

Coexistence of Corticotroph Adenoma and Thyrotroph Hyperplasia in a Dog

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(Received 12 June 2008/Accepted 17 August 2008)

ABSTRACT. Pituitary thyrotroph hyperplasia results from prolonged primary hypothyroidism in humans, mice and rats. In dogs with Cushing's disease, many cases have low serum thyroid hormones concentrations due to euthyroid sick syndrome. A 6-year-old castrated male Beagle diagnosed with Cushing's disease had a high serum thyroid stimulating hormone (TSH) concentration that was treated by hypophysectomy. On histological examination, the resected pituitary gland contained both a corticotroph adenoma and thyrotroph hyperplasia. The TSH-positive cell ratio in this case was greater than that of healthy Beagles. In the present case, the pituitary thyrotroph hyperplasia was probably caused by primary hypothyroidism. In conclusion, this Beagle is the first histological confirmation of the coexistence of a corticotroph adenoma and thyrotroph hyperplasia.

KEY WORDS: canine, corticotroph adenoma, Cushing's disease, pituitary, thyrotroph hyperplasia.

J. Vet. Med. Sci. 71(1): 93–98, 2009

Pituitary-dependent hyperadrenocorticism (PDH), or Cushing's disease, is a common endocrine disease in dogs that accounts for 80–85% of spontaneous hyperadrenocorticism (HAC) cases [9]. Excessive secretion of adrenocorticotrophic hormone (ACTH) by the pituitary gland stimulates the adrenal cortex to secrete high concentrations of cortisol.

Endogenously produced or exogenously administered glucocorticoid has been reported to suppress pituitary thyrotroph function and to decrease serum total thyroxine (T₄), triiodothyronine (T₃) and free T₄ (FT₄) concentrations in dogs [11, 27, 29]. Cushing's disease is a cause of secondary hypothyroidism and euthyroid sick syndrome. Euthyroid sick syndrome refers to the suppression of serum thyroid hormone concentrations in euthyroid animals in response to a concurrent disease [22]. HAC is one of these concurrent diseases, and euthyroid sick syndrome may be found in 60% of dogs with PDH [10]. Changes in hormone concentrations in the canine hypothalamus-pituitary-thyroid axis, as well as euthyroid sick syndrome, have been studied [19, 23, 29, 36], and hypothyroidism in dogs with HAC has been reported [10]. However, pituitary thyroid stimulating hormone (TSH)-secreting adenoma and thyrotroph hyperplasia have not been reported in dogs with Cushing's disease.

In the present study, we report a canine case with Cushing's disease, in which the serum TSH concentration was remarkably elevated and the coexistence of a corticotroph adenoma and thyrotroph hyperplasia was indicated.

A 6-year-old castrated, male Beagle weighing 13.9 kg was referred to the Veterinary Medical Teaching Hospital of

Nippon Veterinary and Life Science University with complaints of polyuria, polydipsia (1.5 liters/day), polyphagia, abdominal enlargement and dorsal alopecia. The dog was lively and appeared to be in good physical condition. There was no abnormality in thoracic radiography, but abdominal radiography revealed hepatomegaly. Routine laboratory examination showed lymphopenia (lymphocyte value: 180/ μ l; reference range of 800–3,800/ μ l) and increased concentrations of glucose (142 mg/dl; reference range of 75–128 mg/dl), cholesterol (> 450 mg/dl; reference range of 111–312 mg/dl), triglyceride (> 500 mg/dl; reference range of 30–133 mg/dl), inorganic phosphate (5.7 mg/dl; reference range of 1.9–5.0 mg/dl), alanine aminotransferase (163 U/l; reference range of 17–78 U/l) and alkaline phosphatase (> 3,500 U/l; reference range of 47–254 U/l). The serum concentrations of urea, creatinine, total protein, albumin, aspartate aminotransferase, calcium, sodium and potassium were within the reference ranges. Urine specific gravity was low (1.013). Urinalysis revealed no other abnormalities. The history, clinical signs and laboratory findings suggested HAC.

The plasma ACTH concentrations and serum cortisol, T₄, FT₄ and TSH concentrations were assayed as described previously [13, 14, 20, 33]. Plasma ACTH concentrations were measured using a solid-phase, 2-site chemiluminescent enzyme immunoassay (Immulite[®] ACTH; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) [14, 33]. The basal plasma ACTH concentrations, collected with a 30-min interval, were 92.9 and 96.5 pg/ml (reference range of 6.0–58.0 pg/ml). The ACTH-stimulation test was performed by collecting blood samples for measurement of the cortisol concentration at 0 and 60 min after intravenous administration of 0.25 mg of synthetic ACTH (Cortrosyn[®]; Daiichi Sankyo, Tokyo, Japan). The serum cortisol concen-

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trations were measured using a competitive immunoassay (Immulite® Cortisol; Diagnostics Products Corporation, Los Angeles, CA, U.S.A.) [13]. The basal and post-ACTH serum cortisol concentrations were 11.6 $\mu\text{g/dl}$ (reference range of 0.5–6.0 $\mu\text{g/dl}$) and 46.4 $\mu\text{g/dl}$ (reference range of 6.0–17.0 $\mu\text{g/dl}$), respectively. The serum total T4 concentrations were measured using a homologous solid-phase chemiluminescent enzyme immunoassay (Immulite® canine total T4; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) [13]. The serum FT4 concentrations were measured using equilibrium dialysis (Nichols Institute Diagnostics, San Juan Capistrano, CA, U.S.A.) [20]. The serum TSH concentrations were measured using a homologous solid-phase, two-site chemiluminescent enzyme immuno-metric assay (Immulite® canine TSH; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) [13]. The serum FT4 concentration was within the reference range (FT4: 17.0 pmol/l; reference range of 6.6–40.0 pmol/l), and the total T4 concentration was decreased (total T4: 8.4 nmol/l; reference range of 13.0–51.0 nmol/l). However, the serum TSH concentration was markedly elevated (3.22 ng/ml; reference range of 0.03–0.38 ng/ml). Since a complication of hypothyroidism was suspected, the serum TSH, total T4 and FT4 concentrations were also measured on the 2nd day (TSH, 3.17 ng/ml; total T4, 8.2 nmol/l; FT4, 17.9 pmol/l) after the first visit. The dog did not present clinical signs suggesting hypothyroidism. Ultrasonography was performed using a 6.5 MHz microconvex transducer (ULOGIQ 500 PRO Series, General Electric Company, Tokyo, Japan) and revealed equal enlargement of the bilateral adrenal glands, enlargement of the liver and no abnormalities in size, shape or echogenicity of the bilateral thyroid glands. PDH was suspected, and an MRI of the pituitary fossa was performed with the dog under anesthesia using a 1.5 Tesla superconducting magnet (VISART, Toshiba Medical System Corporation, Tokyo, Japan). With the dog in sternal recumbence, T1-weighted transverse scans of the skull were performed perpendicular to the skull base, from the rostral

clinoid processes to the dorsum sellae, using the spin-echo method, and we obtained a 350-msec repetition time, 15-msec echo time and 2.0-mm thick consecutive slices. T1-weighted sagittal scans were performed at the center of the third ventricle using the spin-echo method, and we obtained a 400-msec repetition time, 15-msec echo time and 2.2-mm thick consecutive slices. MRI revealed that the pituitary gland was not enlarged (height, 4.7 mm; pituitary height/brain area ratio, 0.25; Fig. 1) [21]. A microadenoma of the pituitary was suspected, and transsphenoidal hypophysectomy was performed on the 27th day after the first visit [24, 25].

For histological examination, the pituitary tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Sections 2 μm thick were stained with hematoxylin and eosin (HE). Immunohistochemical staining was performed using the peroxidase-labeled antibody method with a monoclonal mouse antibody to synthetic ACTH_{1–39} (Dako Japan, Kyoto, Japan) [35] diluted 1:200 in 0.01 M phosphate buffered saline (PBS) containing 3% bovine serum albumin (BSA) and a monoclonal mouse antibody to synthetic TSH β (Advanced ImmunoChemical, Long Beach, CA, U.S.A.) diluted 1:100 in 0.01 M PBS containing 3% BSA. The paraffin-embedded 2- μm sections were deparaffinized in xylene and rehydrated through graded ethanol. Endogenous peroxidase activity was blocked by immersing the sections in 0.3% hydrogen peroxide in methanol for 30 min, and the sections were then incubated with the primary antibody overnight at 4°C. Subsequently, horseradish peroxidase conjugated F(ab')₂ fragments of sheep anti-mouse Ig (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.) diluted in 1:100 in 0.01 M 3% BSA was applied to the sections for 60 min at room temperature. After each reaction, the sections were rinsed for 15 min with 0.01 M PBS. The immunoreaction was visualized in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.01% 3,3-diaminobenzidine tetrahydrochloride, 0.005% H₂O₂ and 0.01% sodium nitrate. The sections were lightly counterstained with methyl green.

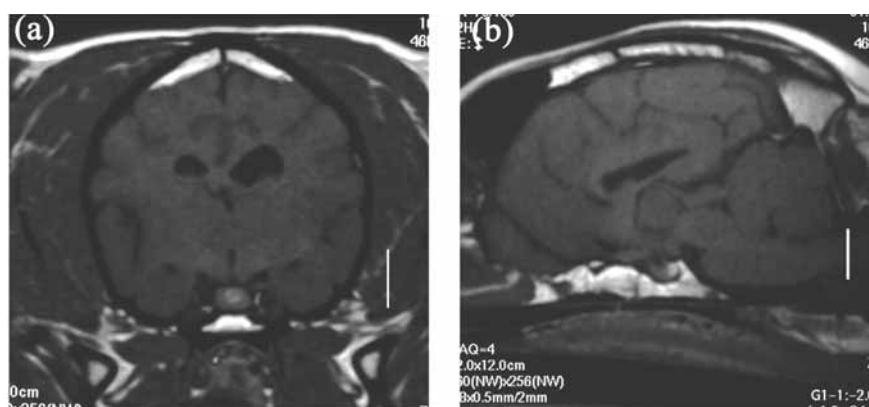


Fig. 1. Gd-T1 weighted images of the pituitary gland in a 6-year-old male Beagle with Cushing's syndrome concurrent with an elevated serum TSH concentration at the time of diagnosis of PDH. (a) Gd-T1 weighted transverse image. The pituitary gland height is 4.7 mm, and the pituitary height/brain area ratio is 0.25. (b) Gd-T1 weighted sagittal image. Bar=10 mm.

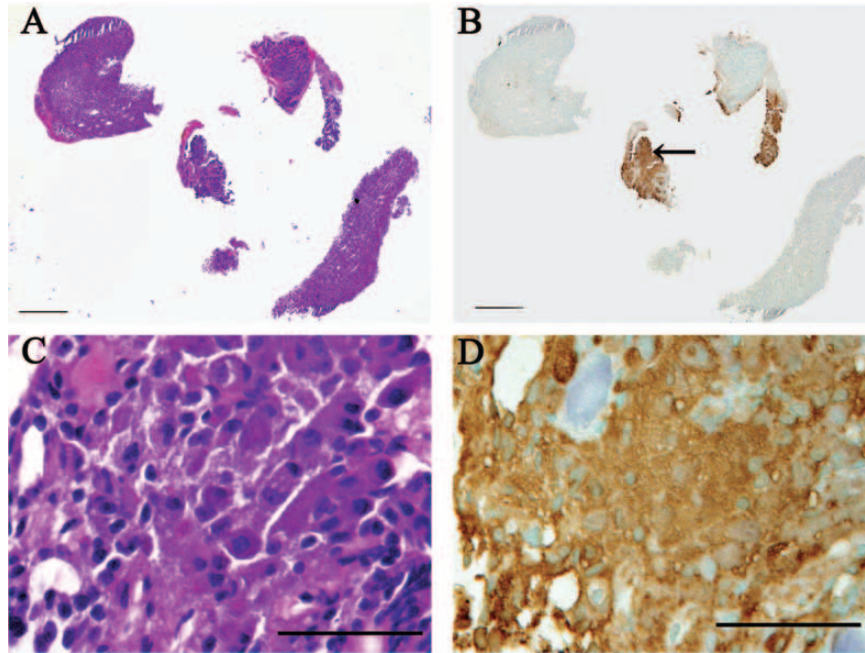


Fig. 2. Immunohistochemistry of resected pituitary tissue using an anti-ACTH antibody. Hematoxylin-eosin staining (A, C) and anti-ACTH immunoreactivity (B, D) in sections of the resected pituitary tissue. The arrow in B indicates C and D. Adenoma cells with strong cytoplasmic immunoreactivity to an anti-ACTH antibody are observed. Bar=500 (A, B) and 50 (C, D) μ m.

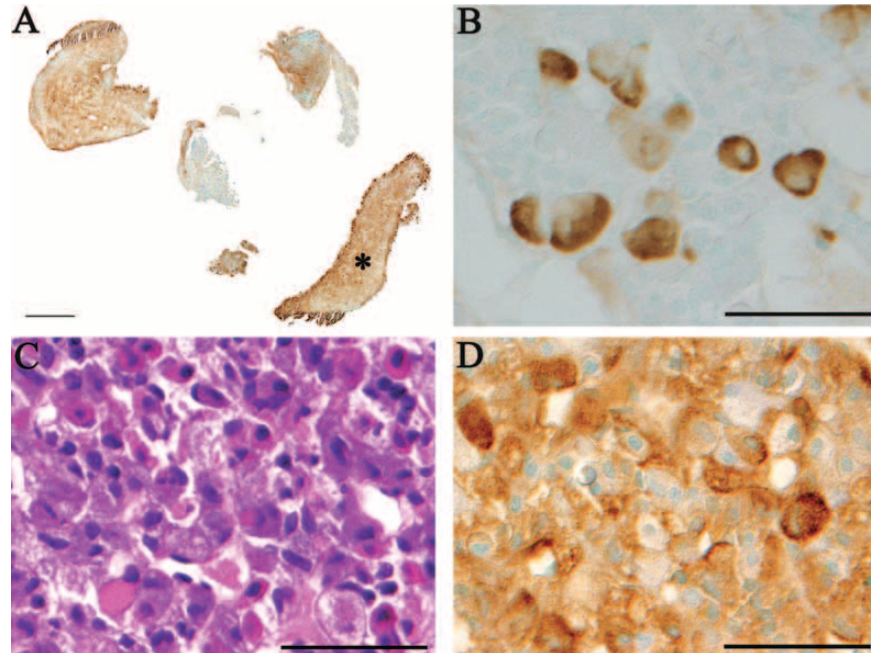


Fig. 3. Immunohistochemistry of resected pituitary tissue using an anti-TSH antibody. Hematoxylin-eosin (HE) staining (C) and anti-TSH immunoreactivity (A, D) in sections of the resected pituitary tissue. The asterisk in A indicates C and D. In the present case, many cells with strong cytoplasmic immunoreactivity to an anti-TSH antibody were observed. Anti-TSH immunoreactivity (B) in a section of anterior lobe of a healthy Beagle's pituitary. Bar=500 (A) and 50 (B, C, D) μ m.

Sections of mouse pituitary already confirmed to be positive for ACTH and TSH were used as a positive control. Sections of pituitary from the present case were incubated with 0.01 M PBS instead of the primary antibodies as a negative control. Pituitary thyrotroph morphometry was performed with the "Image J" software, version 1.36 (<http://rsb.info.nih.gov/ij/>). In 6 healthy Beagles [3 males and 3 females, aged 1–3 years (mean: 1.6 years old) and weighing 8.0–12.0 kg (mean: 10.2 kg)], the numbers of TSH-positive cells in the anterior lobe (AL) were measured. For pituitary thyrotroph morphometry, the AL was divided equally into 5 regions between the dorsal end and ventral end of the pituitary gland in the median section, and 2 fields each were randomly selected from the 5 regions [35]. The number of cells and numbers of TSH-positive cells per field of AL were measured in 10 fields at 400 \times magnification for each dog, and the ratio of TSH-positive cells was calculated. In the present case, the ratio of TSH-positive cells was calculated for three sections 2- μ m thick with a 30- μ m intersection gap.

Histological examination of the resected pituitary gland revealed a basophilic adenoma. HE staining showed that the adenoma cells had a basophilic cytoplasm and irregular nuclei. On immunohistochemical examination, the adenoma was observed to be immunoreactive for ACTH (Fig. 2). In addition, the pituitary gland showed aggregations of TSH-positive cells with variable immunoreactivity for TSH (Fig. 3). In the present case, the aggregations of TSH-positive cells had large and vacuolated cytoplasm that ranged from weak acidophilic to weak basophilic in HE staining.

Table 1. Comparison of the TSH-positive cell ratio

	Healthy Beagles (n=6)	Present case
TSH (ng/ml)	0.30 \pm 0.04	3.22/3.17
Total T4 (nmol/l)	30.7 \pm 5.5	8.4/8.2
FT4 (pg/ml)	25.0 \pm 4.9	17.0/17.9
TSH-positive cell ratio (%)	11.3 \pm 2.9	62.7

All data for healthy beagles are presented as means \pm SD.

The TSH-positive cell ratio was 62.7%, which was greater than that observed in the normal Beagles (Table 1). Taking these results together, we diagnosed this case as having coexistence of a corticotroph adenoma with thyrotroph hyperplasia.

After successful surgery and an uneventful recovery, the dog was released from the hospital on the 19th day after surgery. However, since the entire pituitary was resected, hormone replacement therapy was prescribed that consisted of cortisone acetate and thyroxine, as described previously [15, 24, 25]. Two weeks after hypophysectomy, the basal ACTH concentration was < 9.0 pg/ml. The basal and post-ACTH serum cortisol concentrations were 0.4 μ g/dl and 1.4 μ g/dl, respectively. The serum TSH, total T4 and FT4 concentrations were 0.06 ng/ml, 6.6 nmol/l and 2.6 pmol/l, respectively. Fifteen months after hypophysectomy, the dog was in good physical condition.

A reduction in thyroid hormone concentrations resulting from Cushing's disease is frequently observed. Secondary hypothyroidism results from dysfunctional pituitary thyrotrophs or pituitary thyrotroph hypoplasia, which causes pituitary dwarfism. In dogs, secondary hypothyroidism caused by naturally acquired defects in pituitary thyrotroph function or destruction of pituitary thyrotrophs is uncommon. In contrast, suppression of pituitary thyrotroph function by glucocorticoids is quite common [10]. However, many cases of low thyroid hormone concentrations due to Cushing's disease in dogs have associated euthyroid sick syndrome. Euthyroid sick syndrome is characterized by a low serum total T4 concentration and normal serum TSH concentration, but normal thyroid function [22]. Approximately 60–70% of dogs with HAC are thought to have a decreased basal serum total T4 concentration [9, 10]. Two factors have been identified that may reduce thyroid hormone concentrations in humans and dogs with Cushing's disease. First, although uncommon, pituitary tumors may impair secretion of TSH through the destruction of thyrotrophs by an expanding, space-occupying mass [10]. Second, high levels of cortisol concentrations alter the physiological function of thyroid hormone. There are several proposed mechanisms for the alterations in thyroid hormone concentrations, including the inhibition of TSH secretion, reduced serum protein binding of T4, reduced T3 production and degradation and possibly inhibition of peripheral 5'-deiodination of T4 [11, 19, 27, 29].

TSH is synthesized and secreted only by thyrotrophs in the AL. Thyrotrophs comprise approximately 5% and 10% of anterior pituitary cells in humans [1, 31] and rats [34], respectively. In humans, pituitary thyrotroph hyperplastic cells are large, ovoid, pale with vacuolated chromophobic to slightly acidophilic cytoplasm with several lysosomes and variable TSH-immunoreactivity [1]. On the other hand, thyrotroph adenoma cells usually have a chromophobic appearance, although they stain occasionally as either basophilic or acidophilic. Thyrotroph adenoma cells are often arranged in cords. They frequently appear polymorphous and are characterized by large nuclei and prominent nucleoli [4]. Thyrotrophs are located chiefly in the anteromedial portion of the pituitary gland in humans [31]. On the other hand, there are few reports of canine thyrotrophs. One study demonstrated the localization and percent volume of thyrotrophs in the AL using immunocytochemistry [8] and suggested that canine thyrotrophs were not dispersed equally throughout the AL, but were localized in a chain formation in the centromedian region. In that study, the percent volume of the thyrotrophs in the AL were 19.2 \pm 2.2 (mean \pm SD)% (6 male Beagles) and 27.5 \pm 4.8% (6 female Beagles), which are greater than our results. Accordingly, using criteria for thyrotroph hyperplasia in humans and the percent volume of the thyrotrophs in the AL, the aggregations of TSH-positive cells were diagnosed as thyrotroph hyperplasia in the present case.

Hyperplasia is defined as a cell proliferation induced by a known stimulus and is a controlled process that stops when

the stimulus is removed. Many cases of thyrotroph hyperplasia resulting from prolonged primary hypothyroidism have been reported in humans [2, 6, 17, 28], rats [5, 7, 34] and mice [6, 26]. One study with a limited number of patients found thyrotroph hyperplasia in 7% of all normal pituitaries [32]. Thyrotroph hyperplasia can be explained by negative feedback, in which reduced circulating levels of thyroid hormone result in overstimulation of thyrotrophs directly or indirectly by either thyrotrophin releasing hormone or other hypothalamic factors [31]. Thyrotroph hyperplasia is usually reversible with institution of thyroxine replacement therapy [2, 17, 28]. On the other hand, induction of thyrotroph hyperplasia followed by adenoma is easily obtained in mice and, to a lesser degree, in rats after thyroidectomy [6, 7], propylthiouracil treatment [26] or even chronic iodine deficiency [5].

In the present case, clinical signs suggesting hypothyroidism were not observed, but primary hypothyroidism was suspected because the serum T4 concentrations were decreased and serum TSH concentrations were increased. Primary hypothyroidism is the most common cause of naturally occurring thyroid failure in adult dogs. Two histological forms of primary hypothyroidism predominate in dogs [18]. In the first form, lymphocytic thyroiditis is characterized histologically by a diffuse infiltration of lymphocytes, plasma cells and macrophages within the thyroid gland, resulting in the progressive destruction of follicles and secondary fibrosis. The second form is idiopathic atrophy of the thyroid gland. This form is characterized microscopically by loss of the thyroid parenchyma, which is replaced by adipose tissue. In the present case, we did not perform a histological examination of the thyroid glands, but the low serum T4 concentrations and high serum TSH concentrations indicated primary hypothyroidism [30]. The pituitary thyrotroph hyperplasia in the present case was thought to be due to primary hypothyroidism. There are several reports suggesting the coexistence of Cushing's disease and primary hypothyroidism in dogs [3, 16]; however, no report has histologically demonstrated the coexistence of a corticotroph adenoma and thyrotroph hyperplasia.

In conclusion, this case with Cushing's disease and elevated serum TSH concentrations is the first case report to show the histological confirmation of the coexistence of a corticotroph adenoma and thyrotroph hyperplasia.

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