorders; however, there is definitely an increased risk of exacerbation of these diseases in the postpartum period (19, 20). The mechanism of amelioration may be a generalized suppression of maternal immune reactivity, and it appears that fetal lymphocytes elaborated such a suppressor (21). The loss of fetal suppressor activity postpartum may allow an autoimmune reaction to manifest itself and the onset of lymphocytic hypophysitis postpartum may be attributed to this phenomenon alone.

In all seven cases, the symptoms worsened postpartum and this progression of disease, similar to that of other autoimmune disorders, can be attributed to loss of suppression of immunoreactivity.

In addition to the antipro lactin cell antibodies, other specific pituitary cell antibodies have been identified, such as those against human growth hormone cells in a patient with retarded growth whose mother suffered from Addison’s disease and thyroiditis (22). Antibodies have also been found that do not react with lactotrophs, somatotrophs, growth hormone, or prolactin.
trophs, or thyrotrrophs, and these may represent antigen
ies against FSH and LH secreting cells (22). Thus, it may
well be that antibodies to each of the pituitary cell types
will be identified and that several specific forms of auto-
immune hypophysitis exist, each explaining isolated defi-
ciencies of pituitary hormones. Of course, in each case,
the inflammatory process can lead to destruction of the
organ and may eventually result in panhypopituitarism.

At present, biopsy is the only means of confirming the
presence of lymphocytic hypophysitis. Noninvasive diag-
nosis of this disorder will require further documentation of
the presence of pituitary antibodies in association with the
histologic lesion. Rational treatment for this condition
awaits a better understanding of its pathogenesis.

In summary, the anterior pituitary appears to be a tar-
get for inflammatory autoimmune destruction that may
result in hypopituitarism, especially during pregnancy
and in the postpartum period. Lymphocytic hypophysitis of
pregnancy represents a distinct clinicopathologic entity
and must be considered in the differential diagnosis of
mass lesions of the sella turcica.

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M5B 1W8 Canada.

References

1. Goluboff LG, Ezerin C. Effect of pregnancy on the somatotroph and
the prolactin cell of the human adenohypophysis. J Clin Endocrinol
2. Sheehan HL, Stanfield JP. The pathogenesis of post-partum necro-
3. Child DF, Gordon H, Mashiter K, Joplin GF. Pregnancy, prolac-
4. Kovacs K, Corenblum B, Sirek AMT, Penz G, Ezrin C. Locali-
ation of prolactin in chromophobe pituitary adenomas: study of human
adenohypophysis by immunoperoxidase technique. J Clin Path
5. Godbee RB, Pinkerton PH. Anterior hypophysitis and Hashimoto
6. Hume R, Roberts GH. Hypophysitis and hypopituitarism: report of
7. Egloff B, Fischbach W, von Goumenes E. Lymphomatosis Hype-
ophysitis mit Hypophyseninsuffizienz. Schweiz Med Wochenschr
8. Lack EE. Lymphoid "hypophysitis" with end organ insufficiency. An
9. Gleason TH, Stebbins PL, Shanahan MF. Lymphoid hypophysitis in
a patient with hypoglycemic episodes. Arch Pathol Lab Med
10. Richtsmeyer AJ, Henry RA, Bloodworth JMB Jr, Ehrlich E. Lym-
phoid hypophysitis with selective adrenocorticotropic hormone de-
11. Mayfield RK, Levine JH, Gordon L, Powers J, Galbraith R, Raw SE. Lym-
phoid adenohypophysitis presenting as a pituitary t
12. Doniach I. Histopathology of the anterior pituitary. Clin Endocrin-
Metab. 1977;6:21-32.
13. Appel GB, Holub DA. The syndrome of multiple endocrine gla-
14. Reibord HE, Fisher ER. Ultrastructural features of subacute gran-
momatous thyroiditis and Hashimoto's disease. Am J Clin Path
15. Levine S. Allergic adenohypophysitis: new experimental disease of
16. Bottazzo GF, Pouplard A, Florin-Christensen A, Doniach I.
Autoantibodies to prolactin-secreting cells of human pituitary. Lance
17. Engelberth O, Ježková Z. Autoantibodies in Sheehan's syndrome
18. Volpé R. The role of autoimmunity in hypopituitarism and hyperendo-
rine function with special emphasis on autoimmune thyroid disease
19. Pitkin RM. Autoimmune diseases in pregnancy. Semin Perinatol
21. Froelich CJ, Goodwin JS, Bankhurst AD, Williams RC Jr. Pregnancy, a temporary fetal graft of suppressor cells in autoimmune