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Clinical Relationship between Cholestatic Disease and Pituitary-Dependent Hyperadrenocorticism in Dogs: A Retrospective Case Series

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Background: A high prevalence of cholestatic disease, including gallbladder mucocele (GBM), has been reported in dogs with naturally occurring pituitary-dependent hyperadrenocorticism (PDH).

Hypothesis/Objectives: Differences exist in the clinical features of dogs with PDH and concurrent cholestatic disease, and also is the management of these dogs with trilostane.

Animals: Sixty-five client-owned dogs with naturally occurring PDH.

Methods: This was a retrospective, observational case series. Each dog was treated with trilostane for at least 3 months before the study, and had a good clinical response, as determined by owners. Statistical comparisons of clinical signs, results of routine blood tests, basal and post-ACTH cortisol concentration, and optimal trilostane dosage were made after dogs were separated into the following 3 groups by ultrasonographic imaging: normal on ultrasound (NOU) group, cholestasis group, and GBM group.

Results: The GBM group had more severe clinical signs and significantly different total serum cholesterol concentration and post-ACTH stimulation cortisol concentration at the time of diagnosis. Dogs that weighed <6 kg had a significantly higher prevalence of cholestatic disease than did the other dogs ($P = .003$). The optimal trilostane dosages for the GBM and cholestasis groups were 2.5 and 1.5 times the dosage of the NOU group, respectively ($P < .001$).

Conclusions and Clinical Importance: Gallbladder disease associated with cholestatic disease is correlated with PDH in dogs, in both its clinical features and drug management. These findings may be associated with hypercholesterolemia, unidentified genetic factors, and the hydrophobic nature of trilostane.

Key words: Cholesterol; Female; Mucocele; Trilostane.

Hyperadrenocorticism (HAC) is a common endocrine disease of dogs and is classified as pituitary-dependent hyperadrenocorticism (PDH), adrenal-dependent hyperadrenocorticism (ADH), or iatrogenic depending on the cause of the excessive cortisol.[1–3] Hyperadrenocorticism is characterized by variable clinical signs, and several medical complications may develop secondary to prolonged excess of cortisol. Well-described complications include systemic hypertension, pancreatitis, diabetes mellitus, steroid hepatopathy, pulmonary thromboembolism, and pituitary macrotumor syndrome.[1–8] Patients with PDH can be treated with drugs, surgical procedures, radiation therapy, or a combination of these options.[3,9–12]

Abbreviations:

ACTH	adrenocorticotrophic hormone
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AT	adrenal tumor
BCS	body condition score
GBM	gallbladder mucocele
GGT	gamma-glutamyl transpeptidase
HAC	hyperadrenocorticism
NOU	normal on ultrasound
PDH	pituitary-dependent hyperadrenocorticism
T-chol	total cholesterol
TG	triglyceride

Trilostane[a] is a drug that is now used worldwide in dogs with HAC since its development as a veterinary product in 1998.[12–19] It is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase that inhibits the synthesis of glucocorticoids, mineralocorticoids, and adrenal androgens.[12]

Gallbladder mucocele (GBM) is an immobile abnormal accumulation of mucin accompanied by hyperplasia of mucus-secreting gallbladder epithelium[20–22] that has been suggested to have a possible association with endocrinopathies, especially HAC.[21,23,24] It is highly prevalent in dogs with HAC, with a prevalence 29 times higher than in dogs without HAC.[24] Conversely, only 23% dogs with GBM had HAC.[21] Other studies suggest that the association between HAC and cholestatic disease is less clear.[21,23,24]

Our study aimed to determine the association of the clinical features, pathophysiology, and management of cholestatic disease, including GBM, with PDH in dogs.

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Materials and Methods

Study Population

A total of 121 privately owned dogs with naturally occurring HAC presented to the Veterinary Medical Teaching Hospital, Seoul National University, Seoul, South Korea, from 2010 to 2014 were studied. The diagnosis of HAC was made on the basis of history, physical examination findings, results of routine blood testing, and endocrine function test results.[3] The endocrine tests that were used to obtain a diagnosis were as follows: ACTH stimulation test, low-dose dexamethasone suppression test (LDDST), and urinary cortisol:creatinine ratio (UCCR).[3,25–27] Some patients also were evaluated by high-dose dexamethasone suppression test (HDDST) for differentiation of PDH. All patients underwent abdominal radiography and ultrasonographic examination at the time of diagnosis; some patients also were evaluated by computed tomography (CT).

Of the 121 patients, 18 adrenal tumor (AT) patients that had a confirmed, positive diagnosis of AT by ultrasonographic evaluation and endocrine function tests were excluded.[6,25] Subsequently, of the 103 dogs with PDH, those managed at local veterinary hospitals after diagnosis and those not followed up appropriately were excluded. The remaining 65 patients were followed up until they had a good clinical response, as determined by the owner, with a post-ACTH cortisol concentration of 2.0–9.1 μg/dL. This range of post-ACTH cortisol concentrations has been recommended by the manufacturer of trilostane. Finally, patients with appropriate follow-up procedure and response to therapy were divided into 3 groups based on ultrasonographic findings of the gallbladder at the time of diagnosis: normal on ultrasound (NOU) group (n = 18), cholestasis group (n = 32), and GBM group (n = 15).[21,22,27–31]

Diagnostic Tests

A thorough history and physical examination were performed in all dogs (n = 65). A CBC, urinalysis, and routine biochemical profile including alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) were performed in all dogs. Triglyceride (TG) and total cholesterol (T-Chol) concentrations were determined in some dogs: 83% (n = 15) of the NOU group, 78% (n = 25) of the cholestasis group, and 93% (n = 14) of the GBM group, respectively.[1–3,14] Adrenocorticotropic hormone stimulation tests were performed in all dogs (n = 65), and in some cases, LDDST (n = 22) or UCCR (n = 2) or both were also performed. The diagnosis was confirmed if the results of LDDST or ACTH stimulation testing were consistent with a diagnosis of HAC along with sufficient clinical signs. To perform the ACTH stimulation test, cortisol concentrations were measured before and 1 hour after IV or IM administration of 5 μg/kg synthetic tetracosactrin. The diagnosis was considered consistent with HAC when the post-ACTH cortisol concentration was ≥20 μg/dL. For the LDDST, serum samples were collected before and 4 hours and 8 hours after administration of 0.01 mg/kg IV dexamethasone. A cortisol concentration >1.4 μg/dL in the sample collected at 8 hours was considered compatible with HAC. To perform the UCCR, a first morning urine sample was collected at home at least 2 days after a visit to our hospital, and a diagnosis of HAC was suggested by finding an increased UCCR ($>60 \times 10^{-6}$). Serum cortisol concentrations from collected samples were measured with a chemiluminescence system, validated for use in the dog. The UCCR was used as additional test with the ACTH stimulation test or LDDST.[3,25–27]

Ultrasonographic Evaluation

A thorough ultrasonographic examination of the entire abdomen was performed in all patients at the time of diagnosis.

The differentiation of PDH from AT was based on ultrasonographic features of the adrenal gland and, additionally in some cases, on the results of the LDDST and HDDST. For the HDDST, serum samples were collected before and 4 hours and 8 hours after administration of 0.1 mg/kg IV dexamethasone. Patients with any suspicion of AT during the sonographic examination were excluded from the study. Findings consistent with AT included moderate asymmetry (diameter of larger adrenal gland >2 cm), contralateral adrenocortical atrophy (smaller adrenal gland width <5 mm), destruction of normal tissue architecture, acoustic shadowing, and hyperechoic foci in an enlarged gland, or some combination of these findings. Patients with cortisol suppression (<1.4 μg/dL or <50% of basal cortisol concentration) at 4 hours (LDDST and HDDST) were considered to have PDH.[6,25,32]

After excluding AT patients, we divided the remaining patients into 3 groups depending on the sonographic findings of the gallbladder. The NOU group had no evidence of biliary stasis, with normal-sized gallbladder lumen. The cholestasis group had evidence of mobile gallbladder sludge or calculi with a dilated gallbladder lumen. All patients in this group were defined as not having characteristics of a GBM, which was evaluated by repositioning the patient and by gentle transducer agitation on the ventral abdomen to suspend gallbladder sediment. The GBM group exhibited immobile sludge and a finely stellate or striated pattern within the gallbladder lumen.[21,22,30,31,33–35]

Treatment and Follow-Up

All patients were treated with trilostane q12h or q24h.

Owners were requested to bring their dogs for re-evaluation after 14 days and 1 month after commencement of trilostane treatment. After 1 month, the evaluation schedules of each patient were variable, and depending on their clinical response and endocrine test results at the previous evaluation, re-evaluations at 2- or 4-week intervals were planned. Once patients achieved good clinical response and appropriate post-ACTH cortisol concentrations, the interval of re-evaluations was adjusted to at least once every month. At each re-evaluation, all owners were asked about clinical response since the time of diagnosis. The owners also were questioned about any potential adverse effects from trilostane treatment such as anorexia, weakness, vomiting, or diarrhea, regardless of whether or not these signs could be attributed to the medication.[12,14–16,18] All dogs were examined physically, and a serum biochemical profile and ACTH stimulation test were performed. The ACTH stimulation tests were performed in all groups at the first re-evaluation, between 4 and 6 hours after the administration of trilostane. The range of post-ACTH cortisol concentrations that were considered indicative of proper disease control was 2.0–5.4 μg/dL, or 5.4–9.1 μg/dL, with good clinical response as determined by the owner. These criteria for appropriate post-ACTH cortisol concentrations followed the drug manufacturer's recommendations.

The dosage of trilostane was regulated individually based on post-ACTH cortisol concentrations and clinical signs. The trilostane dosage was kept constant in dogs with good clinical response and an ACTH stimulation test with a result <9.1 μg/dL, and this dosage (in mg/kg) was regarded as the optimal trilostane dosage for statistical analysis among the 3 groups. The dosage of trilostane was decreased by 25–50% in dogs with a post-ACTH cortisol concentration <2.0 μg/dL, regardless of the presence or absence of adverse effects. All patients in the study were followed up for at least 1 more month after achieving proper disease control as described above.

Statistical Analysis

Results were analyzed by an SPSS[b] statistical package (version 23). The population variables that had continuous data for all

groups were verified for normality by Kolmogorov-Smirnov test. The continuous data with normality were compared by a Levene *t*-test or analysis of variance (ANOVA), and those without normality were compared by Mann-Whitney test. The population variables with categorical data for all 3 groups were compared by a chi-square test, Fisher's exact test, or linear-by-linear association test, including determination of odds ratios (OR). In 2 × 2 contingency tables, a chi-square test was used when no cells in the table had an expected value <5, with Fisher's exact test being used when any cell in the table had an expected value <5. Differences were considered significant at values of $P \leq .05$.

Results

Study Population

The sex, breed distribution, mean age, body weight, and body condition score (BCS) of each group are summarized in Table 1. In addition, 29 dogs were male (5 intact, 24 neutered) and 36 (14 intact, 22 spayed) were female, with no significant differences ($P = .056$) evident in the neuter status between the 2 groups. The proportion of female patients was 39% (n = 7) in the NOU group, 56% (n = 18) in the cholestasis group, and 73% (n = 11) in the GBM group, with no significant statistical differences ($P = .057$) evident in the proportion of female patients among each group. There was no significant difference ($P = .471$) between the prevalence of GBM in intact females (6/14, 43%) and spayed females (6/22, 27%). The most frequently represented breeds were Shih Tzu (22%; n = 14), Miniature Schnauzer (15%; n = 10), Yorkshire Terrier (15%; n = 10), Maltese (14%; n = 9), mixed breed (11%; n = 7), Poodle (5%, n = 3), and Pomeranian (5%, n = 3). There were 2 Cocker Spaniels, 2 Miniature Pinschers, 2 Dachshunds, 2 Boston Terriers, and 1 Pekinese. There were no statistical differences in the age ($P = .682$) and BCS ($P = .797$) among the 3 groups. There was a significant statistical difference ($P = .003$) in the body weight between the NOU group (mean ± SD: 8.2 ± 3.4 kg) and the other groups, but no statistical difference ($P = .501$) was identified between the cholestasis group (mean ± SD: 5.9 ± 3.4 kg) and the GBM group (mean ± SD: 5.1 ± 1.7 kg).

Table 1. Demographic characteristics of the study population.

	Gallbladder Lesion		
	NOU	Cholestasis	GBM
n (65)	18	32	15
Age (years)	11.1 ± 2.2	10.5 ± 2.3	10.7 ± 2.3
Sex*	M (11), F (7)	M (14), F (18)	M (4), F (11)
BW (kg)**	8.2 ± 3.4	5.9 ± 3.4	5.1 ± 1.7
BCS (9-pt)	6.2 ± 1.0	6.2 ± 1.1	6.0 ± 1.1
Breeds			
Shih Tzu	8	4	2
Miniature Schnauzer	3	6	1
Yorkshire Terrier	1	6	3
Maltese		6	3
Mixed breed	1	5	1
Poodle		1	2
Pomeranian		2	1
Cocker Spaniel	2		
Miniature Pinscher		2	
Dachshund			2
Boston Terrier	2		
Pekinese	1		

All data are presented with the mean value (±SD).

NOU, normal on ultrasound; BW, body weight; BCS, body condition score; M, male; F, female.

*$P = .057$; **Statistically significant difference: $P = .003$.

Diagnostic Tests

Clinical Signs. The most common clinical signs were polyuria, polydipsia, abdominal distension, alopecia, and lethargy. There were no statistical differences in the presence of any of these 4 clinical signs between the NOU and the cholestasis groups. The OR for clinical signs in the GBM group are presented in Table 2.

The most common clinical signs mentioned above were statistically analyzed according to sex. In the male group, 90% (n = 26) of the dogs presented with polyuria and polydipsia, 62% (n = 18) with abdominal distention, 48% (n = 14) with alopecia, and 45% (n = 13) with lethargy. In the female group, 92% (n = 33) of the dogs presented with polyuria and polydipsia, 81% (n = 29) with abdominal distention, 72% (n = 26) with alopecia, and 61% (n = 22) with lethargy. There was a significant statistical difference between the male group and the female group with regard to the presence of alopecia (*P*, .049; OR, 2.79; 95% confidence interval (CI), 0.99–7.81) but there were no statistical differences regarding the presence of polyuria and polydipsia ($P = 1.000$), abdominal distention ($P = .098$), and lethargy ($P = .191$).

Routine Blood Tests. The CBC results, urinalysis results, ALT, AST, ALP, and TG at diagnosis were similar among the 3 groups, and there were no statistical differences in any variables. The proportion of dogs with increased GGT was 56% (n = 10) in the NOU group, 66% (n = 21) in the cholestasis group, and 93% (n = 14) in the GBM group. There was a significant difference ($P = .024$) in the proportion of dogs with increased GGT among the 3 groups by linear-by-linear association. The portion of dogs with increased T-Chol was 40% (n = 6) in the NOU group, 63% (n = 20) in the cholestasis group, and 93% (n = 13) in the GBM group. There was a significant difference ($P = .003$) in the proportion of dogs with increased T-Chol among the 3 groups by linear-by-linear association.

The proportions of hypertriglyceridemic or hypercholesterolemic patients also were statistically analyzed based on sex and neuter status. Gallbladder mucocele had the effect of increasing T-Chol, which warranted analysis of the non-GBM (NOU + cholestasis) population. There were no statistical differences ($P = .208$) in the proportion of dogs with increased TG between the male and female groups. The proportion of

Table 2. Odds ratio for PU/PD, abdominal distention, lethargy, and alopecia in the gallbladder mucocele (GBM) group against the other group.

Clinical Signs	Number in GBM	VS other Group (Number)	Odds Ratio	95% CI	*P* Value
PU/PD	14/15	VS NOU (16/18)	1.75	0.14–21.43	1.000
		VS Cholestasis (29/32)	1.45	0.14–15.21	1.000
Abdominal distention	14/15	VS NOU (11/18)	8.91*	0.95–83.62	.046
		VS Cholestasis (22/32)	6.36	0.73–55.30	.078
Alopecia	13/15	VS NOU (9/18)	6.50*	1.13–37.48	.026
		VS Cholestasis (18/32)	5.01*	0.98–26.18	.040
Lethargy	12/15	VS NOU (8/18)	5.00*	1.04–24.03	.037
		VS Cholestasis (15/32)	4.53*	1.07–19.19	.032

*Statistically significant difference: $P < .05$.
PU/PD, polyuria and polydipsia; NOU, normal on ultrasound; VS, versus; CI, confidence interval.

hypercholesterolemic dogs was 38% (8/21) in the non-GBM female patients, and 74% (14/19) in the non-GBM male patients. There was a significant difference (*P*, .024; OR, 0.22; 95% CI, 0.06–0.85) in the proportion of dogs with increased T-Chol between the non-GBM male patients and the non-GBM female patients, whereas there was no statistical difference ($P = .133$) between the overall population of male and female patients.

The serum concentrations of T-chol and TG of patients were statistically analyzed based on the neuter status of the female group. The mean ± SD of serum T-chol of patients was 316 ± 99 mg/dL in the intact female group and 406 ± 155 mg/dL in the spayed female group ($P = .086$). The mean concentrations (range) of serum TG of patients were 139 mg/dL (range, 68–315; median, 103) in the intact female group and 246 mg/dL (range, 106–785; median, 199) in the spayed female group. A significant difference was evident ($P = .049$) in the serum TG concentrations of the spayed female group.

Cortisol Concentrations. All data were obtained at the time of diagnosis. The mean concentration (range) of basal cortisol concentrations was 7.6 μg/dL (range, 2.0–20.1; median, 6.5) in the NOU group, 7.5 μg/dL (range, 1.5–16.8; median, 7.5) in the cholestasis group, and 10.2 μg/dL (range, 3.2–20.2; median, 8.6) in the GBM group. There were no significant differences ($P = .154$) in basal cortisol concentrations among the 3 groups. The mean concentrations (range) of post-ACTH cortisol concentrations of patients were 31.0 μg/dL (range, 16.1–50.0; median, 32.2) in the NOU group, 34.9 μg/dL (range, 12.4–50.0; median, 33.9) in the cholestasis group, and 39.9 μg/dL (range, 24.6–50.0; median, 40.2) in the GBM group. There was a significant difference ($P = .030$) between the GBM group and the remaining dogs, but no statistical difference was found between the NOU group and the cholestasis group. The box plots of basal cortisol concentration and post-ACTH cortisol concentration are presented in Figure 1.

Ultrasonographic Evaluation

In all groups, the most common findings during abdominal ultrasonographic evaluations were hepatomegaly (85%, n = 55), gallbladder lesions (72%, n = 47), urinary bladder lesions (54%, n = 35), hepatic nodules (49%, n = 32), and pancreatic lesions (40%, n = 26). There were no significant differences among the 3 groups concerning these lesions. The urinary bladder lesions included thickened and irregular bladder walls, irregular bladder margins, or hyperechoic material within the lumen of the bladder. The pancreatic lesions included heterogenous echotexture, edematous change, and hyperechoic or hypoechoic changes.

Treatment and Follow-Up

The percentages of patients treated q24h and q12h were as follows: NOU group: 56 and 44%; cholestasis group: 34 and 66%; and GBM group: 27 and 73%. There was no significant difference ($P = .168$) in the medication frequency among the 3 groups. Additionally, there was no change in the status of q12h patients to q24h patients. The conversion of q24h patients to q12h patients was as follows: 2 (25%) in the NOU group, 5 (21%) in the cholestasis group, and 1 (9%) in the GBM group ($P = .650$).

The initial dosage of trilostane ranged between 1.0 and 5.0 mg/kg/d. The mean initial trilostane dosage was 2.1 mg/kg/d (range, 1.0–5.0; median, 2.0) in the NOU group, 2.0 mg/kg/d (range, 1.0–5.0; median, 2.0) in the cholestasis group, and 2.9 mg/kg/d (range, 1.0–5.0; median, 2.0) in the GBM group. The most frequent dosages were 1 q12h (mg/kg, 34%, n = 22), 2 q24h (mg/kg, 20%, n = 13), 1 q24h (mg/kg, 18%, n = 12), and 2 q12h (mg/kg, 12%, n = 8). The choice of initial trilostane dosage was made after a subjective assessment of each patient. There was a significant difference ($P = .022$) between the GBM group and the other groups, and no statistical difference was found between the NOU group and the cholestasis group.

The mean ± SD for the optimal trilostane dosage of patients was 3.1 ± 1.3 mg/kg/d in the NOU group, 4.6 ± 1.7 mg/kg/d in cholestasis group, and 7.8 ± 2.3 mg/kg/d in the GBM group. There was a significant difference ($P < .001$) among the 3 groups. The box plots of the initial dosage and optimal trilostane dosages are presented in Figure 1.

Discussion

We identified a strong association between PDH and cholestatic disease with regard not only to clinical

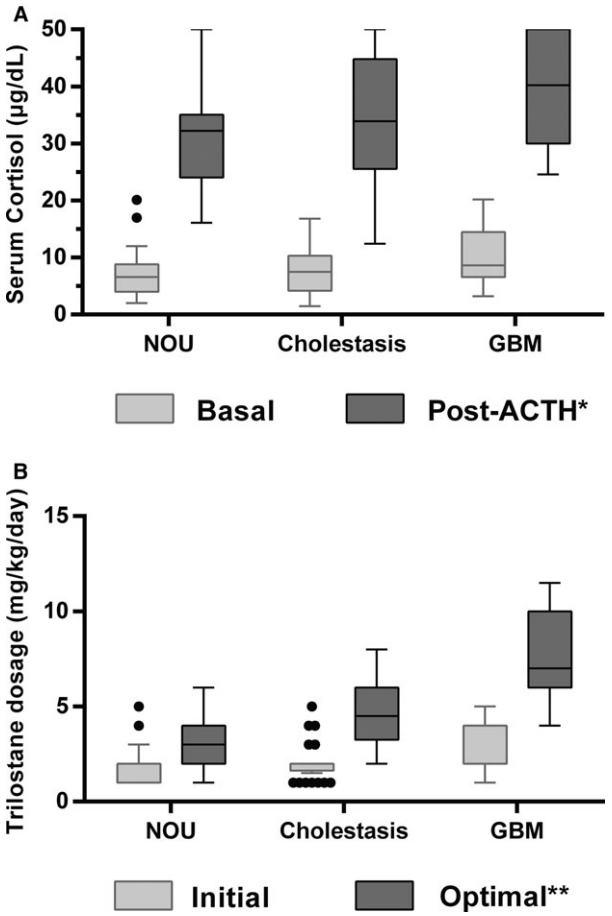

Fig 1. The box-and-whiskers plots comparing serum cortisol concentration (**A**) and required trilostane dosage (**B**) among 3 groups. The top end of each box represents the 75th percentile of data, and the bottom end represents the 25th percentile. The horizontal line through each box is the median. The whiskers on the top and bottom of the boxes indicate the highest data point still within 1.5 interquartile range (IQR) of the upper quartile, and the lowest data point still within 1.5 IQR of the lower quartile. Any data not included between whiskers are presented as outliers. NOU, normal on ultrasound; IQR, interquartile range. Statistically significant difference: $*P = .03$; $**P < .001$

features and clinicopathologic findings but also to trilostane management. Cholestatic disease, including GBM, must be considered a crucial complication of PDH with regard to major clinical signs and increased cortisol concentrations. This finding is supported by other data from our study, including the OR of major clinical signs in the GBM group, and a higher post-ACTH cortisol concentration in the GBM group. The pathophysiology of GBM occurrence in PDH patients may be related to cholesterol metabolism and female sex, and there may be a tendency of breed predisposition. Furthermore, we found that PDH dogs with cholestatic disease require higher trilostane dosages than do those without cholestatic lesions.

Several previous studies suggested various GBM risk factors, including dysmotility of the gallbladder, cholelithiasis, cholecystitis, mucus hypersecretion, and hyperlipidemia, as well as hypercholesterolemia, endocrine disease, genetic factors, and breed predisposition. However, the precise etiology has not been definitively identified.[21–23,30,34,36–38]

Shetland Sheepdogs, Cocker Spaniels, and Miniature Schnauzers are predisposed to GBM. Meanwhile, PDH tends to occur in smaller dogs, and approximately 75% of PDH dogs weigh <20 kg.[1–3,12,14,15] In our study, 100% of dogs weighed <20 kg, and 63 of 65 dogs were toy or small breeds. The body weight (kg) of the NOU group was significantly higher than that of the cholestasis group and the GBM group whereas the BCS of the 3 groups were almost equal. Assuming a cutoff value as a risk factor of <6 kg, only 17% (n = 3) of the NOU group weighed <6 kg, whereas 56% (n = 18) of the cholestasis group and 67% (n = 10) of the GBM group weighed <6 kg. In addition, there was a strong statistical significance ($P = .003$) among the 3 groups. These results suggest that there may be numerous, uninvestigated, breed predispositions toward cholestatic disease in PDH dogs. The insertional mutation of the ABCB4 gene in various canine breeds affected with GBM has been reported.[37] In humans, several genes related to gall stone formation have been identified, including ABCB4, ABCB11, ABCG5/G8, ARDB3, APOA1, APOB, and CCK1R.[39–43] These genes regulate biliary secretion of phospholipids and cholesterol, gallbladder motility, and bile salt synthesis. In our study, Yorkshire Terriers (OR, 12.00; CI, 1.18–122.27; *P*, .033) with PDH had a significantly higher prevalence of cholestatic disease than did Shih Tzus with PDH. However, this finding does not necessarily indicate that Yorkshire Terriers have a significant breed predisposition toward GBM prevalence among all dogs with PDH. The cohort of our study was small, and we were unable to detect breeds at significant risk of cholestatic disease.

In humans, gallstones and gallbladder polyps are the most common gallbladder diseases, whereas GBM is considered a rare condition. Gallstone disease in humans has variable risks and genetic factors including female sex, pregnancy, and estrogen therapy. Female predisposition is a result of hormonal effects, including bile cholesterol hypersaturation and gallbladder dysmotility induced by estrogen and progesterone.[41–49] Previously, no specific risk factors related to the sex of dogs have been correlated with GBM.[20–22,24,50] Nonetheless, our study suggested that a relationship between sex and cholesterol might exist in GBM dogs with PDH. There was a significant linear association between hypercholesterolemia and the development of cholestatic disease. A high hypercholesterolemia prevalence (93%) within the GBM group is thought to have 2 major causes, dysregulated lipid metabolism caused by hypercortisolism and decreased bile excretion of cholesterol caused by GBM.[1–3,31,50,51] Bile excretion is the prime pathway of cholesterol elimination from the body.[51–53] The gallbladder ejection fraction is significantly decreased in dogs with gallbladder sludge or GBM.[31] In our study, although female dogs were thought to have decreased bile excretory ability compared to male dogs because of more severe cholestatic

progression, there was a lower proportion of hypercholesterolemia in females than in males, with statistical significance in non-GBM patients. Because nearly half of the female patients were intact, statistical analysis based on their neutered status was performed in our study. The results however were insufficient to suggest any specific role for female sex hormones.

The increase in serum cortisol concentration in dogs with variable conditions including nonadrenal illness, exercise, and the physiologic changes associated with shelter conditions, anesthesia and postsurgery are well known,[54–57] whereas few studies have investigated the relevance of HAC diagnostic test results in nonadrenal illness.[58] In our study, higher post-ACTH cortisol concentrations were thought to have 2 possible causes, including stress-associated cholestatic disease (including GBM) and a more severe form of HAC. In 1 study, there was no significant difference in post-ACTH cortisol concentrations between clinically normal dogs and dogs with nonadrenal disease, whereas LDDST and UCCR results did have significant differences.[57] Furthermore, only 8 of the 59 (14%) dogs with nonadrenal disease had high serum cortisol concentrations after ACTH stimulation. In our study, although basal cortisol concentrations among the 3 groups were not statistically different ($P = .154$), which may be because of the small study population, a significant difference was evident in the post-ACTH cortisol concentrations among the 3 groups. The mean post-ACTH cortisol concentrations between the NOU and GBM groups differed by nearly 9 μg/dL. Incidentally, the upper limit of detection for serum cortisol concentration was set at 50 μg/dL, resulting in a ceiling effect that caused a number of samples (n = 13) to be constrained by this upper limit. Approximately 11% (n = 2) of the NOU group, 19% (n = 6) of the cholestasis group, and 33% (n = 5) of the GBM group had post-ACTH cortisol concentrations of 50 μg/dL, with further evaluation as to the true extent of their increased cortisol concentrations not possible. A more pronounced difference between the NOU and cholestasis groups may have been detected if instrumental limitations on cortisol concentrations had not been present. Overall, in consideration of the high proportion (47/65, 72%) of dogs with cholestatic disease in our study, including that of GBM in PDH dogs, the wide distribution range of the post-ACTH cortisol concentrations in PDH dogs cannot be accounted for by HAC complications. Thus, the higher cortisol concentrations of the GBM group may have been because of both the stress of concurrent cholestatic disease and more severe progression of HAC, with the latter most likely being implicated by our results.

Trilostane is well known for its lipid-soluble character. Therefore, it is strongly recommended to administer the medication with or after meals, which can stimulate bile secretion from the gallbladder.[12–15,59] It also is well known that dogs with GBM have a potential risk of nutritional deficiency of lipid-soluble substances because of extrahepatic biliary obstruction.[60–62] The high optimal trilostane dosage of the GBM group in this study can be explained by 2 causes. The first is the higher cortisol production of the GBM group as compared to the other groups, which likely was induced by advanced PDH. This conclusion is supported by a higher post-ACTH cortisol concentration than in the other groups, but there were no significant differences in basal cortisol concentrations among the 3 groups. The second is decreased ability of bile secretion induced by concurrent cholestatic disease.[21,31,33] We believe the severity of PDH is not sufficient to explain the high trilostane dosage in the GBM group. Our results suggest that the occurrence of cholestatic disease can be a major reason for the wide distribution of optimal trilostane dosage in HAC patients.[15,18,19] Consequently, proper client education is required when treating PDH patients that also have cholestatic disease, especially GBM. Meanwhile, we attempted to find completely resolved cases of GBM after treatment initiation, based on a study of nonsurgical resolution of GBM in dogs.[63] Regrettably, we had no completely resolved cases, but 4 of 15 GBM patients did have partial improvement based on ultrasonographic gallbladder examination after 6 months of GBM and PDH management. Moreover, only 1 of 4 required a decreased dosage of trilostane, which is not sufficient for analysis.

Our study had a number of limitations. The first was the small study population and the limitation of a retrospective study design. For instance, as described previously, the TG and T-chol tests were performed in only 83% (n = 54) of patients. To our knowledge, no specific animal model of GBM exists, which makes it difficult to perform a prospective study. The second limitation was a biased breed distribution. In Korea, toy breeds and small breeds generally are more popular than medium-to-large breeds, and the preference of some individual breeds is notable.

In conclusion, our study suggests that GBM is a crucial complication of PDH related to the severity of hypercortisolism. Dogs with PDH and GBM have more severe clinical signs and higher post-ACTH cortisol concentrations than do those without GBM. The pathophysiology of GBM formation with PDH may include breed, genetic, and female sex predisposition, which may be associated with cholesterol metabolism and bile secretion. In addition, there was a significant difference in the required trilostane dosage, which is likely because of the lipid-soluble characteristic of trilostane. Cautious monitoring and proper client education about the risk of cholestasis and GBM formation should be carried out in dogs with HAC.

Footnotes

[a] Vetoryl, Arnolds Veterinary Products, Shrewsbury, UK
[b] SPSS 23.0 for Windows; SPSS Inc., Chicago, IL

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Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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