Hypovitaminosis D Is Associated with Negative Outcome in Dogs with Protein-Losing Enteropathy: A Retrospective Study of 43 Cases

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Abstract

Background: Hypovitaminosis D previously has been shown to be prevalent among dogs with protein-losing enteropathy (PLE). The hypothesis of this study was that low 25-hydroxyvitamin D (25(OH) D) serum concentrations could be a risk factor for a negative outcome in dogs with PLE. We collected and analyzed serum vitmain D concentrations archived at -80 degrees Celsius from 43 dogs diagnosed with PLE from 2005 to 2014. Post-diagnostic communication with the referring veterinarians allowed us to determine the outcome of the PLE dogs. Dogs that died from PLE within four months after diagnosis comprised the negative-outcome group, n=22, and dogs that were living or that died due to another disease at the end point of the study one year after diagnosis made up the good-outcome group, n=2. Serum samples taken at the time of diagnosis were analyzed for ionized calcium (iCa) concentrations and serum 25(OH)D concentration.

Results: Canine chronic enteropathy clinical activity index (CCECAI) scores, age at PLE diagnosis and iCa concentrations were not significantly different between dog groups. A significantly greater ($p<0.001$) number of PLE dogs treated with a hydrolyzed or elimination diet alone showed good outcome as compared to the PLE negative-outcome group. Median serum 25(OH)D concentration was significantly ($p=0.017$) lower in dogs with negative outcome versus PLE dogs with good outcome. Using logistic regression analysis, 25(OH) D serum concentration was shown to be a statistically significant factor for outcome determination. Cox regression analysis yielded a hazard ratio of 0.974 (95% CI 0.949, 0.999) per each nmol/l increase in serum 25(OH)D concentration.

Conclusions: Low-serum 25(OH)D concentration in PLE dogs was significantly associated with poor outcome. Further studies are required to investigate the clinical efficacy of vitamin D (cholecalciferol) as a potential therapeutic agent for dogs with PLE.

Glossary of Abbreviations
ACTH: Adrenocorticotropic Hormone
BCS: Body Condition Score
CCECAI: Canine Chronic Enteropathy Clinical Activity Index
cPLI: Canine Pancreatic Lipase Immunoreactivity
GI: Gastrointestinal Disease
HR: Hazard Ratio
IBD: Inflammatory Bowel Disease
iCa: Ionized Calcium
IL: Intestinal Lymphangiectasia
25(OH)D: 25-Hydroxyvitamin D
iCA: Ionized Calcium
PLE: Protein-Losing Enteropathy
RIA: Radioimmunoassay
TLI: Trypsin-Like Immunoreactivity

Introduction

PLE in dogs is a clinical syndrome characterized by loss of protein through the intestines.[1] There are three major causes for PLE in dogs including inflammatory bowel disease (IBD), primary intestinal lymphangiectasia (IL) and intestinal lymphoma.[1] Apart from dogs diagnosed with intestinal lymphoma, which generally show poor response to chemotherapy and short survival times, dogs with PLE secondary to IBD or primary IL have a variable prognosis.[1-5] Few reports describe prospective treatment trials of dogs with PLE since mortality is high despite intense immunosuppressive and nutritional treatment protocols.[2,3] Possible life-threatening complications include intractable diarrhea, extreme malnutrition and thromboembolic disease.[6] Risk factors associated with poor outcome have not been well-characterized in PLE dogs. Several breeds are predisposed to the development of PLE, with Yorkshire Terriers having a better outcome in some instances,[4] while in Rottweilers the disease generally carries a poor prognosis.[1] In addition, there is evidence that biomarkers, such as

serum C-reactive protein, serum canine pancreatic lipase immunoreactivity and fecal alpha-1 proteinase inhibitor concentrations, are more commonly elevated in dogs having the shortest survival times.[7,8]

Electrolyte disturbances, such as low total and ionized calcium concentrations and hypomagnesemia, also have been reported in some canine PLE cases.[9,10] It is hypothesized that the ionized hypocalcemia in IBD cases could be caused by reduced vitamin D or calcium absorption, reduced dietary intake and/or vitamin D receptor polymorphisms in impaired vitamin D metabolism.[11] Furthermore, low-serum concentrations of 25(OH)D recently have been described in dogs with chronic enteropathies,[12] and have been shown to be associated with negative outcome.[13] We, therefore, sought to investigate the presence of low iCa and 25(OH)D serum concentrations in dogs with PLE and whether these variables are associated with negative outcome.

Methods

Aim, Design and Study Setting

The aim of the current study was to assess the prevalence of decreased 25(OH)D serum concentrations in dogs with PLE caused by IBD. In addition, we investigated whether 25(OH)D could serve as a prognostic indicator of outcome.

This was a retrospective study that included 43 cases seen at the Royal Veterinary College of the University of London from 2005 to 2014.

Animals

The medical records of dogs referred to the Queen Mother Hospital for Animals (QMHA) at the Royal Veterinary College between 2005 and 2014 were reviewed retrospectively to identify dogs with a clinical diagnosis of PLE. The diagnosis of PLE was made if all of the following applied: (1) history of chronic gastrointestinal (GI) disease, including weight loss, vomiting, diarrhea, and decreased appetite; (2) panhypoproteinemia, with serum albumin less than 2.8 g/dL and serum globulin less than 2.1g/dL; reference ranges from 2.8 to 3.9 and 2.1 to 4.1g/dL, respectively; (3) diagnostic tests including performance of complete blood count, biochemistry profile, urinalysis, abdominal ultrasound, adrenocorticotropic hormone (ACTH) stimulation test, serum trypsin-like immunoreactivity (TLI), and canine pancreatic lipase immunoreactivity (cPLI) serum assays to reflect the presence or absence of primary GI disease versus extra-intestinal disease; (4) histopathological confirmation of IL or IBD with secondary IL; (5) exclusion of hepatic dysfunction by serum bile acid stimulation test; and (6) absence of proteinuria. Proteinuria was excluded in all dogs on the basis of a negative urine dipstick or a urine protein:creatinine ratio of <0.5. In addition, at the time of PLE diagnosis, all dogs had to have a CCECAI[14] recorded by the clinician, and a serum sample frozen within 30 minutes after collection and stored at -80 degrees Celsius until later analysis.

Clinical Data

Follow-up communication with referring veterinarians was made to determine post-diagnostic outcome of PLE dogs. In accordance with previoulsy published studies, dogs were divided into two groups: The first group consisted of dogs that had died from their illness or were euthanized due to intractable clinical disease within four months after diagnosis[4] (negative-outcome group), and the second group consisted of PLE dogs that were alive or had died due to non-PLE disease at least one year after diagnosis (good-outcome group). Individual treatments of dogs also were categorized into two groups: Group 1 dogs comprised those that received either an elimination diet (a commercial single-protein veterinary therapeutic diet the dog had not been given before) or a hydrolyzed diet (commercial hydrolyzed ingredient veterinary therapeutic diet) on an exclusive basis (diet group); Group 2 dogs consisted of dogs that were prescribed an elimination or hydrolyzed diet in conjunction with immunosuppressive drugs, including combination therapy with prednisolone, cyclosporine and/or azathioprine.

Measurement of Ionized Calcium (iCa) and Serum 25(OH)D Concentrations

Vitamin D status was assessed by the measurement of serum concentrations of 25-hydroxyvitamin D (25[OH]D), which is the most widely used approach to analyze whole body vitamin D status.[15] At the time of diagnosis, dogs had samples collected for biochemical and hematological analysis. Residual serum samples were then frozen at -80° Celsius within 30 minutes after collection until future analysis. Ionized calcium concentrations were measured using an ion-specific electrode, and 25(OH)D was measured using commercial radioimmunoassays (RIA) that have been validated for use in veterinary medicine.[17] Samples were shipped on dry ice to the Michigan State University Diagnostic Center for Population and Animal Health (DACPAH)[a] for batch analysis. Serum 25(OH)D and iCa concentrations previously have been shown to be stable under these conditions[16] (and have been personally communicated by DACPAH staff).

Statistical Analysis

Differences between dog groups were assessed using a Mann-Whitney U test for numerical data or Fisher's exact test for categorical data, respectively. Correlations were analyzed using Spearman's rank-order correlation tests. Breed, age, serum albumin concentrations, CCECAI scores, treatment group, iCa concentrations, and 25(OH)D concentrations were entered into a univariate logistic regression analysis. Factors that were significantly associated with outcome in the univariate logistic regression analysis were then assessed in a multivariable logistic regression. Kaplan-Meier estimator and Cox regression analyses were used to illustrate and estimate the effect of 25(OH)D serum concen-

tration on survival times after diagnosis. Hazard ratio (HR) and 95% confidence interval (CI) were reported. Statistical analyses were performed with SPSS version 22 and GraphPad Prism 7 statistical software, with $p<0.05$ considered statistically significant.

Results

Forty-three PLE dogs were included in the study with 21 dogs having good outcomes and 22 dogs having negative outcomes. In the negative-outcome group, median survival time was 19 days (range from 1 to 301 days). In the good-outcome group, 13/22 dogs were still alive at four months, while nine dogs had been euthanized due to non-PLE related illnesss. Median survival time in this latter group was 1,095 days (range from 515 to 3,130 days).

In the good-outcome group, the median age was 5.2 years (range from 1 to 11 years), with six neutered males, three intact males, nine neutered females, and three intact females making up this group. Median age in the negative-outcome group was 6.7 years (range from 0.9 to 13.7 years). Histopathology in this group was consistent with IBD in 13 dogs, IBD with IL in four dogs, and IL only present in five dogs. There was no statistically significant difference in age or breed distribution between the two PLE dog groups (p=0.35 and p=0.42, respectively). Median body condition score (BCS)[b] was not different between the two groups: the good-outcome group averaged a 4.5 BCS (range of 1 to 6), and the negative-outcome group averaged a BCS of 3.8 (range of 1 to 5), p=0.5.

The percentage of dogs receiving immunosuppressive drugs between outcome groups was significantly different, with the negative-outcome dogs receiving more immunosuppressive drugs ($p<0.001$). A greater number of dogs treated with diet alone were in the good-outcome (13/22) group versus PLE dogs in the negative-outcome group (2/21, $p<0.001$).

Median serum albumin concentration was 17g/l (reference range from 28 to 35), with no difference observed between the outcome groups (good-outcome group: median 19, range from 12 to 26; negative-outcome group: median 16, range from 10 to 27, p=0.23). Serum albumin concentration was not correlated with either iCa, 25(OH)D or CCECAI (r^2=1.15, r^2=0.21, and r^2=0.004, respectively).

The median 25(OH)D concentration was 23 nmol/L (range from 0 to 81 nmol/L, reference range from 60 to 215 nmol), being significantly lower in the negative-outcome group (16.5 nmol/L, range from 0 to 66 nmol/L) versus the good-outcome group (37 nmol/L, range from 6 to 81 nmol/L, p=0.017) (Figure 1). Hypovitaminosis D was present in 17 dogs (81%) of the good-outcome group and was not statistically different (p=0.65) than its occurrence in the 20 dogs (91%) of the negative-outcome group (reference range from 60 to 215 nmol). Higher 25(OH)D serum concentration at PLE diagnosis indicated a better prognosis for survival

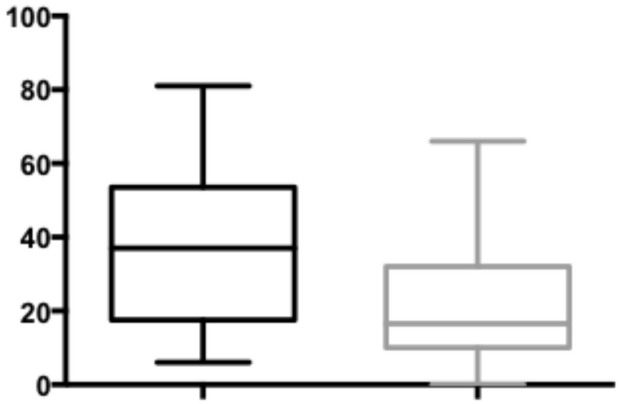

Figure 1. Box and Whisker plots representing 25(OH)D serum concentrations between protein-losing enteropathy (PLE) dogs in the poor-outcome group versus the good-outcome group. 25(OH)D serum concentration in the poor-outcome group: median 16.5 nmol/L, range from 0-66 nmol/L; good-outcome group: median 37 nmol/L, range from 6-81 nmol/L, p=0.017).

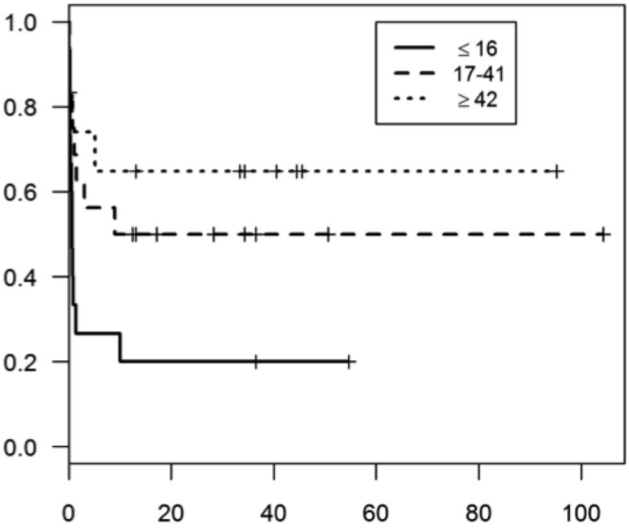

Figure 2. Kaplan-Meier curve and Cox regression using >16 nmol/l, 117-41 nmol/l, and >42 nmol/l as cutoff points for 25(OH)D serum concentration. Higher 25(OH)D serum concentration at diagnosis indicated a better survival of protein-losing enteropathy (PLE) dogs with an hazard ratio of 0.974 (95% CI 0.949, 0.999) per each nmol/l increase in vitamin D.

with a hazard ratio of 0.974 (95% CI 0.949, 0.999) for each nmol/l increase in 25(OH)D serum concentration (Figure 2).

Serum iCa concentrations were measured at the time of diagnosis in 41 of the 43 patients. The median serum iCa concentration in the combined cohorts of PLE dogs was 1.22 mmol/L (range from 0.79 to 1.45 mmol/L, (reference range from 1.25 to 1.45 mmol/L). In the good-outcome group (n=21), the median serum iCa concentration was 1.25 mmol/L (range from 0.79 to 1.35 mmol/L) with 10 dogs having iCa concentration below the reference range. In the negative-outcome group (n=20), the median serum iCa concentration was 1.18 nmol/L (range from 0.84 to 1.45 mmol/L),

with 13 dogs having iCa concentrations below the reference range. There was a moderate positive correlation between serum iCa and 25(OH)D concentrations (r=.52, p<0.0005).

The CCECAI scores between the good-outcome group versus the negative-outcome group were not statistically significant. The results for the negative-outcome group were median 8, range from 4 to 19, and for the good-outcome group were median 7, range from 4 to 13; p=0.6. There was no correlation between CCECAI scores or BCS and 25(OH)D concentrations (CCECAI: r=0.043, p=0.786; BCS: r=0.069, p=0.465). Treatment with immunosuppressive drugs and low-serum 25(OH)D concentration at diagnosis were the only factors associated with negative outcome (univariate logistic regression: p=0.006 and p=0.024, respectively). 25(OH)D serum concentration was the only significant (p=0.033) risk factor in the multivariable logistic regression analysis, with an increase of 25(OH)D level reducing the odds of having a poor outcome (odds ratio=0.96, 95% confidence interval: 0.93 to 0.997).

Discussion

Decreased iCa serum concentrations previously have been described with PLE possibly due to malabsorption of vitamin D in dogs with severe mucosal disease.[10] This study shows for the first time that low 25(OH)D serum concentrations and low iCa serum concentrations are highly prevalent in a cohort of PLE dogs and that decreased 25(OH)D serum concentrations are significantly associated with negative outcome.

There was a significant correlation between treatment group (diet versus diet + drugs) and outcome of PLE patients. The majority of patients in the good-outcome group were managed solely with nutritional therapy, while the majority of patients in the poor-outcome group were treated with diet and immunosuppressive drug protocols. The fact that BCS was not different between the PLE groups also indicates that poor nutritional status alone was not predictive of outcome. In addition, we could not find a correlation between serum albumin concentration and iCa, serum 25(OH)D concentrations or CCECAI. This indicates that loss of vitamin D-binding protein alone is probably not the sole factor for decreased serum 25(OH)D concentrations in these dogs. Furthermore, it may indicate that serum 25(OH)D concentration is an important metabolite to measure in these patients, as serum albumin alone may not be predictive for outcome.

Several studies have described dogs with GI disease, and low total and iCa serum concentrations often are prone to hypocalcemia even after clinical improvement.[9,18,19] This possibly could be due to serum vitamin D levels not being corrected and/or increased fraction of serum ionized calcium. In humans with vitamin D deficiency, survival is significantly better in patients with normal vitamin D levels compared to severely ill patients with vitamin D deficiency.[20] Future studies investigating vitamin D status in dogs should be performed using the gold standard tests as well as standard quality control schemes for laboratories, such as the vitamin D external quality assurance scheme (DEQAS).[c]

[a] Michigan State University Diagnostic Center for Population and Animal Health, Meridian Charter Township, MI
[b] WSAVA Body Condition Score for Dogs (*wsava.org/sites/default/files/Body%20condition%20score%20chart%20dogs.pdf*)
[c] *deqas.org*

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