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ORIGINAL ARTICLE

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Comparison of serum 25(OH) vitamin D, parathormone and immunity marker concentrations between dogs with transmissible venereal tumour and healthy dogs

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Abstract

Background: 1,25-Dihydroxyvitamin D (1,25(OH)$_2$ D) is vital in the homeostasis of calcium and bone health as well as in the prevention of many disorders such as neoplasms. Epidemiological data show that low concentrations of both 1,25(OH)$_2$D and its precursor 25(OH) vitamin D (25(OH)D) are associated with an increased risk of a variety of human tumours.

Objectives: To investigate 25(OH)D, parathormone (PTH) and immunity marker concentrations in dogs with transmissible venereal tumour (TVT).

Methods: 25(OH)D, PTH and various biochemical and immunity markers were evaluated in dogs with TVT ($n = 26$) and in healthy ($n = 30$) dogs.

Results: 25(OH)D concentrations were significantly lower in dogs with TVT in comparison with healthy dogs. In contrast, PTH, immunoglobulin G and interleukin (IL)-9 concentrations were higher in the dogs with TVT. Other variables, including IL-10, interferon γ, calcium and inorganic phosphate, were not statistically different between the two groups.

Conclusions: Decreased serum 25(OH)D concentration may be a risk factor for the development of canine TVT; however, cause-and-effect remains incompletely understood. Further studies are required to elucidate the exact role of 25(OH)D in canine TVT and whether vitamin D supplementation may be useful prophylactically or as an adjunct to chemotherapy.

KEYWORDS
25-hydroxyvitamin D, cytokines, dogs, transmissible venereal tumour

1 | INTRODUCTION

Calcitriol or 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) is the natural ligand for the vitamin D receptor (VDR) and has a classical role as the main regulator of bone metabolism and calcium homeostasis. Unlike humans, dogs are not able to efficiently or sufficiently synthesize vitamin D from cholesterol in response to sunlight (How et al., 1994; Nascimento-hama et al., 2022; Selting et al., 2016; Weidner & Verbrugghe, 2017). This lack of cutaneous production means that, in healthy dogs, serum vitamin D concentrations generally reflect levels in the diet (Corbee, 2020; Hurst, Homer, & Mellanby, 2020; Hurst, Homer, Gow, et al., 2020; Kritikos et al., 2018; Laing et al., 1999). Following

absorption from the gut, vitamin D is converted to 25(OH)D in the liver and then to the active metabolite $1,25(OH)_2D$ in the kidney (Gerber et al., 2004).

Low 25(OH)D levels are common in the face of disease and may arise from reduced vitamin D absorption or increased metabolism. For example, deprivation of sunlight, low food intake, low dietary vitamin D content, inadequate intestinal absorption, leakage of vitamin D from the gut, and reduced renal production of the active form (Corbee, 2020).

In addition to its primary role in calcium metabolism, vitamin D has several other important functions, including regulation of cell proliferation, differentiation, apoptosis, immune regulation and genome stability. Fundamental epidemiological investigations have shown that low serum 25(OH)D concentrations are associated with an increased risk for a variety of cancers in humans (Abbas et al., 2008; McCullough et al., 2010). In dogs, studies have demonstrated reduced amounts of 25(OH)D in patients with mast cell tumours (MCT), malignant mammary tumours in obese female dogs, hemangiosarcoma, lymphoma and neoplastic spirocercosis. In addition, expression of the VDR has been demonstrated in some canine neoplasms suggesting the potential use of calcitriol for therapeutics approaches (Davies et al., 2012; Nascimento-hama et al., 2022; Russell et al., 2010; Sánchez-Céspedes et al., 2021; Selting et al., 2016; Weidner et al., 2017). Indeed, evidence for a protective influence of vitamin D on tumour occurrence has been reported for several systemic cancers in humans, particularly urinary bladder, colon, prostate and breast cancer (Gorham et al., 2005; Konety et al., 2001; McCullough et al., 2010; Nascimento-hama et al., 2022; Yin et al., 2009).

Transmissible venereal tumour (TVT) is a horizontally transmitted contagious round cell neoplasia of dogs that is unique in oncology because it was the first tumour to be transmitted experimentally (Cohen, 1985). It is a naturally occurring tumour transmitted from animal to animal during copulation by viable tumour cells. It mainly affects the external genitalia and occasionally the internal genitalia, but in some cases can be found in extra genital sites (Cohen, 1985). Several successful therapeutic approaches for TVT have been described (Amber et al., 1990; Rogers et al., 1998) but the relationship between vitamin D and canine TVT has not yet been investigated. The objective of the present study was to compare serum 25(OH)D levels and various biochemical and immunity markers between healthy dogs and dogs with TVT.

2 | MATERIALS AND METHODS

2.1 | Animals

An observational study design was used. Thirty healthy dogs and 30 dogs with newly diagnosed TVT were recruited. Age (<1-year old, 1 to 5-year old and >5-year old), weight, sex, breed size (small/large), husbandry condition (indoor/outdoor), type of diet (home cooked/commercial) and body condition score were recorded. For each dog with TVT, a control dog with approximately similar characteristics was selected. Based on history, clinical examination, routine hematological, serum biochemistry and urinalysis panels, exclusion criteria for both case and control groups were as follows: receiving corticosteroids within 2 weeks of enrolment, pregnancy, hypercalcaemia, malignancy, hyperparathyroidism, hypoparathyroidism, hepatic disorders, renal disorders, diabetes mellitus, receiving any supplements containing vitamin D, calcium or both or concurrent systemic or infectious disease. Dogs with TVT were diagnosed by cytology of fine needle aspirate or swab/roll-prep samples of suspected lesions. In all cases, a board-certified clinical pathologist performed cytologic analysis.

2.2 | Sample collection

Blood samples were taken from jugular vein. A volume of 2.5 mL blood was collected into tubes containing EDTA for a complete blood count using a veterinary cell counter (Nihon Kohden, Celltac α, MEK 6450k). A Giemsa-stained blood smear was used to confirm differential leukocyte counts, which were performed on 100 white blood cells. A volume of 7.5 mL blood was collected into a plain tube, and once coagulated, was centrifuged at room temperature at 1800 *g* for 10 min. The serum was then decanted and used for biochemical analysis. The surplus serum was stored at −20°C for further analysis.

2.3 | Biochemical analysis

Levels of 25(OH)D (kit performance: assay range: 1–350 ng/mL, sensitivity: 0.53 ng/mL), parathyroid hormone (PTH) (kit performance: assay range: 10–2000 ng/L, sensitivity:4.99 ng/L), interleukin (IL)-9 (kit performance: assay range: 0.5–300 ng/L, sensitivity: 0.28 ng/L), IL-10 (kit performance: assay range: 2–800 pg/mL, sensitivity: 1.02 pg/mL), immunoglobulin G (IgG) (kit performance: assay range: 0.05–20 mg/mL, sensitivity: 0.03 mg/mL) and interferon γ (IFN-γ) (kit performance: assay range: 5–1500 ng/L, sensitivity: 2.58 ng/L) were measured with dog-specific ELISA kits (Bioassay Technology Laboratory kits, China) using an ELISA automatic washer and reader (BioTek, ELx-50; BioTek, ELx-800, USA). The intra- and inter-assay coefficients of variation (CV) of ELISA kits were <8% and <10%, respectively. Other variables, including albumin (Alb), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), calcium (Ca), inorganic phosphorus (Ph), total bilirubin (BR), cholesterol (CHO), creatinine (Cre), triglyceride (TG), glucose (Glu) and urea, were measured with commercial kits (Pars Azmoon, Tehran, Iran) using a biochemical auto analyser (Biotecnica, BT 1500, Rome, Italy). The inter-assay and intra-assay CV of all measurement methods was ≤5%. Control serum (Centronorm, Centronic GmbH, Germany) was used for assessing measurement accuracy.

2.4 | Statistics

Statistical analysis was performed using SPSS software (version 22). The outcome variables with Shapiro–Wilk values of $p > 0.05$ were

TABLE 1 Mean ± standard error (SE) of measured variables in transmissible venereal tumour (TVT) ($n = 26$) and control ($n = 30$) groups of dogs.

Variables	Control	TVT group	*p*-Value
25(OH)D (ng/mL)	36.4 ± 3.1	29.5 ± 5.8	0.028
PTH (ng/L)	289.9 ± 12.6	357.8 ± 22.4	0.01
IL-9 (ng/L)	41.3 ± 2.2	61.9 ± 9.6	0.036
IL-10 (ng/L)	54.4 ± 4.7	84.2 ± 15.5	0.101
IFN-γ (ng/L)	59.92 ± 8.0	72.59 ± 9.22	0.307
IgG (mg/mL)	2.7 ± 0.25	4.2 ± 0.62	0.008
Cholesterol (mg/dL)	206.42 ± 8.5	174.7 ± 8.8	0.015
Triglyceride (mg/dL)	86.9 ± 12.6	67.2 ± 5.7	0.241
Urea (mg/dL)	47.4 ± 3.8	38.6 ± 3.7	0.105
Creatinine (mg/dL)	1.5 ± 0.05	1.3 ± 0.06	0.007
Bilirubin (mg/dL)	0.26 ± 0.005	0.27 ± 0.005	0.458
Glucose (mg/dL)	135 ± 6.8	145 ± 7.3	0.297
Total protein (g/dL)	6.56 ± 0.17	6.74 ± 0.18	0.478
Albumin (g/dL)	3.30 ± 0.05	3.33 ± 0.08	0.732
ALT (U/L)	29.5 ± 1.4	33.3 ± 2.4	0.191
AST (U/L)	31.4 ± 2.0	31.0 ± 3.6	0.528
ALP (U/L)	103 ± 12	112 ± 10	0.584
GGT (U/L)	3.3 ± 0.3	4.3 ± 0.4	0.036
Calcium (mg/dL)	9.38 ± 0.32	9.2 ± 0.34	0.750
Phosphorus (mg/dL)	4.6 ± 0.19	4.7 ± 0.21	0.658

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; IFN-γ, interferon γ; IgG, immunoglobulin G; IL, interleukin.

considered to be normally distributed (IFN-γ, TP, Alb, Ph, Glu, ALP, ALT, GGT and urea). All other variables were considered as parametric data following logarithmic transformation (25(OH)D, PTH, IL-9, IL-10, IgG, Ca, AST, BR, CHO, TG and Cre). All data were expressed as mean ± standard error and the differences between trial groups were analysed using the parametric *t* test. Statistical significance was set at $p \leq 0.05$.

3 | RESULTS

3.1 | Animals

Three dogs were eliminated from the study based on the existence of concurrent inflammatory diseases, leaving 27 dogs with newly diagnosed TVT and 30 healthy controls.

Each group comprised the following: age (<1-year old: $n = 2$, 1 to 5-year old: $n = 24$ and >5-year old: $n = 5$), sex (male $n = 17$, female $n = 13$), breed size (small breed: $n = 5$ and large breed: $n = 25$), husbandry condition (indoor: $n = 6$ and outdoor: $n = 24$), type of diet (combined homemade and commercial food) and BCS (underweight: $n = 9$, optimal: $n = 18$ and overweight: $n = 3$). The weight range was 7–30 kg for dogs in the TVT group and 6–32 kg in the healthy dogs.

3.2 | Biochemical analysis

The concentrations of measured variables and the results of statistical comparisons between trials groups are presented in Table 1. Serum 25(OH)D concentrations were significantly lower in the dogs with TVT in comparison with the control dogs ($p = 0.028$). Serum PTH concentrations were significantly higher in the TVT group ($p = 0.01$). Serum concentrations of IL-9 and IgG were also significantly higher in the TVT group ($p = 0.036$ and $p = 0.008$, respectively). Serum amounts of IL-10 and IFN-γ were not significantly different between groups ($p > 0.05$). Among the serum biochemical variables evaluated, GGT activity was significantly higher in the TVT group ($p = 0.036$), whereas CHO ($p = 0.015$) and Cre ($p = 0.007$) levels were higher in the control group. There were no significant differences between groups concerning the other measured variables (Alb, TP, ALT, AST, ALP, Ca, ph, BR, TRI, urea and Glu).

4 | DISCUSSION

The primary aim of this study was to compare serum 25(OH)D concentrations between dogs with TVT and healthy dogs. As has been found in dogs with other cancers (Davies et al., 2012; Nascimento-hama et al., 2022; Russell et al., 2010; Sánchez-Céspedes et al., 2021; Weidner

et al., 2017), the results demonstrated that serum 25(OH)D concentrations were significantly lower in dogs with TVT than in healthy dogs. This suggests that low serum 25(OH)D might be a risk factor for the occurrence and development of this neoplasm and/or, that the presence of TVT may result in a lowering of serum 25(OH)D levels. In support of former hypothesis, previous studies have suggested that calcitriol supplementation can prevent canine transitional cell carcinoma, canine osteosarcoma and MCT (Barroga et al., 1998; Kaewsakhorn et al., 2005; Malone et al., 2010) and have described a synergistic effect of calcitriol and cisplatin on inhibition of proliferation in canine tumours in vitro (Goulão et al., 2018; Rassnick et al., 2008). Vitamin D supplementation has also been shown to have anti-neoplastic effects in both humans (Abbas et al., 2008; Gorham et al., 2005; Lappe et al., 2007; Osborne & Hutchinson, 2002) and in dogs (Barroga et al., 1998; Kaewsakhorn et al., 2005; Toyota et al., 1996) in a wide range of epithelial, mesenchymal and hematopoietic malignancies. This antitumour effect of vitamin D is partly due to the direct increased binding of the nuclear VDR that promotes tumour cell death through apoptosis. In addition, antiproliferative and antiangiogenic effects of calcitriol are described which limit invasive growth by influencing epidermal growth factor (Abu el Maaty & Wolfl, 2017; Nascimento-hama et al., 2022; Pandolfi et al., 2017; Rassnick et al., 2008).

This study also found that PTH and 25(OH)D had an inverse relationship, and that dogs with TVT had higher PTH levels compared with healthy dogs. These findings are similar to those of a previous study (Parker et al., 2017). Increased PTH concentrations are likely to be a compensatory response to low vitamin D levels via stimulation of 1a-hydroxylase activity, ultimately increasing calcitriol synthesis with a subsequent increase in intestinal calcium and phosphorus absorption, and reduction of 24-hydroxylase activity (Parker et al., 2017). In another study, low vitamin D levels in dogs with cancer were associated with low Ca levels (Davies et al., 2012) but in the present study, serum concentrations of both Ca and Ph were not significantly different between TVT-affected dogs and healthy dogs.

Although control of both Ca and Ph is dependent upon the actions of 25(OH)D and PTH, maintenance of tight control is critical, thus, the lack of Ca and Ph changes in this study are likely to be explained by compensatory mechanisms via increased PTH and its effects on bone and kidney.

Human studies have shown that, as well as being markers of inflammation, IFN-γ, IL-9, IL-10 and IgG have important and complex functions in relation to malignancy (Shalaby et al., 1985). In contrast with the findings in some human malignancies (Shalaby et al., 1985), there was no significant difference found in IFN-γ or IL-10 levels between the two groups. However, IL-9 and IgG were significantly higher in the TVT group. Previous studies have also reported an inverse relationship between 25(OH)D and IL-9 and IgG (Shalaby et al., 1985). As has been found in humans (Zhang et al., 2012) higher expression and production of IgG may reflect its involvement in tumour growth and proliferation. In fact, some cancer cells are capable of synthesizing IgG, which helps to potentiate their malignancy, a possibility that needs further investigation in relation to TVT in dogs. IgG might even have utility as a prognostic indicator in canine TVT, as has been reported in oesophageal squamous cell carcinomas (Zhang et al., 2012).

IL-9 has apposing roles in tumour immunity (Wan et al., 2021). On one hand, it acts as a lymphocyte growth factor promoting hematopoietic tumour progression. Conversely, in solid tumours, IL-9 can inhibit tumour development via its actions on innate or adaptive immune responses (Wan et al., 2020). The exact role of IL-9 in pathogenesis of TVT needs to be elucidated (Wang et al., 2019).

In the present study, levels of cholesterol and creatinine were significantly lower, and the activity of GGT was significantly higher, in the TVT group than the control group. The levels of these variables were nevertheless all within reference range, thus, although statistically different, this was not deemed clinically important. An association of GGT activity has been found with metastatic lesions and stress-related corticosteroid responses (Willard & Twedt, 2012). Lower creatinine levels may be related to reduced muscle mass in the patients with neoplasia (Meuten & Sample, 2022). Hypocholesterolemia has also been reported in selected malignancies, and in patients with malnutrition (Nelson, 2012); however, the significance and mechanisms of these changes in dogs with TVT are not clear.

The main limitation of this study is that only total calcium levels were measured. Despite normal total calcium levels, we cannot completely exclude the possibility of ionized hypercalcaemia, the biologically active form. In addition, the use of diagnostic imaging as part of the health screening would have helped to further rule out occult disease.

In summary, although the results of this study concluded that 25(OH)D, IgG and IL-9 levels are increased in dogs with TVT, cause-and-effect remains incompletely understood and further studies are needed to improve our understanding of the role of vitamin D in dogs with TVT. Similarly, more information is required to ascertain whether vitamin D supplementation could have a role with regards to treatment or prophylaxis (Russell et al., 2010; Selting et al., 2016; Weidner & Verbrugghe, 2017).

AUTHOR CONTRIBUTIONS

Investigation; data curation; validation; writing original draft: Helia Kamali Sadeghian. *Conceptualization; data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; writing – review and editing*: Mehrdad Mohri.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflicts of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and

the appropriate ethical review committee approval has been received (3/51077). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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PEER REVIEW

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