

Efficacy of Transsphenoidal Surgery on Endocrinological Status and Serum Chemistry Parameters in Dogs with Cushing's Disease

Yasushi HARA^{1)*}, Takahiro TESHIMA¹⁾, Takahiro TAODA²⁾, Hirokazu ISHINO¹⁾, Yoshinori NEZU¹⁾, Yasuji HARADA¹⁾, Takuya YOGO¹⁾, Hiroyuki MASUDA¹⁾, Akira TERAMOTO⁴⁾ and Masahiro TAGAWA¹⁾

¹⁾Divisions of Veterinary Surgery, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino-shi, Tokyo 180-8602, ²⁾Department of Small Animal Surgery 2, School of Veterinary Medicine, Kitasato University, 35-1 Higashi 23-bancho, Towada-shi, Aomori 034-8628 and ³⁾Department of Neurosurgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan

(Received 21 August 2009/Accepted 16 November 2009/Published online in J-STAGE 9 December 2009)

ABSTRACT. Postoperative changes in endocrinological status and serum chemistry during the 4 years after transsphenoidal surgery (TSS) in 25 dogs with Cushing's disease were investigated in a prospective study. In all 25 dogs, Cushing's disease was diagnosed from resected pituitary tissues as a corticotroph adenoma in the anterior lobe of the pituitary. Prior to TSS, all 25 dogs showed hypercortisolemia. After TSS, the ACTH stimulation test showed continued low serum cortisol concentrations in 21 dogs (84%). In addition, the serum thyroid stimulating hormone concentrations decreased sequentially, while the serum T4 concentrations tended to increase due to the postoperative hormone substitution therapy utilized to avoid secondary hypothyroidism. In regard to serum chemistry, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and total cholesterol are commonly increased in canine Cushing's disease. In this study, ALP, ALT and total cholesterol were increased in 23 cases (92%), 19 cases (76%) and 20 cases (80%), respectively. However, postoperatively, these concentrations gradually decreased. The postoperative serum concentrations of ALP at 1 year, that of ALT at six months to 2 years and that of total cholesterol over the course of the 4 years decreased significantly compared with the concentrations before TSS. These results show that TSS is an effective treatment for canine Cushing's disease and for long-term improvement of hypercortisolemia. Moreover, TSS is effective in improvement of hypercortisolism, such as increased concentrations of serum ALP, ALT and total cholesterol.

KEY WORDS: canine, Cushing's disease, hypophysectomy, transsphenoidal surgery (TSS), pituitary tumor.

J. Vet. Med. Sci. 72(4): 397-404, 2010

Cushing's disease is a common endocrinopathy in dogs that is caused by corticotroph adenoma that secretes adrenocorticotrophic hormone (ACTH). In humans, the first choice of treatment for Cushing's disease is transsphenoidal surgery (TSS) [6, 10, 21]. If a patient with Cushing's disease treated by TSS does not present remission or if the pituitary adenoma is difficult to resect, then radiotherapy is selected [14, 31]. In contrast, in the treatment of canine Cushing's disease, most dogs are treated with mitotane and trilostane [1, 5, 8, 13, 16, 27, 38, 40, 43]. However, in human medicine, the use of mitotane and trilostane to decrease cortisol excess is limited and is selected only to improve hypercortisolemia before TSS, when re-operation is too difficult, when the localization of the corticotroph adenoma is not obvious, or as a short-term treatment until radiotherapy [6, 10, 21].

In veterinary clinical medicine, the prevalence of advanced imaging modalities for diagnosis, such as computer tomography and magnetic resonance imaging (MRI), has enabled detailed visualization of the pituitary. Moreover, follow-up investigations of canine Cushing's disease treated by TSS have shown effective outcomes [23, 24]. A few reports have demonstrated long-term survival, disease-

free fractions, complications of TSS and the effects of long-term alternations in endocrinological status in canine Cushing's disease [23, 24]. However, few studies have investigated the effects of TSS on long-term alternations in serum chemistry. Therefore, in the present study, endocrinological status and serum chemistry over the course of 4 years after TSS in 25 dogs with Cushing's disease were examined.

MATERIALS AND METHODS

Animals: Twenty-five dogs with Cushing's disease referred to the Nippon Veterinary and Life Science University over an 8-year period (2000-2008) underwent TSS. Crossbred and purebred dogs were both represented (Table 1). Seventeen of the dogs were male (6 castrated), and 8 were female (5 spayed). The age at the time of surgery ranged from 4 to 11 years (median of 8 years), and the body weights of the dogs ranged from 3.2 to 33.4 kg (median of 11.1 kg).

In most of the dogs, the characteristic clinical signs of canine hypercortisolism were obvious, including polyuria, polydipsia, polyphagia, truncal obesity, muscle atrophy and skin changes such as alopecia and calcinosis cutis.

Diagnosis of Cushing's disease: Cushing's disease was diagnosed by clinical signs, routine laboratory examination, endocrine examination (ACTH stimulation test), abdominal ultrasonography and MRI of the pituitary [25, 50, 51].

* CORRESPONDENCE TO: HARA, Y., Division of Veterinary Surgery, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino-shi, Tokyo 180-8602, Japan.
e-mail: hara@nvlu.ac.jp

Table 1. Clinical characteristics of the patients included in this study

No.	Breed	Age (Years)	Gender ^{a)}	Weight (kg)	P/B ratio	Pre-cortisol ^{b)} ($\mu\text{g/dl}$)	Post-cortisol ^{c)} ($\mu\text{g/dl}$)
1	Bull Terrier	5	FS	17.5	0.430	4.3	34.3
2	Cavalier KC Spaniel	7	MC	11.1	0.491	3.9	31.7
3	West Highland White Terrier	11	FS	8.3	0.698	3.8	70.2
4	Shiba	10	M	21.9	0.487	2.7	26.7
5	Beagle	8	M	17.0	0.691	10.7	77.6
6	Shiba	7	M	10.9	0.335	6.3	51.2
7	Beagle	11	M	11.6	0.366	5.7	24.4
8	Shetland Sheepdog	5	M	13.8	0.705	15.8	79.2
9	Golden Retriever	6	MC	33.4	0.271	5.5	45.1
10	Shiba	8	F	11.2	0.286	7.6	53.2
11	Yorkshire Terrier	11	FS	3.2	0.388	9.3	32.8
12	Cavalier KC Spaniel	7	FS	6.8	0.327	21.3	73.4
13	Miniature Dachshund	9	F	6.6	0.366	2.2	30.7
14	Mongrel	8	MC	10.9	0.450	21.5	27.4
15	Pembroke Welsh Corgi	4	F	11.2	0.381	10.9	50.0
16	Beagle	6	M	12.5	0.470	6.2	40.7
17	Mongrel	10	MC	14.6	0.264	2.6	39.6
18	Yorkshire Terrier	6	MC	5.8	0.372	8.5	67.3
19	Mongrel	8	M	10.2	0.323	8.5	59.6
20	Miniature Dachshund	10	M	9.1	0.390	7.1	50.0
21	Shetland Sheepdog	10	FS	7.7	0.288	7.1	51.9
22	Beagle	6	MC	13.9	0.249	11.6	46.4
23	Mongrel	8	M	16.1	0.240	7.2	24.8
24	Miniature Dachshund	9	M	8.6	0.530	19.9	56.4
25	Mongrel	11	M	9.5	0.373	15.7	50.6

a) Gender: F=female intact, FC=female spayed, M=male intact, MC=male castrated. b) Cortisol: Pre-operative serum basal cortisol concentration. c) Cortisol: Pre-operative serum post-ACTH cortisol concentration; values were obtained at 60 min after intravenous administration of 0.25 mg of synthetic ACTH. Reference range: Pre-cortisol, 0.5–6.0 $\mu\text{g/dl}$; post-cortisol, 6.0–17.0 $\mu\text{g/dl}$.

Pituitary imaging: The pituitary size and localization of the gland in relation to surgical landmarks were assessed by MRI in all 25 dogs. MRI was performed on anesthetized dogs using a 1.5-Tesla superconducting magnet. T1-weighted transverse images were made before and after an intravenous bolus injection of 0.1 mmol contrast medium (Omniscan[®]; gadodiamidehydrate; Daiichi Sankyo, Tokyo, Japan) per kg body weight. Enlarged pituitaries were distinguished from non-enlarged pituitaries by the ratio between the height of the pituitary gland and the area of the brain (P/B ratio), as described previously [29]. Enlarged pituitaries have a P/B ratio greater than 0.31, and non-enlarged pituitaries have a ratio equal to or less than 0.31.

Surgery and postoperative medication: TSS was performed [37], and the resected pituitary tissue was diagnosed in each case as a corticotroph adenoma in the anterior lobe of the pituitary by immunohistochemical analysis, as described previously [50, 51]. Postoperative hormone substitution therapy was performed as described previously [25, 36, 37]. Briefly, hydrocortisone (Hydrocortone, Banyu Pharmaceutical Co., Ltd., Tokyo, Japan; 1 mg/kg, i.v., TID) and desmopressin (Desmopressin, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan; 0.2 $\mu\text{g/kg}$, s.c., or 1 drop into the conjunctival sac, SID-BID) were administered directly after removal of the pituitary gland. When the dogs had resumed drinking and eating, oral substitution therapy was started

with prednisolone (Predonine, Shionogi & Co., Ltd., Osaka, Japan; 0.5 mg/kg, p.o., BID) and levothyroxine sodium (Thyradin-S, Aska Pharmaceutical Co., Ltd., Tokyo, Japan; 20 $\mu\text{g/kg}$, p.o., BID). Desmopressin acetate was administered for approximately 2 weeks and continued if polyuria due to central diabetes insipidus persisted.

Endocrine tests and hormone determination: An ACTH stimulation test was performed by collecting blood samples for measurement of the serum cortisol concentration at 0 and 60 min after intravenous administration of 0.25 mg of synthetic ACTH (Cortrosyn[®]; Daiichi Sankyo) [22]. The serum cortisol, total T4 and thyroid stimulating hormone (TSH) concentrations were assayed as described previously [19, 28, 42]. All ACTH stimulation tests were performed at 3 to 4 hr after administration of the morning hormone substitution therapy.

Follow-up: All dogs were re-evaluated at 1, 2, 4, 12 and 24 weeks and 1, 2, 3 and 4 years after TSS. Re-evaluation included a serum chemistry profile (Fuji DRI-CHEM 7000V, Fujifilm Corporation, Tokyo, Japan) including alkaline phosphatase (ALP), alanine aminotransferase (ALT), total cholesterol, basal and post-ACTH serum cortisol, serum TSH and serum total T4 concentrations.

Remission was defined as a basal serum cortisol concentration <6 $\mu\text{g/dl}$, post-ACTH serum cortisol concentration <17 $\mu\text{g/dl}$ and resolution of the clinical signs of hypercorti-

solism. Residual disease was defined as an early postoperative (< 2 months after TSS) basal cortisol concentration $\geq 6 \mu\text{g/dl}$, post-ACTH serum cortisol concentration $\geq 17 \mu\text{g/dl}$ and/or presence of remnant pituitary tumor tissue on early postoperative MRI. Postoperative mortality was defined as death within 4 weeks after surgery irrespective of the cause of death. Recurrence was defined as a basal cortisol concentration $\geq 6 \mu\text{g/dl}$, post-ACTH serum cortisol concentration $\geq 17 \mu\text{g/dl}$ or recurrence of clinical signs of hypercortisolism after initial complete remission.

Statistical analysis: Survival fraction was analyzed by the Kaplan-Meier estimate procedure. The survival period was defined as the interval between the date of surgery and the date on which the dog was last known to be alive or the date of its death due to any cause. Dogs that were still alive or died of causes unrelated to hypophysectomy or hypercortisolism at the time of follow-up were counted as censored cases.

Results are presented as least square means (LSM) \pm standard error. Changes in serum cortisol, TSH and total T4 concentrations and serum chemistry profiles (ALP, ALT and total cholesterol concentrations) were analyzed by repeated-measures analysis of variance using the SAS version 9.1 software (SAS Institute, Inc., Cary, NC, U.S.A.), and the differences among LSMs were analyzed using Tukey-Kramer post hoc tests. Differences were considered significant when $P < 0.05$.

RESULTS

Follow-up: One dog died within 4 weeks after surgery. The dog (dog 11) died 26 days after TSS due to traumatic thoracic vertebral fractures. Hypercortisolism went into remission in 21 dogs. In 3 dogs (dogs 3, 8 and 20), there was residual disease based on the post-ACTH serum cortisol concentrations at 1 week after TSS. In 1 dog (dog 18), signs of hypercortisolism recurred with high post-ACTH serum cortisol concentrations at 3 months after TSS. The follow-up results are presented by curves of the estimated survival fraction. The 1-year estimated survival rate was 92% (95% confidence interval [CI], 81–100%), while the 2-, 3- and 4-year estimated survival rate was 81% (CI, 65–98%; Fig. 1).

ACTH-stimulation test: The basal and post-ACTH serum cortisol concentrations before TSS were $9.4 \pm 1.2 \mu\text{g/dl}$ (reference range of 0.5–6.0 $\mu\text{g/dl}$ [18]) and $49.7 \pm 9.2 \mu\text{g/dl}$ (reference range of 6.0–17.0 $\mu\text{g/dl}$ [18]), respectively. After TSS, the basal and post-ACTH serum cortisol concentrations decreased significantly compared with those before TSS (Fig. 2A and 2B).

Serum TSH and total T4 concentrations: The serum TSH concentration before TSS was $0.14 \pm 0.01 \text{ ng/ml}$ (reference range of 0.03–0.38 ng/ml). After TSS, the serum TSH concentration decreased significantly until 4 years postoperatively compared with that before TSS (Fig. 3A). The serum total T4 concentration before TSS was $0.72 \pm 0.14 \mu\text{g/dl}$ (reference range of 1.2–3.9 $\mu\text{g/dl}$). After TSS, the serum total T4 concentrations tended to increase. The serum total

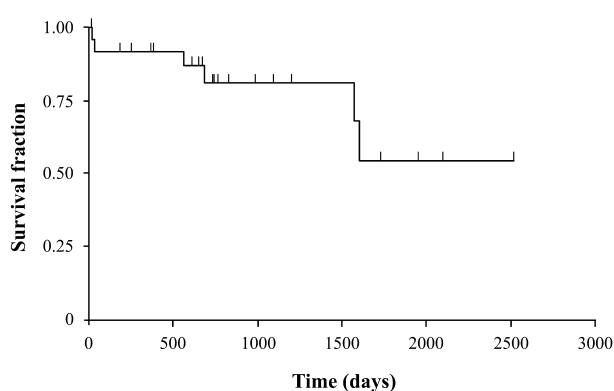


Fig. 1. Survival curve after transsphenoidal surgery in 25 dogs with Cushing's disease. Censored cases (dogs that died from unrelated causes or that were still alive at the time of follow-up) are represented by vertical bars.

T4 concentration at 3 months after TSS increased significantly compared with that before TSS (Fig. 3B).

Serum chemistry profile: The serum concentrations of ALP, ALT and total cholesterol before TSS were $1,306 \pm 349 \text{ U/l}$ (reference range of 47–254 U/l [51]), $154 \pm 30 \text{ U/l}$ (reference range of 17–78 U/l [51]), and $443 \pm 33 \text{ mg/dl}$ (reference range of 111–312 mg/dl [51]), respectively. After TSS, the serum concentrations of ALP and ALT gradually decreased. The serum concentrations of ALP at 1 and 2 years after TSS were significantly decreased compared with that before TSS (Fig. 4A). The serum concentrations of ALT at 3 months to 2 years were significantly decreased compared with that before TSS (Fig. 4B). The serum concentrations of total cholesterol after TSS were significantly decreased compared with that before TSS (Fig. 4C).

DISCUSSION

Cushing's disease is a complex of physical and biochemical abnormalities resulting from chronic exposure to cortisol excess caused by an ACTH-secreting corticotroph adenoma. Moreover, dogs with Cushing's disease may have serious and life-threatening secondary complications and concomitant diseases because of the long-term cortisol excess [18, 39].

The low-dose dexamethasone test (LDDS) has been considered the "gold standard" for differentiation of canine hypercortisolism. In contrast, the ACTH stimulation test is the most commonly used "screening" test for confirming a diagnosis of canine hypercortisolism because it is safe, simple and not time-consuming. In previous reports, the ACTH stimulation test was shown to be more specific than the LDDS, but the LDDS proved to be more sensitive than the ACTH stimulation test [18, 26, 53]. In regard to diagnostic accuracy, a previous study as shown the diagnostic accuracies of the ACTH stimulation test and the LDDS to be 84–93 and 58–92%, respectively [18]. In the present study, we used the ACTH stimulation test without the LDDS to diag-

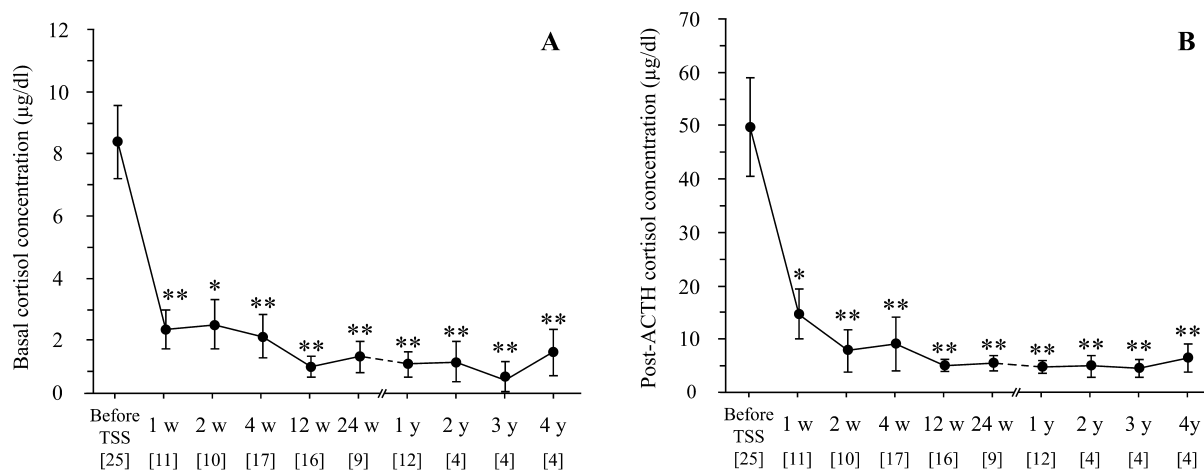


Fig. 2. Changes in serum cortisol concentrations after transsphenoidal surgery. The basal cortisol concentrations (A). The post-ACTH cortisol concentrations (B). *, $P < 0.05$ vs. before TSS. **, $P < 0.01$ vs. before TSS. Time after transsphenoidal surgery (TSS): w, week; y, year. The number of cases is shown below each time point.

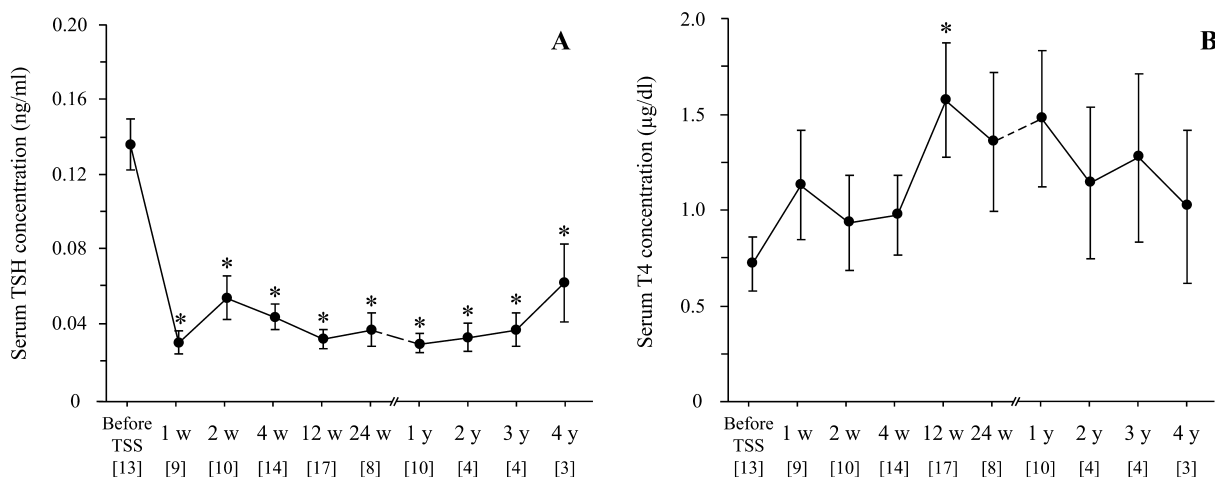


Fig. 3. Changes in serum TSH and total T4 concentrations after transsphenoidal surgery. The serum TSH concentrations (A). The serum total T4 concentrations (B). *, $P < 0.05$ vs. before TSS. Time after transsphenoidal surgery (TSS): w, week; y, year. The number of cases is shown below each time point.

nose hypercortisolism in the 25 dogs for 2 reasons. First, the post-ACTH serum cortisol concentrations in all 25 dogs were over $22 \mu\text{g/dl}$, and values of $22 \mu\text{g/dl}$ or higher are consistent with a diagnosis of hypercortisolism. Second, abdominal ultrasonography was performed on all 25 dogs, and the results revealed that the bilateral adrenal glands were equally enlarged and that the width of the left adrenal in each dog was over 7.5 mm. In one study, this parameter showed a sensitivity of 81% and a specificity of 100% in detecting adrenal enlargement in canine hypercortisolism [7]. We agree that the LDDS added to the ACTH stimulation test and abdominal ultrasonography enhances the diagnosis of hypercortisolism, but no screening test shows 100% sensitivity and specificity. In the present study, the postoperative ACTH stimulation test showed continued low serum

cortisol concentrations in 21 dogs (84%) during the period of examination. These results indicated that resection of the ACTH-secreting corticotroph adenomas that caused cortisol excess by TSS reduced the serum cortisol concentrations and decreased the production and secretion of cortisol from the adrenal cortex. In previous reports, the production and secretion of cortisol was shown to decrease quickly after TSS, and moreover, the responses of ACTH and cortisol to exogenous corticotropin releasing hormone were slight [33, 34].

In the previous reports, remission and recurrence were defined by the urine cortisol-to-creatinine ratio (UCCR) in duplicate at 24 hr after glucocorticoid medication and resolution/return of clinical signs of hypercortisolism [23, 24]. This is the most commonly used method in humans to define

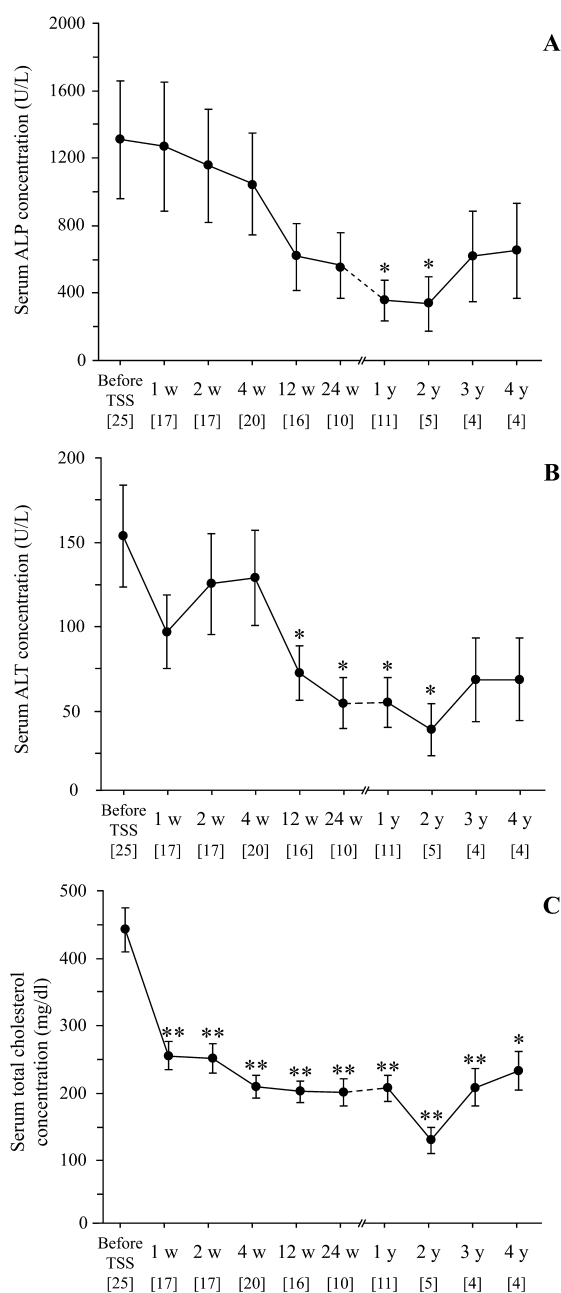


Fig. 4. Changes in serum chemistry profiles after transsphenoidal surgery. The serum ALP concentrations (A). The serum ALT concentrations (B). The serum total cholesterol concentrations (C). *, $P < 0.05$ vs. before TSS. **, $P < 0.01$ vs. before TSS. Time after transsphenoidal surgery (TSS): w, week; y, year. The number of cases is shown below each time point.

remission or recurrence. However, in the present study, we used the basal and post-ACTH serum cortisol concentrations instead of the UCCR because we were not able to collect urine samples at 24 hr after every prednisolone

administration in each case. Postoperatively, the basal and post-ACTH serum cortisol concentrations are affected by hormone substitution therapy because the hypothalamus-pituitary-adrenal axis is affected by prednisolone and desmopressin. The response of the adrenal glands to ACTH stimulation may be inhibited by oral prednisolone medication, and remnant pituitary ACTH secretion may be stimulated by desmopressin. In the present study, we performed the ACTH-stimulation test without discontinuation of hormone substitution therapy, so the results may have been influenced by administration of the prednisolone or desmopressin medication. However, the ACTH stimulation tests were performed under the same conditions in all dogs at 3 to 4 hr after administration of the morning hormone substitution therapy, so comparisons between the pre- and post-TSS values are valid.

Based on our definition in the present study, 3 recurrent cases (12%) and 1 residual disease case (4%) were observed, but we were unable to investigate each case. There are many reports of postoperative results with regard to long-term survival and the recurrence fraction in humans [6]. However, in canine Cushing's disease, only two reports, by the same group of researchers, describe postoperative results with regard to long-term survival and the recurrence fraction in significant numbers of Cushing's disease dogs [23, 24]. In the present study, the dogs that died of unrelated causes or that were still alive at the time of follow-up were counted as censored cases. The present study included 25 dogs, and the 1-year estimated survival rate was 92%, while the 2-, 3- and 4-year estimated survival rate was 81%. These results are similar to those of previous reports [23, 24]. Comparison of these results with the results of medical treatment with mitotane or trilostane is difficult. However, approximately half of the dogs with Cushing's disease treated with mitotane have shown recurrence within a year of initiation of treatment [24]. In regard to trilostane, the dose of trilostane was adjusted in most of the dogs and was increased in 30–43% of the cases [1, 38, 44]. Based on the comparison with these previous reports of medical treatments, TSS may be a more effective treatment of canine Cushing's disease.

In humans, only the pituitary adenoma tissue is resected by TSS. However, in dogs, selective resection is difficult, and the whole pituitary, including normal tissue, is resected. Therefore, postoperative hormone substitution therapy, using glucocorticoid (prednisone) and thyroxine (levothyroxine sodium) is necessary [32, 35]. In the present study, decreasing TSH concentrations were observed after TSS, but the T4 concentrations were increased compared with the levels before TSS. This was due to administration of levothyroxine sodium when the dogs resumed drinking and eating to avoid secondary hypothyroidism. Thus, adequate hormone substitution therapy resulted in none of the 25 dogs showing clinical symptoms suggesting secondary hypothyroidism.

Serum chemistry abnormalities commonly include mild to marked elevations in liver enzymes, such as ALP, ALT

and γ -glutamyl transpeptidase, and in cholesterol, triglycerides and glucose and a decrease in the blood urea nitrogen level [18, 20]. In particular, the concentrations of ALP and total cholesterol are increased in more than 90% of dogs with Cushing's disease [18, 52]. In the present study, before TSS, the ALP, ALT and total cholesterol concentrations were increased in 23 cases (92%), 19 cases (76%) and 20 cases (80%), respectively. Two factors may increase ALP in dogs with Cushing's disease [3, 17, 45, 49, 52]. First, as a result of hepatic glycogen deposition and vacuolization impinging on the biliary tract, the rate of ALP production increases. Second, the induction of a specific hepatic isoenzyme by either endogenous or exogenous glucocorticoids may increase ALP. This corticosteroid-induced isoenzyme of ALP, which is unique to the dog, is increased markedly by the excess of either endogenous or exogenous glucocorticoids, and it is the most common factor for increased ALP concentrations in dogs with Cushing's disease [3, 4, 18]. In addition, ALT activity is commonly increased in dogs with Cushing's disease [3, 4, 18]. This is usually a mild increase, which is believed to occur secondary to damage caused by swollen hepatocytes, glycogen accumulation or interference with hepatic blood flow [3, 4].

There are few studies that have investigated the effects of mitotane on canine Cushing's disease in regard to improvement of hepatic enzymes. In the treatment of canine Cushing's disease with mitotane, the recovery of hepatic enzymes may require 6 months to several years [41]. In regard to trilostane, one study found that half of the dogs with elevated pretreatment serum ALP concentrations treated with once-daily trilostane showed no significant decrease during treatment [44]. In another study investigating twice-daily administration of trilostane, there was a significant decrease in the mean serum ALP concentration at the 6 month re-evaluation, but there were several dogs whose serum ALP concentrations persisted outside the normal range [43]. This can be explained by the short duration of trilostane activity. It is known that the action of trilostane begins shortly after administration, but lasts for only a few hours. With once-daily administration, we would expect the cortisol concentration to increase during the day [44]. The short period of suppression is sufficient to lead to an improvement in the clinical condition but may not result in hematologic and biochemical improvements. In the present study, elevated ALP and ALT concentrations decreased after TSS, but the mean ALP concentration persisted outside the normal range. It is likely that TSS led to normocorticism and restored hepatic function, resulting in lowering of the endogenous corticosteroid-induced isoenzyme of ALP. However, prednisolone was administered after TSS as an exogenous glucocorticoid, which may be the reason why the ALP concentration did not restore completely to the normal range. Therefore, we speculated that the recovery period of hepatic enzymes might be influenced by not only the degree of hepatopathy resulting from hypercortisolemia prior to TSS, but also by the hormone substitution therapy after TSS. From the changes of hepatic enzymes after TSS, long

term recovery is required to improve the liver enzymes in a manner to the effects of mitotane and trilostane, and administration of medication, such as ursodeoxycholic acid, before and after TSS may enhance improvement of the liver enzymes.

In the present study, the total cholesterol concentration decreased significantly after TSS. This can be explained by 2 factors [15, 18, 30, 48]. First, lipolysis was improved by the resolved hypercortisolemia as a result of TSS. Second, the effect of administration of levothyroxine sodium may prevent secondary hypothyroidism after TSS because thyroid hormone stimulates the uptake of cholesterol to the intracellular fraction.

In conclusion, we have confirmed that TSS is an effective treatment for canine Cushing's disease to improve hypercortisolemia. Moreover, TSS may be effective in improvement of serum chemistry abnormalities, such as increased concentrations of serum ALP, ALT and total cholesterol.

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