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Comparison of serum vitamin D concentrations between dogs with atopic dermatitis and healthy dogs: Pilot study

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Summary

Atopic dermatitis (AD) is a genetically predisposed inflammatory and pruritic skin disease that is common in dogs. Vitamin D is involved in the skin's barrier function, immune response and the production of antimicrobial peptides. However, since vitamin D has immunomodulatory properties, its deficiency can lead to dermatological problems. The aim of the present study was to determine serum vitamin D concentrations in dogs with AD, compare serum vitamin D concentrations in healthy dogs and dogs with AD, and reveal the relationship between clinical scores and serum vitamin D concentrations. Blood and skin scraping samples were taken from dogs diagnosed with AD clinically using Favrot criteria for diagnosis and differential diagnosis. The canine atopic dermatitis extent and severity index (CADESI-4) and the pruritus visual analog scale (pVAS) were measured in dogs diagnosed with AD. In addition, serum vitamin D concentrations were determined in serum samples taken from these dogs. Serum vitamin D concentrations in dogs with AD and healthy dogs were compared by statistical analysis. Although serum vitamin D concentration was higher in dogs with AD compared to the control group, it was not found to be statistically significant ($p > 0.05$). It is thought that the high serum vitamin D concentrations are due to changes in cytokine levels in the course of AD affecting vitamin D levels.

Keywords: atopic dermatitis, canine, 25-hydroxyvitamin D

Atopic dermatitis (AD) is a genetically predisposed inflammatory and pruritic skin disease that is common in dogs (5, 26, 27). It is reported that allergens involved in the etiology of canine AD are house dust mites, plants, arthropods, epithelial debris, foods, and mold (5). It has also been reported that the incidence of AD increases in cases where the immune system is weak, the skin barrier is damaged, and microbial colonization on the skin is high (5, 25, 38). Although its pathogenesis is not fully known, it has been shown to be associated with immunoglobulin E (IgE) directed against environmental allergens (25). The primary clinical indication in canines afflicted with atopic dermatitis is pruritus, with subsequent manifestations including erythema, papules, pustules, crusting, peeling, alopecia, hyperpigmentation, and lichenification (5, 25, 38). Pruritus and lesions are usually seen on the face, auricle, neck, armpits, forelegs, hind legs, lower abdomen, perineum, and tail (5).

The canine atopic dermatitis extent and severity index (CADESI-4) and the pruritus visual analog scale (pVAS) were reported to be used to determine the clinical severity of the disease (12, 22, 32). Diagnosis is made after eliminating skin diseases (fungal, bacterial, parasitic) with clinical signs similar to those of AD based on anamnesis, clinical findings (evaluation of Favrot criteria and pruritus score), and the results of allergen-specific IgE serum tests (6, 11, 15, 37, 39).

Vitamin D is a fat-soluble vitamin that exists in two main forms: ergocalciferol (vitamin D_2), produced by plants, and cholecalciferol (vitamin D_3), obtained from animal foods (8, 17, 18, 24, 34). Unlike other vitamins, most of vitamin D can be produced from pro-vitamin D_3 (7-dehydrocholesterol) via ultraviolet B sunlight (4). However, it has been reported that dogs have a lower capacity to produce cholecalciferol in the skin compared to other mammals (8).

It has been reported that vitamin D affects not only the musculoskeletal system, but also the gastrointestinal, cardiovascular, and integumentary systems (4). Vitamin D also plays a role in the barrier function of the skin, immune response, and the production of antimicrobial peptides (AMP) (17). It is reported that active vitamin D reduces the release of immunoglobulin (Ig) E by inhibiting B-lymphocyte function (4). In addition, it is known that it causes changes in the function of immune-related cells by reducing T helper (Th) 1 cytokine secretion (17). However, since vitamin D has immunomodulatory properties, its deficiency can lead to dermatological problems (4, 24). In AD, which is common in dogs, Th2-related cytokines such as interleukin (IL)-4, IL-5 and IL-13 predominate, leading to IgE production (13). Vitamin D plays a role in alleviating the inflammatory response by reducing IL-4 production and increasing antimicrobial peptides (17). Thus, the incidence or severity of diseases such as AD may increase (31). It has been reported that the mean serum 25-hydroxyvitamin D [25(OH)D] level in patients with AD is lower than in healthy dogs, but this difference is not statistically significant (7, 14, 19, 21, 28, 36).

The aim of the present study was to determine serum vitamin D concentrations in dogs with AD, compare serum vitamin D concentrations in healthy dogs and dogs with AD, and reveal the relationship between clinical scores and serum vitamin D concentrations.

Material and methods

Ethics statement. Ethical approval was obtained from the Local Ethics Committee for Animal Experiments, Dicle University (13-09/27.06.2024).

Animal material. The animal material of the study consisted of 11 dogs, aged 1-3 years, of different breeds (2 Golden Retrievers and 9 mixed-breed dogs) and sexes (5 females and 6 males), presented to the Veterinary Clinic of Dicle University Faculty of Veterinary Medicine with complaints of pruritus. On clinical examination, these dogs showed no signs of systemic infection other than pruritus and primary and secondary skin lesions. No positive findings were detected in parasitological and microbiological analyses, and the dogs were diagnosed with AD according to the Favrot criteria I (11).

The control group consisted of 11 healthy dogs, aged 1-3 years, of different breeds (3 shepherd dogs, 1 Golden Retriever, and 7 mixed-breed dogs) and sexes (4 males and 7 females), which were determined to be clinically healthy based on clinical examination. The nutritional regimens of both the control and patient groups were kept the same. The animal owners were informed about the study, and written informed consent was obtained from them.

Clinical examination. Routine examinations (auscultation, palpation, and inspection) were performed in dogs to identify dermatological findings and to rule out non-dermatological problems (such as fever, diarrhea, vomiting, etc.). As a result of the examinations, 11 dogs with no issues other than dermatological complaints were diagnosed with AD using the Favrot criteria (11). The Canine Atopic Dermatitis Extent and Severity Index and pVAS were used to assess the clinical severity of AD in dogs included in the study.

Sample collection. Blood samples were taken from the vena cephalica antebrachii of healthy dogs and dogs with AD included in the study into tubes with and without anticoagulant. Skin scrapings and sterile swab samples were taken from the lesioned areas of the dogs with atopic dermatitis.

Blood analysis. Hematological analyses of blood samples taken in anticoagulant tubes were performed with a hematology device (Mindray BC-2800 Vet, Hasvet, Türkiye). Samples taken in tubes without anticoagulant were centrifuged at 3000 rpm for 10 minutes after being allowed to clot at room temperature. The serum samples obtained were stored in storage tubes at –20°C until analysis to measure vitamin D concentrations.

Parasitological and microbiological analysis. Parasitological and microbiological analyses were performed by taking skin scrapings and sterile swabs from the lesioned areas of dogs suspected of atopic dermatitis. Scraping samples were studied in potassium hydroxide under a microscope to detect *Demodex* spp. and *Sarcoptes scabiei*. Each swap sample intended for microbiological analysis was incubated according to the required conditions (3). As a result of the analyses, 11 AD dogs with no bacteriological or mycological growth and no parasites detected in parasitological scrapings were included in the study.

Hill's atopy index application. Canine atopic dermatitis extent and severity index and pVAS scores were measured in the dogs with AD using the Hill's Atopy Index application (35).

Serum vitamin D concentration measurement. Serum Vitamin D concentrations were measured by the High-Performance Liquid Chromatography (HPLC) method using the Agilent Infinity II 1260 HPLC instrument. The results are given in µg/L.

Statistical analysis. Serum vitamin D concentrations in dogs with atopic dermatitis and healthy dogs were compared by statistical analysis. Statistical analysis was performed with SPSS 24.0 (SPSS Inc., Chicago, USA). Since the mean eosinophil values for the patient and control group dogs showed a normal distribution, the Mann-Whitney U test was used for statistical analysis. Since serum vitamin D concentrations did not show a normal distribution, statistical analysis was performed using the Independent Samples Test.

Results and discussion

Clinical examination findings. Severe alopecia, erythema, and lichenification were detected in the clinical examinations of the 11 dogs with complaints of pruritus and diagnosed with AD.

Hematological examination findings. The percentage of eosinophils was found to be significantly ($p < 0.05$) higher in dogs with AD (Fig. 1).

The canine atopic dermatitis extent and severity index and pruritus findings. The mean CADESI-4 and pruritus scores of the dogs with AD were measured as 45.18 and 4.10, respectively (Fig. 2).

Serum vitamin D concentration findings. Serum vitamin D concentrations in healthy dogs and dogs

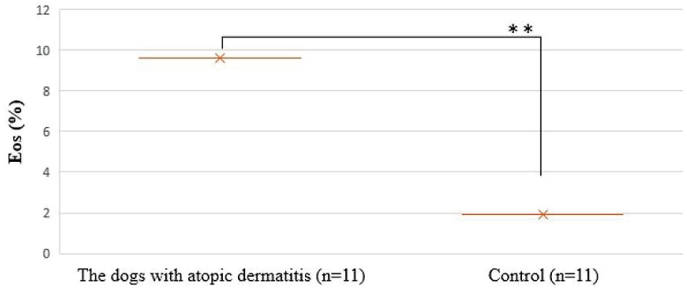

Fig. 1. Eosinophil percentage findings for patient and control group dogs **(p < 0.05)

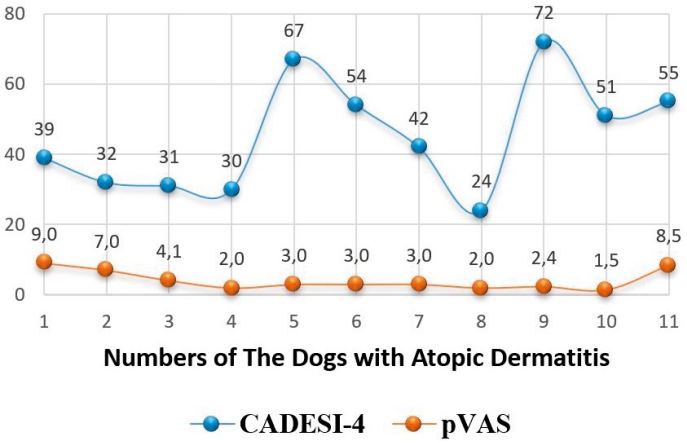

Fig. 2. CADESI-4 and pVAS scores of dogs with AD

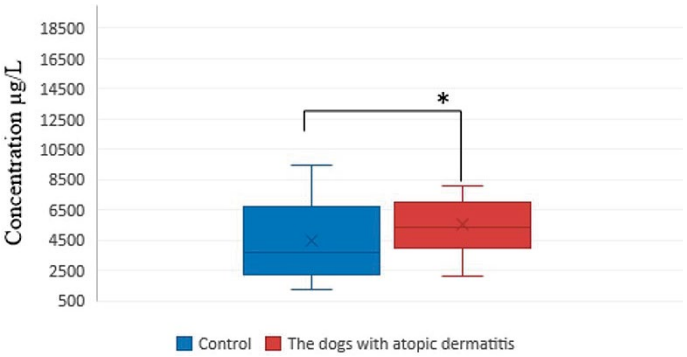

Fig. 3. Serum vitamin D concentration in the dogs with AD and the control group dogs *(p > 0.05)

with AD were determined and statistically evaluated. Although serum vitamin D concentration was higher in the dogs with AD compared to the control group, the difference was not found to be statistically significant (p > 0.05) (Fig. 3). In addition, it was determined that there was no relationship between CADESI-4 and pVAS scores of the dogs with AD and serum vitamin D levels.

Atopic dermatitis is a chronic, recurrent, inflammatory, and allergic skin disease characterized by pruritus (17, 19). Lesions associated with AD include alopecia, erythema, and lichenification in addition to pruritus (23). In dogs with AD, lesions are most commonly seen in the face, auricle, lower abdomen, inguinal and perineal regions, and distal extremities (10). It is known that AD in dogs occurs as a result of exposure to environmental allergens, house dust, house and shelter mites, pollen, mold, and hair (5).

Researchers (5, 13, 20, 25) report that primary and secondary skin lesions can be seen together with pruritus in dogs with AD. In this study, findings of erythema, alopecia, and lichenification were detected together with itching in clinical examinations of the dogs with AD, and this is consistent with the results of other studies (5, 13, 20, 25).

Vitamin D, considered a hormone, is classified as ergosterol (vitamin D_2) and cholecalciferol (vitamin D_3) (8). Vitamin D has important functions in many systems, especially the bone and skeletal system in metabolism (1). Vitamin D has been reported to play a role in the production of antimicrobial peptides, skin barrier functions, immune response, and alleviating the inflammatory response by reducing the production of IL-4. In addition, since some ILs play a role in the pathogenesis of AD, vitamin D is thought to affect the activity of AD (9, 31). Although the role of vitamin D in canine AD is still not fully known, it has been reported that it may be related to low vitamin D levels (9).

Rose et al. (30) measured serum 25(OH)D concentrations in dogs and cats with various diseases and reported that 22 of 79 diseased dogs had inadequate serum 25(OH)D concentrations. Pineda et al. (29) conducted a study to determine whether plasma vitamin D concentrations in dogs with mammary tumors were related to altered mineral metabolism or clinical features and reported that plasma 25(OH)D concentrations in diseased dogs were higher than in controls, but this was not statistically significant. Allison et al. (2) reported that there was no significant difference between serum 25(eOH)D concentrations in shelter dogs and healthy dogs. Hernandez et al. (16) reported that serum 25(OH)D concentrations were lower in dogs with histoplasmosis than they were in healthy dogs, but statistical analysis revealed that it was not significant (p > 0.05).

Other researchers (7, 14, 21, 28, 36) compared the mean serum 25(OH)D concentration in AD patients with the mean serum 25(OH)D concentration in healthy individuals. According to the results of statistical analysis they performed, they reported that the serum 25(OH)D concentration for AD patients was lower than the values for healthy dogs, but the difference was not statistically significant (p > 0.05) (7, 14, 21, 28, 36).

Kovalik et al. (19) measured the mean serum 25(OH)D concentration in 20 dogs diagnosed with AD and 30 healthy dogs. They reported that the mean serum 25(OH)D concentration in dogs with AD was lower than that in healthy dogs, but they emphasized that there was no significant difference (p > 0.05) between the two groups in statistical analysis.

In this study, the mean serum 25(OH)D concentration in dogs with AD (n = 11) and healthy dogs (n = 11) were measured. The mean serum 25(OH)D concentration in dogs with AD was found to be higher than that in

healthy dogs, but statistical analyses showed that there was no significant difference ($p > 0.05$) between the two groups. It is thought that the higher serum 25(OH)D concentrations in the dogs with AD compared to the healthy dogs in this study are probably due to the variability of IL levels in the course of AD (33).

This study showed that serum 25(OH)D concentrations in dogs with AD were not significantly ($p > 0.05$) different from those in healthy dogs and that serum 25(OH)D concentrations were not associated with increased disease severity.

In conclusion, it was determined that although serum 25(OH)D concentrations in dogs diagnosed with AD were elevated, this observation did not attain statistical significance. It is hypothesized that elevated serum 25(OH)D levels are attributable to alterations in cytokine levels and IgE concentration affecting vitamin D levels during the progression of AD. To achieve greater statistical power, the sample size should be increased. It would also be useful to determine the serum concentrations of cytokines released in the course of AD and compare them with serum 25(OH)D concentrations.

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