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Risk factors for low plasma thyroxine and high plasma thyroid-stimulating hormone concentrations in dogs with non-thyroidal diseases

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ABSTRACT. The aim of the current study was to identify independent risk factors for thyroid axis alterations in dogs with non-thyroidal diseases. In this retrospective cross-sectional study, data and plasma samples from 207 dogs with non-thyroidal diseases was used. The involvement of various factors (disease severity, sex, age, breed, category and duration of disease, and medication) in the alteration of plasma thyroxine (T4) or thyroid-stimulation hormone (TSH) concentrations was analyzed using multivariate logistic regression. Among the 207 dogs analyzed, 99 (47.8%) had low plasma T4 concentrations, while 45 (21.7%) had high TSH concentrations. Intact male sex [odds ratio (OR), 3.25; 1.67–6.35; $P<0.001$], Labrador Retrievers (OR, 18.70; 2.32–151.00; $P=0.006$), moderate (OR, 2.39; 1.21–4.74; $P=0.012$) and severe diseases (OR, 6.84; 2.27–20.70; $P<0.001$) were associated with increased risk for low plasma T4 concentrations. Meanwhile, intact male (OR, 3.93; 1.51–10.30; $P=0.005$), spayed female (OR, 4.22; 1.59–11.20; $P=0.004$), older age (OR, 2.73; 1.28–5.84; $P=0.009$), and Miniature Dachshunds (OR, 5.39; 2.38–12.20; $P<0.001$) had increased risk for high plasma TSH concentrations. Disease severity had been determined as an independent risk factor for canine NTIS. In addition, sex, age and breed were also associated with thyroid axis alterations in dogs with non-thyroidal diseases.

KEY WORDS: canine, hypothyroidism, logistic regression, non-thyroidal illness syndrome

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Euthyroid sick syndrome or non-thyroidal illness syndrome (NTIS) has been defined as alterations in thyroid hormone metabolism resulting from non-thyroidal diseases. Accordingly, human patients with NTIS have lower serum T3 and T4 concentrations compared to healthy individuals [8]. These changes in the thyroid axis have been believed to be a compensatory reaction to pathologic conditions through which energy expenditure is optimized [36]. Although the mechanisms for the development of NTIS have yet to be elucidated, multiple factors have been hypothesized, such as suppression of thyrotropin-releasing hormone (TRH) or thyroid-stimulating hormone (TSH) secretion, alterations in thyroid hormone metabolism, and changes in thyroid hormone-binding protein [8, 39].

NTIS is also well known in dogs. Low serum T4 concentrations have been observed in dogs with various non-thyroidal diseases [15, 20, 28, 35, 38]. Severe diseases have been found to more potently decrease serum T4 concentrations [15], even to the point wherein levels fall below the reference range. Surgery and anesthesia also decrease serum T4 concentrations [42], while various drugs, such as glucocorticoids, phenobarbital, sulfonamides, and non-steroidal anti-inflammatory drugs (NSAIDs), can lower serum T4 concentration in dogs [6, 7, 16, 41]. In such situations, serum thyroid hormone concentrations may become very low, which can complicate thyroid function evaluation. Although dogs with NTIS usually have normal or low serum TSH concentrations, some dogs have TSH levels above the reference range [15, 20, 29, 32, 35]. In these dogs, concurrent low T4 and high TSH levels aggravate the risk for misdiagnosis of hypothyroidism.

However, discriminating between NTIS and hypothyroidism is sometimes challenging in dogs [22]. Thus, identifying risk factors associated with the low serum T4 concentrations helps veterinarians estimate the pretest probability of NTIS in dogs. Although many diseases or drugs may have been associated with low serum T4 concentrations in dogs as previously described,

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the influence of factors, such as sex, breed, and age, in the development of canine NTIS remains largely unclear. These non-pathological factors have been known to affect circulating thyroid hormone concentrations [12, 30]. Given the multiple risk factors involved in thyroid axis abnormalities among dogs with non-thyroidal diseases, the potential for confounding should be considered during the evaluation of risk factors for altered thyroid test results. However, to date, such analyzes have not been conducted. The present study therefore analyzes the association between these factors and thyroid axis abnormalities in dogs with non-thyroidal diseases using multivariate analysis to identify independent risk factors for altered thyroid test results in dogs.

MATERIALS AND METHODS

Samples and data collection

The present retrospective cross-sectional study included data from dogs diagnosed with non-thyroidal diseases at a single referral veterinary hospital between March 2015 and May 2017. Dogs were diagnosed with non-thyroidal diseases through physical examination, blood tests, urinalysis, radiography, echography, cytotechnology, computerized tomography, and magnetic resonance imaging, as necessary. Dogs diagnosed with hypothyroidism and diseases related to the thyroid gland (e.g., thyroid tumor) were excluded. Dogs showing clinical signs that may be observed during hypothyroidism (e.g., dermatologic signs, peripheral neuropathy, and megaesophagus) were also excluded given that the exclusive diagnosis of hypothyroidism may result in a substantial bias in plasma T4 and TSH concentrations. As an exception, dogs diagnosed with hyperadrenocorticism were included, although they can show dermatologic signs similar to hypothyroidism (e.g., bilateral symmetric alopecia). Upon study enrolment, none of the dogs had been evaluated for blood thyroid hormones or TSH concentrations.

The medical database was reviewed for all enrolled dogs to obtain data regarding animal information (sex, neuter status, age, and breed); disease severity, category, and duration; and glucocorticoid or NSAID use within the previous month. The medical database was also reviewed for classify diseases severity, which was classified into mild, moderate, and severe according to a previous report [15]. Dogs with mild disease shows clinical signs but healthy enough to be treated as outpatient, dogs with moderate disease is sick enough to require hospitalization, and dogs with severe disease need intensive care [15]. After biochemical measurements for clinical diagnosis, the rest of the heparinized plasma samples were stored at −30°C until further analyzes for T4 and TSH concentrations. All dog owners provided written consent regarding the use of their samples and data for research purposes.

Assays

Plasma T4 and TSH concentrations were measured using an automated analyzer developed for dogs (FUJI DRI-CHEM IMMUNO AU-10V, Fujifilm, Tokyo, Japan). Inter-assay coefficient of variation was 0.9–5.7% and 1.0–7.6% for plasma T4 and TSH concentrations, respectively. Spike tests resulted in recoveries of 81–98% for T4 and 105–115% for TSH. The measured values showed very good correlation with values determined by IMMULITE Canine Total T4 and IMMULITE Canine TSH (Siemens, Munich, Germany) ($y=0.99x + 0.08$, $r=0.978$, $n=128$ for T4; $y=0.97x + 0.01$, $r=0.93$, $n=128$ for TSH). The reference ranges provided by the manufacturer is 1.3–2.9 μg/d*l* for T4, and ≤0.5 *n*g/m*l* for TSH.

Statistical analysis

Dogs with plasma T4 concentrations <1.3 μg/d*l* or plasma TSH >0.5 *n*g/m*l* were regarded as low T4 or high TSH, respectively. The dogs were divided into two groups based on median age and disease duration. Dog breeds containing less than 10 individuals were categorized under other breeds. Likewise, disease categories containing less than 10 individuals were categorized under other diseases. Potential risk factors for low T4 or high TSH among dogs were initially examined using univariate analysis (Fisher's exact test). Factors having a P value <0.1 during univariate analysis were subsequently entered into a multivariate binary logistic regression model with a backward stepwise procedure based on Akaike's Information Criterion. Odds ratios (OR) for each factor were reported with 95% confidence intervals. P values <0.05 were considered statistically significant. All statistical analyzes were carried out using EZR (Saitama Medical Center, Jichi Medical University) [14], which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.0.2).

RESULTS

Among the 207 dogs enrolled, 111 were males (64 intact and 47 castrated), 96 were females (44 intact and 52 spayed), and the median age was 11 years. Dog breeds included 40 Miniature Dachshunds, 15 Labrador Retrievers, 13 Toy Poodles, 10 Miniature Schnauzers, 17 mongrels, and 112 other breeds. Disease severity was categorized as mild in 121 dogs, moderate in 60, and severe in 26. A total of 35 dogs were diagnosed with gastrointestinal diseases, 23 with liver and pancreatic diseases, 14 with respiratory diseases, 11 with urological diseases, 11 with hyperadrenocorticism, 13 with endocrine diseases other than hyperadrenocorticism, 13 with multicentric lymphoma, and 87 with other diseases (29 cutaneous tumors, 28 miscellaneous tumors, 9 neurologic diseases, 6 intraoral melanomas, 5 infectious diseases, 4 hematological diseases, 2 dental diseases, 2 orthopedic problems, and 2 immunologic diseases). The median disease duration was 12 weeks. Moreover, 61 and 16 had been administered glucocorticoids and NSAIDs within the previous month, respectively.

Among the 207 dogs analyzed, 108 (52.2%) had low plasma T4 concentrations (<1.3 μg/d*l*), while 45 (21.7%) had high plasma TSH concentrations (>0.5 *n*g/m*l*). Concurrent low plasma T4 and high TSH concentrations were observed in 31 dogs (14.9%). Univariate analysis revealed that sex, breed and the severity of diseases were significantly associated with increased risk for low

plasma T4 concentrations (Table 1). In contrast, sex, age, and breed were significantly associated with increased risk for high plasma TSH concentrations in dogs with non-thyroidal diseases (Table 2).

Variables that had *P* values <0.1 during univariate analysis were analyzed using multivariate logistic regression analysis to identify independent risk factors for low plasma T4 concentrations, among which five variables were selected for the final logistic regression model (Table 3). Intact male sex [odds ratio (OR), 3.25; 1.67–6.35; *P*<0.001], Labrador Retrievers (OR, 18.70; 2.32–151.00; *P*=0.006), moderate (OR, 2.39; 1.21–4.74; *P*=0.012) and severe diseases (OR, 6.84; 2.27–20.70; *P*<0.001) were significantly associated with increased risk for low plasma T4 concentrations. Likewise, variables that had *P* values <0.1 during univariate analysis were analyzed using multivariate logistic regression analysis to identify independent risk factors for high plasma TSH concentrations, among which four variables were selected for the final logistic regression model (Table 4). Accordingly, intact male (OR, 3.93; 1.51–10.30; *P*=0.005), spayed female (OR, 4.22; 1.59–11.20; *P*=0.004), older age (OR, 2.73; 1.28–5.84; *P*=0.009) and Miniature Dachshunds (OR, 5.39; 2.38–12.20; *P*<0.001) were significantly associated with increased risk for high plasma TSH concentrations.

DISCUSSION

The present study revealed that disease severity and non-pathological factors (sex, age, and dog breeds) were independently associated with the risk for abnormal T4 or TSH concentrations. To the best of our knowledge, this has been the first report to analyze risk factors for abnormal plasma T4 or TSH concentrations in dogs with non-thyroidal diseases using multivariate analysis.

The results in the present study showed that among dogs with non-thyroidal diseases, a substantial proportion (52.2%) had low plasma T4 concentrations, while 21.7% had high plasma TSH concentrations. The prevalence of abnormal T4 or TSH levels was higher than that in the previous studies. For example, serum T4 concentrations were below the reference range in 69 of 223 dogs (30.9%) [15] and 68 of 196 dogs (34.7%) [20] with varying severity of non-thyroidal diseases. On the other hand, 5 of 16 (31%) [29], 4 of 33 (12%) [32], 5 of 66 (8%) [35], 18 of 223 (8.1%) [15], and 6 of 181 (3.1%) [20] euthyroid dogs with non-thyroidal diseases had high TSH concentration. The higher prevalence of dogs with abnormal T4 or TSH might be related with differences in the cut-off values, or differences in severity of diseases.

Table 1. Univariate analysis of risk factors for low plasma thyroxine (T4) concentrations in dogs with non-thyroidal diseases

		T4≥1.3 μg/ml	T4<1.3 μg/ml	*P* value
n		99	108	
Sex	Intact male	43	21	0.004
	Castrated male	23	24	
	Intact female	14	30	
	Spayed female	28	24	
Age	>11 years	42	59	0.095
	≤11 years	57	49	
Breed	Miniature Dachshund	21	19	0.003
	Labrador Retriever	1	14	
	Toy Poodle	10	3	
	Miniature Schnauzer	5	5	
	Mongrel and other breeds	62	67	
Disease severity	Mild	71	50	<0.001
	Moderate	23	37	
	Severe	5	21	
Disease category	Gastrointestinal diseases	20	15	0.507
	Liver and pancreatic diseases	13	10	
	Respiratory diseases	4	10	
	Urological diseases	5	6	
	Hyperadrenocorticism	4	7	
	Other endocrine diseases	8	5	
	Multicentric lymphoma	7	6	
	Other diseases	38	49	
Disease duration	>12 weeks	51	52	0.677
	≤12 weeks	48	56	
Medication	Glucocorticoids	27	34	0.369
	NSAIDs	5	10	
	No glucocorticoids or NSAIDs	67	64	

NSAIDs, non-steroidal anti-inflammatory drugs.

Table 2. Univariate analysis of risk factors for high plasma thyroid-stimulating hormone (TSH) concentrations in dogs with non-thyroidal diseases

		TSH ≤0.5 *n*g/m*l*	TSH >0.5 *n*g/m*l*	*P* value
n		162	45	
Sex	Intact male	45	19	0.002
	Castrated male	41	6	
	Intact female	41	3	
	Spayed female	35	17	
Age	>11 years	69	32	<0.001
	≤11 years	93	13	
Breed	Miniature Dachshund	19	21	<0.001
	Labrador Retriever	11	4	
	Toy Poodle	12	1	
	Miniature Schnauzer	7	3	
	Mongrel and other breeds	113	16	
Disease severity	Mild	95	26	0.100
	Moderate	43	17	
	Severe	24	2	
Disease category	Gastrointestinal diseases	28	7	0.642
	Liver and pancreatic diseases	21	2	
	Respiratory diseases	10	4	
	Urological diseases	10	1	
	Hyperadrenocorticism	8	3	
	Other endocrine diseases	9	4	
	Multicentric lymphoma	10	3	
	Other diseases	66	21	
Disease duration	>12 weeks	80	23	0.867
	≤12 weeks	82	22	
Medication	Glucocorticoids	52	9	0.122
	NSAIDs	10	6	
	No glucocorticoids or NSAIDs	101	29	

TSH, thyroid-stimulating hormone; NSAIDs, non-steroidal anti-inflammatory drugs.

Table 3. Multivariate binary logistic regression analysis of risk factors for low plasma thyroxine (T4) concentrations in dogs with non-thyroidal diseases

Variable	OR	95% CI	*P* value
Intact male[a)]	3.25	1.67–6.35	<0.001
Labrador Retriever[b)]	18.70	2.32–151.00	0.006
Toy Poodle[b)]	0.24	0.06–1.02	0.053
Moderate disease[c)]	2.39	1.21–4.74	0.012
Severe disease[c)]	6.84	2.27–20.70	<0.001

a) vs Intact female, b) vs Mongrel and other breeds, c) vs Mild disease. OR, odds ratio; CI, confidence interval.

Table 4. Multivariate binary logistic regression analysis of risk factors for high plasma thyroid-stimulating hormone (TSH) concentrations in dogs with non-thyroidal diseases

Variable	OR	95% CI	*P* value
Intact male[a)]	3.93	1.51–10.30	0.005
Spayed female[a)]	4.22	1.59–11.20	0.004
Age >11 years[b)]	2.73	1.28–5.84	0.009
Miniature Dachshund[c)]	5.39	2.38–12.20	<0.001

a) vs Intact female, b) vs Age ≤11 years, c) vs Mongrel and other breeds. OR, odds ratio; CI, confidence interval.

The current study found that disease severity was associated with increased risk for low plasma T4 concentrations. This was consistent with the previous studies wherein serum T4 concentrations were found to be inversely proportional to disease severity [15, 20]. The present study revealed that disease severity was a risk factor for low T4 concentrations independent of other factors, such as sex, age, and breed.

Among humans, male sex has been reported to be associated with higher blood T4 concentrations, although with some inconsistent results [1, 10]. In contrast, one study showed that male dogs without diseases have significantly lower serum T4 concentrations [12], whereas another reported no difference in serum T4 concentrations between male and female dogs [30]. Castration or the discontinuation of testosterone increased serum T4 concentrations in dogs [13], which is consistent with the result that androgen decreases serum T4 concentration [40]. In addition, progesterone increases the affinity between T4 and binding proteins, thereby increasing serum T4 concentrations [40]. Our data are consistent with the aforementioned evidence showing lower T4 concentrations in intact male dogs.

The present results showed that Labrador Retrievers had increased risk for low plasma T4 concentrations. Among dogs without diseases, particular breeds, such as the Greyhound [11], Alaskan sled dogs [24], Saluki [34], Whippets [37], Sloughis [21], Basenjis [33], English Setters and Golden Retrievers [12], have been known to have lower serum T4 concentrations compared to other breeds. In addition, smaller dog breeds have higher serum T4 levels [30]. Such breed-specific or body size-related differences in blood T4 reference ranges might have resulted in the higher prevalence of low T4 concentrations among Labrador Retrievers with non-thyroidal diseases in the present study.

In general, blood TSH concentrations are either normal or decreased during NTIS [39]. However, some dogs with non-thyroidal illness develop increased serum TSH concentrations [15, 20, 29, 32, 35], which can further complicate the differentiation between NTIS and hypothyroidism. One possible explanation for the increase in TSH concentrations during NTIS is the elevation of TSH secretion when recovering from disease states. A number of human patients have showed transient TSH elevation during their recovery from NTIS [2, 4]. Our results showed that dogs with severe diseases tended toward not having high plasma TSH concentrations. This is consistent with the hypothesis that animals with high TSH levels are in the recovery phase of NTIS. Unfortunately, the present study was unable to validate this hypothesis given that an objective assessment of "dogs in the recovery phase" was impossible. Further studies that include serial TSH measurements in dogs under treatment might be warranted.

In the present study, intact male and spayed female dogs showed increased risk for high TSH levels. This was contradictory to the results in humans that indicated higher TSH levels in healthy females than that in males [1, 19, 25, 26]. In addition, sex hormones did not alter TSH levels in dogs [9, 13]. These evidences do not support the present result; however, the present data may raise the possibility that TSH levels in dogs with non-thyroidal diseases are affected by sex.

Our results suggested that older dogs had increased risk for high TSH concentrations. In human patients with non-thyroidal diseases, age had no influence on TSH levels [43]. On the other hand, older humans [3, 25] and dogs [12] without diseases had higher serum TSH concentrations. Changes in the thyroid's responsiveness to feedback regulation and decreased biologic activity of TSH with age have been suggested [12, 31].

In the present study, Miniature Dachshunds had significantly increased risk for high plasma TSH concentrations. Considering that TSH is secreted episodically [17], substantial individual variations are inevitable. However, some dog breeds, such as Collies, Samoyeds, and Keeshonds, had a higher TSH reference range than other dog breeds [12]. However, the mechanisms for the differences in TSH reference ranges among breeds have not been elucidated. In healthy humans, TSH reference limits have been influenced by ethnicity [3, 27]. Serum TSH levels within an individual have been tightly regulated based on a set-point, while several single nucleotide polymorphisms have been associated with serum TSH levels [5, 18]. Therefore, similar to plasma T4 concentrations, breed-specific differences in TSH reference ranges could result in increased risk for high plasma TSH concentrations in Miniature Dachshunds.

A limitation of the present study was our inability to definitely exclude dogs with hypothyroidism. Given that clinical signs of canine hypothyroidism may be nonspecific, it is possible that concurrent hypothyroidism was present in dogs with non-thyroidal disease. However, thyroid function tests for the exclusion of hypothyroidism prior to study inclusion would have caused substantial bias in the data. In the present study, dogs showing clinical signs characteristic of hypothyroidism were excluded. In addition, the prevalence of canine hypothyroidism is very low (0.2%) [23]. Thus, the current study had a low likelihood of including dogs with true hypothyroidism. Another limitation was small number of dogs included in this study. Larger sample size could have enabled us to analyze the risk factors for concurrent low T4 and high TSH concentrations. This might have provided more valuable information.

In conclusion, disease severity was independently associated with the risk for NTIS in dogs. Moreover, sex, age and breed affected the prevalence of low plasma T4 or high TSH concentrations in dogs with non-thyroidal diseases. These factors might increase the risk for low plasma T4, and possibly concurrent low plasma T4 and high TSH concentrations, which could result in a misdiagnosis of hypothyroidism in dogs. Accordingly, veterinary practitioners should be aware of the aforementioned risk factors for low T4 and high TSH concentrations when evaluating thyroid function in dogs with non-thyroid diseases.

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