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Research Article

Association between Serum Vitamin D, Inflammatory Biomarkers, and Clinical Severity in Dogs with Chronic Dermatological Diseases

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ABSTRACT

Introduction: Chronic dermatological diseases are common in dogs and are often linked to persistent inflammation, immune dysregulation, and reduced quality of life. Vitamin D has important immunomodulatory and anti-inflammatory properties. This study aimed to investigate the association between serum vitamin D levels, inflammatory biomarkers, and clinical severity in dogs with chronic dermatological diseases.

Materials and methods: This case-control study included 30 dogs with chronic dermatological diseases and 30 clinically healthy dogs as controls, conducted between March 2025 and May 2026. The mean age of dogs was 4 years (1.8-7.5 years), and the mean body weight was 19.1 kg (8.5-30.5 kg). Of the 15 male dogs, 10 were neutered, and of the 15 female dogs, 12 were spayed. The clinical severity of dermatological diseases was assessed by the Canine atopic dermatitis extent and severity index, 4th iteration, and the Pruritus visual analog scale. Blood samples were collected on day 1 from all dogs for serum Vitamin D, C-reactive protein (CRP), and interleukin-6 (IL-6) measurements. Complete blood counts were obtained, and the neutrophil-to-lymphocyte ratio (NLR) was calculated on day 1. At the end of the study period, dogs affected by dermatological diseases and healthy dogs were compared for serum vitamin D levels and inflammatory biomarkers.

Results: Dogs with chronic dermatological diseases had significantly lower serum vitamin D levels and higher CRP (12.4 mg/L), IL-6 (42.6 pg/mL), and NLR (4.8) than controls. Lower vitamin D status in dogs with chronic dermatological diseases correlated with higher clinical disease severity and higher pruritus scores. Vitamin D concentrations negatively correlated with CRP, IL-6, and NLR, whereas CRP and IL-6 positively correlated with clinical severity in dogs with chronic dermatological diseases. The current results indicated that lower serum vitamin D levels were significantly associated with more severe dermatological manifestations after adjusting for age, body weight, sex, and neuter status.

Conclusion: The current results demonstrated that decreased serum vitamin D levels were associated with increased systemic inflammation and increased clinical severity in dogs with chronic dermatologic diseases. Thus, serum vitamin D levels and inflammatory biomarkers can serve as an adjunctive indicator of disease severity in dogs.

1. Introduction

Chronic dermatological diseases are among the most common conditions in small animal veterinary practice,

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affecting a high percentage of the canine population[1,2]. Diseases such as atopic dermatitis, pemphigus foliaceus, and sebaceous adenitis are characterized by persistent inflammation, immune dysregulation, and a considerable impact on the quality of life of affected animals and their owners[1,2]. Beyond local cutaneous manifestations, emerging evidence in human medicine suggests that chronic inflammatory skin disorders may trigger a more systemic inflammatory response beyond the skin barrier[3]. Chronic dermatological diseases can trigger systemic inflammatory responses, affecting patient monitoring, prognosis, and the discovery of new therapeutic targets in veterinary dermatology[3].

Beyond its well-established role in calcium and phosphorus homeostasis, vitamin D is now recognized as a potent immunomodulatory agent with anti-inflammatory properties[4]. The active metabolite of vitamin D, 1,25-dihydroxyvitamin D, mediates its effects through the vitamin D receptor (VDR), which is expressed on different immune cells, including macrophages, dendritic cells, and T lymphocytes[4]. Thus, vitamin D regulates innate and adaptive immune responses, induces tolerance, and inhibits excessive inflammatory cytokine production[4]. Serum 25-hydroxyvitamin D [25(OH)D] is currently recognized as an important marker for assessing overall vitamin D status in humans and dogs[5,6]. Low vitamin D levels have been associated with increased severity of atopic dermatitis, psoriasis, and other chronic inflammatory skin diseases in human dermatology[7]. However, the association between vitamin D status and chronic dermatological diseases has not yet been fully characterized in veterinary medicine. Previous studies in dogs have suggested a possible link between vitamin D and skin health[8,9]. For instance, a study in Japanese Akita dogs found that serum vitamin D concentrations were significantly lower in dogs with dermatological disease than in healthy dogs, and that skin depigmentation, a characteristic feature of some immune-mediated dermatoses, was associated with lower vitamin D concentrations[10]. C-reactive protein (CRP) and interleukin-6 (IL-6) are among the most widely studied biomarkers of systemic inflammation in veterinary medicine[11]. The CRP is an acute-phase protein synthesized primarily by the liver in response to pro-inflammatory cytokines, particularly IL-6, and serves as a sensitive, albeit non-specific, indicator of ongoing inflammatory activity[11,12]. The IL-6 is a pleiotropic cytokine that plays a central role in the initiation and amplification of the inflammatory cascade, including the induction of acute-phase proteins, recruitment of immune cells, and modulation of both innate and adaptive immune responses[13]. In chronic inflammatory conditions, persistent low-grade elevation of these biomarkers reflects a sustained systemic inflammatory state that may contribute to disease progression and complications beyond the primary affected organ[13]. In veterinary dermatology, no significant differences in serum pro-inflammatory cytokine concentrations (including IL-6) were detected between healthy dogs and dogs with atopic dermatitis or pemphigus foliaceus, despite the expectation of systemic inflammation in moderate-to-severe disease[14]. Similarly, the neutrophil-to-lymphocyte ratio (NLR), a readily available hematological marker of systemic inflammation, has emerged as a useful indicator of inflammatory burden in different chronic disorders[15].

In veterinary dermatology, however, the role of these inflammatory biomarkers in chronic skin diseases remains incompletely understood. Given the growing evidence supporting a systemic inflammatory component in canine chronic dermatological disorders, evaluating CRP, IL-6, and NLR alongside vitamin D status may provide a more comprehensive picture of disease severity and help identify animals at risk of systemic complications. Thus, the present study aimed to comprehensively assess the potential utility of vitamin D and inflammatory markers as adjunctive indicators of disease severity in canine dermatology.

2. Materials and Methods

2.1. Ethical approval

The study protocol was approved by the Institutional Animal Care and Use Committee at the Science and Research Branch, Islamic Azad University, Tehran, Iran, before starting the study (IAUSR 55.2-1-2532-99-12). All dog owners provided written informed consent prior to enrollment.

2.2. Study design

This case-control study was conducted at a veterinary teaching hospital in Tehran, Iran, between March 2025 and May 2026. A minimum of 27 dogs per group was required to detect a difference in serum Vitamin D concentrations with 80% power at a two-sided α of 0.05. To account for potential data loss, 30 dogs per group were enrolled. Thus, a total of 60 client-owned dogs were enrolled in the current study, of which 30 dogs were diagnosed with chronic dermatological diseases (experimental group) by a certified veterinarian, and 30 dogs were clinically healthy (control group), which were examined by routine clinical examinations such as skin scraping, tape impression, and Wood's lamp examination. Body temperature, respiratory rate, and heart rate were checked, and all were within normal ranges. The current study included dogs with a confirmed diagnosis of one or more chronic dermatological conditions lasting at least 3 months. Eligible diagnoses included canine atopic dermatitis, diagnosed according to Favrot's criteria[16], pemphigus foliaceus, sebaceous adenitis, and chronic *Malassezia* dermatitis. Dogs were excluded when they had received systemic glucocorticoids, cyclosporine, or other immunomodulatory agents, tropical glucocorticoids, antihistamines within the previous 4 weeks, vitamin D supplementation within the previous 8 weeks, had concurrent systemic diseases such as renal, hepatic, and endocrine disorders, and had been diagnosed with flea allergy dermatitis, sarcoptic mange, flea allergy dermatitis, sarcoptic mange, or demodicosis. Sarcoptic mange was excluded by negative results on multiple deep skin scrapings and response to acaricidal therapy, while demodicosis was excluded by negative deep skin scrapings performed at multiple affected sites. Healthy control dogs

were recruited from the same hospital population at routine wellness examinations. Dogs were deemed healthy based on normal physical examination, absence of dermatological lesions, and normal complete blood count and serum biochemical analysis. Healthy dogs were selected to ensure that their age and sex distribution closely matched that of the dogs with dermatological disease. In other words, the groups were adjusted for age and sex to facilitate more equitable comparisons. Age matching was performed using four categories, including < 2 years, 2-5 years, 5-8 years, and > 8 years. Sex matching was used to align the male-to-female ratio of the disease group. The final distribution was 16 males and 14 females in the experimental group and 15 males and 15 females in the control group. In addition, dogs with a history of chronic dermatological disease and any history of immunomodulatory treatment were excluded from the experiment.

2.3. Signalment and baseline characteristics

The experimental group comprised 30 dogs of different breeds, including Labrador Retriever (n = 7), German Shepherd (n = 5), Golden Retriever (n = 4), French Bulldog (n = 3), Pug (n = 3), and mixed breeds (n = 8). The mean age was 4.2 years (1.5-8.0), and the mean body weight was 18.5 kg (7.8-32.0). The experimental group included 16 males (12 neutered) and 14 females (11 spayed). The control group consisted of 30 dogs of similar breeds, including Labrador Retriever (n = 6), German Shepherd (n = 6), Golden Retriever (n = 5), French Bulldog (n = 3), Pug (n = 2), and mixed breeds (n = 8). The mean age of dogs was 4 years, ranging from 1.8 to 7.5 years, and the mean body weight of dogs was 19.1 kg, ranging from 8.5 to 30.5 kg. Of the 15 male dogs, 10 were neutered, and of the 15 female dogs, 12 were spayed.

2.4. Clinical severity assessment

Clinical severity of the dermatologic disease was assessed using two validated scoring systems, including the Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-4)[17] and the Pruritus Visual Analog Scale (PVAS)[15]. CADESI-4 assesses 20 body sites commonly affected in atopic dogs, with three lesion types (erythema, lichenification, and alopecia/excoriation), each scored from 0 to 3. The total score ranged from 0 to 180, and proposed cutoffs have been established to classify disease severity as mild (10), moderate (35), or severe (60) scores. Dog owners rated pruritus severity using an enhanced Visual Analog Scale (PVAS). The pruritus visual analog scale (PVAS) was a 10 cm horizontal line with descriptive anchors at either end, on which owners marked the perceived level of their dog's pruritus[18]. Scores ranged from 0 (no pruritus) to 10 (severe, continuous pruritus). A score of less than 2 indicated clinical remission of pruritus. A single veterinary dermatologist certified by the Science and Research Branch, Islamic Azad University, Tehran, Iran, performed all clinical assessments to limit inter-observer variability.

2.5. Sample collection

Blood samples were collected on the day of enrollment, immediately after diagnosis confirmation, and before initiating any treatment, following a minimum 12-hour fast. Samples (5 mL) were obtained from all dogs via cephalic or jugular venipuncture. Blood was collected in EDTA tubes for complete blood count and serum separator tubes for biochemical and biomarker analysis, including serum vitamin D, CRP, IL-6, and NLR. Serum was separated by centrifugation at 1500 × g for 10 minutes within 30 minutes of collection and frozen at -80°C for further analysis.

2.5.1. Serum vitamin D

Serum vitamin D concentrations were measured by a validated chemiluminescent immunoassay (CLIA) according to the manufacturer's instructions (Padtangostar Isar, Iran), and results were reported as ng/mL[19]. All samples were analyzed in duplicate, and the mean of duplicate measurements was used for statistical analysis.

2.5.2. C-Reactive protein

Serum CRP concentrations were measured by a canine-specific immunoturbidimetric assay with a detection limit of 0.5 mg/L, and the results were expressed as mg/L. The assay had an intra-assay coefficient of variation (CV) of < 8% and an inter-assay CV of ≤ 13%, which are within the acceptable limits for canine CRP measurement based on biological variation data[20].

2.5.3. Interleukin-6

Serum IL-6 concentrations were analyzed with a commercially available canine-specific sandwich ELISA kit (Padtangostar Isar, Iran). The lowest detectable concentration of the assay was 7.8 pg/mL with intra-assay and inter-assay coefficients of variation of <10% and <12%, respectively.

2.5.4. Complete blood count and neutrophil-to-lymphocyte ratio

Complete blood counts were acquired in an automated hematology analyzer (Sysmex Corporation, Japan) within 2 hours of collection. The NLR was calculated as the absolute neutrophil count divided by the absolute lymphocyte count in an automated hematology analyzer (Sysmex Corporation, Japan). The reference ranges for NLR in healthy dogs have been reported as 1.3-7.1[21].

2.6. Statistical analysis

Statistical analysis was performed using commercial software (SPSS version 29.0). Normality of the data distribution was tested by the Shapiro-Wilk test. Normally distributed data were expressed as mean ± standard deviation (SD), while non-normally distributed data were expressed as median and interquartile range (IQR). An independent-samples t-test was utilized to compare normally distributed variables, and the Mann-Whitney U test was used to compare non-normally distributed variables; chi-square or Fisher's exact test was used to compare categorical variables. Spearman's rank correlation coefficient was used to analyze the correlation between serum vitamin D levels, inflammatory biomarkers, and

clinical severity scores in the study with non-normally distributed results. Multivariable linear regression analysis was used to assess the independent association between serum vitamin D concentration and clinical severity (CADESI-4 score) after adjusting for potential confounding variables, including age, body weight, sex, and neuter status. Variables with $p < 0.10$ were considered for multivariable model inclusion. A p-value less than 0.05 was considered statistically significant for comparing the results.

3. Results

3.1. Study population

A total of 60 dogs were included in the control and experimental groups; the most frequent diagnoses in the experimental group were canine atopic dermatitis (n = 18, 60%), pemphigus foliaceus (n = 5, 16.7%), sebaceous adenitis (n = 4, 13.3%), and chronic *Malassezia* dermatitis (n = 3, 10%). The mean duration of dermatological signs before enrollment was 8.4 months (3-24 months). No significant differences were found between the groups in age, body weight, sex distribution, and neuter status ($p > 0.05$).

3.2. Clinical severity scores

Dogs in the experimental group had a mean CADESI-4 score of 42.6 with a median of 38, and an IQR between 30-56, indicating a moderate-to-severe range (Table 1). The mean PVAS score was 5.8 ± 1.9 (median: 5.7; IQR: 4.5-7.2), indicating moderate-to-severe pruritus. Control dogs had CADESI-4 and PVAS scores of 0 per the inclusion criteria.

3.3. Serum vitamin D level

Serum vitamin D levels of dogs were significantly lower ($p < 0.05$) in the experimental group (median: 68.4 ng/mL, IQR: 52.1-81.7) than in the control group (median: 92.6 ng/mL, IQR: 78.3-108.4; Table 2).

3.4. Inflammatory parameters

Dogs in the experimental group had significantly higher levels of all measured inflammatory biomarkers compared to controls (Table 2; $p < 0.05$). Serum CRP concentrations (12.4 mg/L), IL-6 levels (42.6 pg/mL), and NLR (4.8) were significantly higher in the experimental group than in the control group ($p < 0.05$).

3.5. Correlation analyses

Correlation analysis revealed significant inverse correlations between serum vitamin D levels and all measured inflammatory biomarkers (Table 1; $p < 0.05$). Specifically, vitamin D was negatively correlated with CRP, IL-6, and NLR. Conversely, inflammatory biomarkers were significantly positively correlated with clinical severity scores. The CRP was positively correlated with CADESI-4 and PVAS scores ($p < 0.05$). The IL-6 was positively correlated with CADESI-4 ($r = 0.51$, $p < 0.05$) and PVAS scores ($r = 0.44$, $p < 0.05$). Furthermore, NLR was positively correlated with CADESI-4 ($r = 0.46$, $p < 0.05$) and PVAS scores ($r = 0.39$, $p < 0.05$). In addition, serum vitamin D levels were negatively correlated with CADESI-4 ($r = -0.58$, $p < 0.05$) and PVAS scores ($r = -0.49$, $p < 0.05$).

Table 1. Correlation coefficients between serum vitamin D, inflammatory biomarkers, and clinical severity scores

Variables	CADESI-4	PVAS	Serum vitamin D	CRP	IL-6	NLR
CADESI-4	—	0.72[a]	-0.58[a]	0.56[a]	0.51[b]	0.46[b]
PVAS	—	—	-0.49[a]	0.48[a]	0.44[b]	0.39[b]
Serum vitamin D	—	—	—	-0.52[a]	-0.47[a]	-0.41[b]
CRP	—	—	—	—	0.61[a]	0.54[a]
IL-6	—	—	—	—	—	0.49[a]

CADESI-4: Canine atopic dermatitis extent and severity index, 4th iteration, PVAS: Pruritus visual analog scale, CRP: C-reactive protein, IL-6: Interleukin-6, NLR: Neutrophil-to-lymphocyte ratio. [a,b] Different superscript letters indicate the significance of the correlation between the variable in the corresponding row and the variable in the corresponding column at $p < 0.05$.

Table 2. Comparison of serum vitamin D and inflammatory biomarkers between dogs with chronic dermatological diseases and healthy dogs

Variables	Dogs with chronic dermatological diseases (n = 30)	Healthy dogs (n = 30)	p-value
Serum vitamin D (ng/mL)	68.4[a] (52.1-81.7)	92.6[b] (78.3-108.4)	< 0.05
CRP (mg/L)	12.4[b] (8.7-18.9)	3.2[a] (2.1-5.8)	< 0.05
IL-6 (pg/mL)	42.6[b] (28.3-67.4)	15.8[a] (9.6-24.1)	< 0.05
NLR	4.8[b] (3.6-6.2)	2.9[a] (2.3-3.7)	< 0.05

n: Number of dogs in each group, CRP: C-reactive protein, IL-6: Interleukin-6, NLR: Neutrophil-to-lymphocyte ratio. [a,b] Mean different superscript letters within a column differ significantly ($p < 0.05$). Data are presented as median (interquartile range).

3.6. Multivariable linear regression analysis

Lower serum vitamin D level independently predicted higher CADESI-4 scores; for every 10 ng/mL decrease in serum vitamin D, CADESI-4 score increased by approximately 3.4 points, holding other variables constant. The final model accounted for 41% of the variance in CADESI-4 scores (adjusted $R^2 = 0.41$), and this proportion was statistically significant ($p < 0.05$). None of the other covariates, such as age, body weight, sex, or neuter status, were statistically significant in the multivariable model ($p > 0.05$). After controlling for covariates, lower serum vitamin D levels were significantly associated with higher PVAS scores ($p < 0.05$).

4. Discussion

In the present study, dogs with chorionic dermatological disease had markedly lower serum vitamin D concentrations and significantly higher inflammatory biomarkers, including CRP, IL-6, and NLR, than the healthy dogs. These current results were consistent with previous studies linking vitamin D insufficiency to systemic inflammation in different dog species[22]. The present findings are consistent with the existing comprehension of the immunomodulatory function of vitamin D4. One of the most well-characterized vitamin D modulatory effects, conserved in both dogs and humans, is the attenuation of pro-inflammatory cytokine production[22]. Observational studies have found an inverse association between serum 25(OH)D3 levels and antigen-stimulated TNF-α concentrations in dogs, consistent with the current study[22,23]. Vitamin D exerts immunomodulatory effects by binding to the VDR, which is expressed on almost all immune cell types, leading to decreased production of pro-inflammatory cytokines and increased production of anti-inflammatory cytokines[24]. In the context of dermatological disease, this immune regulation may explain the association between reduced vitamin D status and increased systemic inflammation and clinical severity in the current study.

The current results were consistent with previous canine studies. In a survey of Japanese Akita dogs, serum vitamin D levels were notably lower in the experimental dogs with skin disease (72.13 ± 4.09 ng/mL) than in healthy dogs (84.90 ± 3.36 ng/mL)[10]. Importantly, the mean serum vitamin D concentration in healthy dogs in the present study (92.6 ng/mL) was similar to that in healthy Akita dogs[10], and the experimental group value (68.4 ng/mL) was similar to that in the experimental group of Casini et al.[10]. In addition, a study in dogs with atopic dermatitis found that dogs that responded well to prednisolone therapy had remarkably higher pretreatment vitamin D concentrations than poor responders[14]. These findings were consistent with the current results, which indicated that lower vitamin D levels were associated with more severe disease and suggested that vitamin D status might influence treatment outcomes in canine dermatological diseases.

The CRP is a composite marker for systemic inflammation in dogs, and concentrations > 30mg/L are considered to be consistent with systemic inflammation[11]. The median CRP concentration observed in the experimental group (12.4 mg/L) was consistent with early, mild, or resolving systemic inflammation. This finding was expected since the dogs included had chronic, manageable dermatological conditions rather than acute systemic illnesses. The strong positive correlation between CRP and CADESI-4 confirmed that even small increases in CRP indicated an inflammatory burden in dermatologic disease. IL-6 is a major pro-inflammatory cytokine and has been implicated in the pathogenesis of atopic dermatitis in both humans and dogs[11]. A single study of anal sac cytokine concentrations in atopic dogs did not indicate notable differences in IL-6 between healthy and affected dogs[25]. The present findings indicated that increased serum IL-6 in dogs with chronic dermatological disease supported a systemic inflammatory component of these diseases. The NLR, while a non-specific marker of inflammation, has been established as a prognostic indicator in canine oncology[26]. Mechanistically, vitamin D acts on dendritic cells via VDR to induce a tolerogenic phenotype, promoting regulatory T cell differentiation and suppressing effector T cell responses[23]. The inverse associations between vitamin D and CRP, IL-6, and NLR observed in the present study provided clinical evidence supporting these mechanistic pathways in canine dermatologic disease[22]. Unlike humans, dogs cannot synthesize vitamin D efficiently in the skin because of low cutaneous 7-dehydrocholesterol concentrations, and serum vitamin D levels in dogs tend to remain stable across seasons[27].

5. Conclusion

The current study concluded that decreased serum vitamin D levels in dogs were correlated with increased systemic inflammation and increased clinical severity of chronic dermatological diseases. Serum vitamin D and inflammatory parameters, including CRP, IL-6, and NLR, may be useful as supplementary indicators of disease severity in dogs. Housing conditions (indoor versus outdoor) and sun exposure were not documented, and diet was not investigated; therefore, controlled trials are needed to determine whether vitamin D supplementation can modify inflammatory responses and clinical outcomes in affected dogs with dermatological diseases.

Declarations

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Hamidreza Shojaei designed the study and coordinated the project. Mohammad Reza Deylami supervised the study, performed statistical analyses, and revised the manuscript as corresponding author. Pouya Mirali and Mohammad Sadegh Khanbabaii contributed to clinical examination and sample collection. Maedeh Taghinezhad performed laboratory analyses, including vitamin D, CRP, IL-6, and NLR measurements. Mohammad Talebi Daloei assessed clinical severity using CADESI-4 and PVAS. Mohammad Reza Mollaei assisted with data curation and manuscript drafting. Erfan Karimi contributed to laboratory investigations and data interpretation. All authors read and approved the final edition of the manuscript.

Competing interests

The authors declared no known competing financial interests or personal relationships that could have appeared to influence the study reported in the present manuscript.

Ethical considerations

All procedures followed ethical principles, and the

authors confirmed that they followed the journal's ethical publication guidelines. The authors stated that this article has not been submitted elsewhere and that this is the first time it has been submitted to the present journal. The authors confirm that the present manuscript has been checked for plagiarism, misconduct, and fabrication. The authors declare and accept full responsibility for the originality of this paper before submission and after acceptance. The authors declare that they did not use any AI tools or applications to conduct, write, or prepare any part of the study.

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