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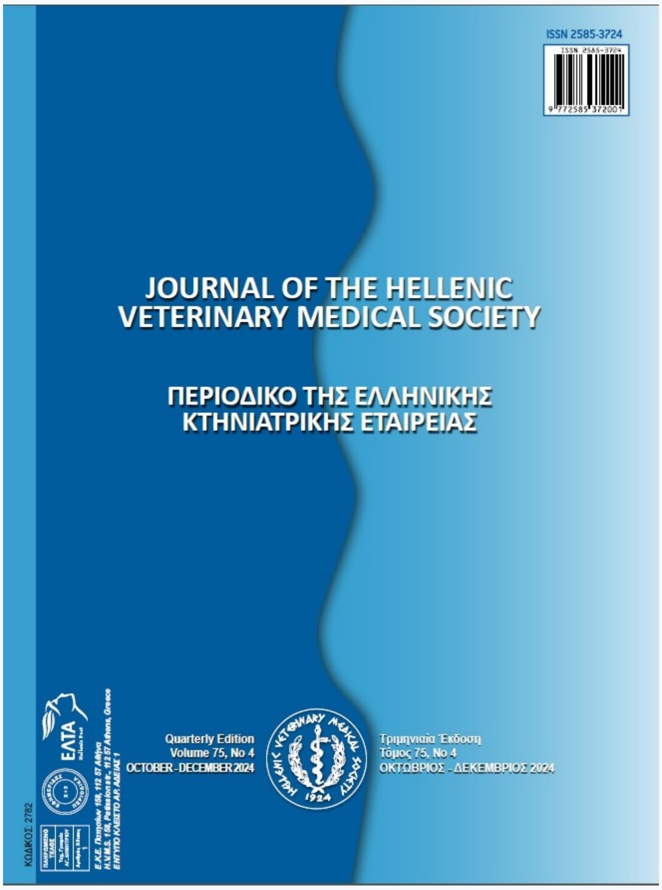

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Research article
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Serum ascorbic acid and calcidiol status in dogs with graded periodontitis

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ABSTRACT The relationship between oral health and vitamins has recently attracted much attention from a dental perspective. In human dentistry, certain vitamins are well-known as the essential component in the preservation of periodontal tissue integrity and deficient conditions can predispose individuals to periodontitis. However, similar studies in veterinary medicine are scarce. The main objective of our study was an evaluation of serum levels of ascorbic acid and calcidiol in healthy dogs and those with periodontitis. Sixty adult dogs were divided into 3 groups based on the depth of the periodontal pocket: periodontally healthy (pocket depth less than 2 mm), moderate periodontitis (pocket depth between 3 and 5 mm) and severe periodontitis (pocket depth more than 5 mm). Serum ascorbic acid levels in dogs with severe periodontitis were significantly lower than periodontally healthy dogs and those with moderate periodontitis (1.86±0.40, 3.93±0.84 and 3.52±0.66 respectively). Serum calcidiol levels in periodontally healthy dogs were significantly higher than groups with periodontitis (33.49±18.43, 18.08±10.05 and 13.81±6.87 respectively). The results of this research clarified that the grade of periodontitis, gingivitis and plaque indices and the age of dogs are inversely associated with the serum levels of ascorbic acid and calcidiol. It can be considered that maintaining adequate levels of serum ascorbic acid and calcidiol may be beneficial in preventing the progression of gingivitis to periodontitis and managing periodontitis in dogs.

Keywords: Small animal; Periodontal disease; Vitamin C; Vitamin D

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INTRODUCTION

Periodontitis has been documented as the commonest disease in dogs, which significantly affects the quality of life (Thatcher, 2020). This condition is initiated by dental plaque microorganisms and manifests when uncontrolled gingivitis progresses to the loss of gingiva, periodontal ligament and alveolar bone, creating deep periodontal 'pockets' as hallmarks of periodontitis which may eventually cause tooth loss (Kinane et al., 2017). Due to the release of inflammatory cells and side products in response to bacteremia, this disease is associated with liver, kidney, cardiac and thromboembolic disorders, myocardial and cerebral infarction, chronic bronchitis and pulmonary fibrosis (Stella et al., 2018).

Among the predisposing factors involved in the pathogenesis of human periodontitis, the influential role of nutrition is often overlooked (Varela-López et al., 2018). Vitamins have a pivotal effect on periodontal health, and the deficiency of these micronutrients may affect the fundamental periodontal defense contributors, including the integrity of the dental-gingival barrier and the turnover of its cells (Pavithra et al., 2017). Among vitamins, those that affect bone metabolism, the immune system, and antioxidant capacity could be promising candidates for preventing or controlling periodontitis (Varela-López et al., 2018).

Vitamin C has critical roles in antioxidation, improvement of chemotaxis and phagocytosis, as well as maintaining the connective tissues' integrity (Varela-López et al., 2018). Furthermore, this micronutrient is effective in killing bacteria by natural killer cells and regulates the expression of pro-inflammatory and anti-inflammatory cytokines (Gordon et al., 2020). Vitamin D has crucial roles in bone metabolism, immunomodulation, and antimicrobial defense (O'Brien et al., 2018) (Hu et al., 2022). In addition, this micronutrient acts as an anti-proliferative and anti-inflammatory agent (Cagetti et al., 2020).

In human periodontology, many studies showed the positive efficacy of vitamins C and D in maintaining periodontal health (Varela-López et al., 2018) (Cagetti et al., 2020). Also, an increased risk of gingivitis, periodontitis and tooth loss associated with insufficient levels of vitamins C and D has been proposed (Woelber et al., 2016) (Hu et al., 2022). However, to the authors' knowledge, there have not been similar studies in small animal periodontology; although some researchers have hypothesized that periodontal disease in dogs is associated with the deficiency of vitamins C and D (Stella et al., 2018) (Özavci et al., 2019). Therefore, this study aims to investigate the levels of ascorbic acid (because most of the blood vitamin C is in the form of ascorbic acid (Hishiyama et al., 2006)) and calcidiol (as the most reliable indicator of blood vitamin D status (Parker et al., 2017)) in dogs with different grades of periodontitis.

MATERIALS AND METHODS

Animals: This study was carried out according to the standard animal experimentation protocol of the Veterinary Ethics Committee of Urmia University (Ref: IR-UU-AEC-3/67). Sixty intact adults, mesocephalic client-owned dogs of both sexes, were recruited. According to the owners' report, all the studied dogs were apparently healthy, fed a commercial diet, and not taking vitamin supplements. Exclusion criteria affecting vitamins C and D status or periodontitis grading included age under two years and over ten years, significant systemic disease (e.g., hepatopathy, renal failure, or diabetes mellitus), pregnancy or lactating, clinically substantial infectious diseases, and recent dentistry procedures that were assessed through clinical and paraclinical examinations.

Study design: The animals were divided into three groups based on the grade of periodontitis. For this purpose, first, the dogs were anesthetized according to common protocols, then using the Williams probe by a trained examiner the periodontal pocket depth of the maxillary 4th premolar and the 1st molar, as the most frequently affected teeth were measured (Wallis and Holcombe, 2020). The periodontal pocket depth was measured by inserting the probe into the gingival sulcus, gently walking the probe along the bottom of the pocket, and recording the deepest distance from the cementoenamel junction to the bottom of the pocket (Harvey et al., 2008). Dogs were included in groups one (periodontally healthy), two (moderate periodontitis) and three (severe periodontitis) if pocket depth was less than 2, 3 to 5 and more than 5 mm, respectively (Barbudo-Selmi et al., 2004). The body condition score (BSC) for each dog was recorded on a 5-point scale (1=emaciated, 2=underweight, 3=ideal, 4=overweight, 5=obese) (Stella et al., 2018).

In addition, gingivitis and plaque indices were also recorded. The gingivitis index was graded as follows: 0=No inflammation: normal gingiva; 1=Mild inflammation: slight oedema, slight color change, no bleeding on probing; 2=Moderate inflammation: oedema, redness, surface glaze, bleeding on probing during 30-seconds; 3=Severe inflammation: bleeding spontaneously or immediately on probing (Kouki et al., 2013). The plaque index was graded as follows: 0=No plaque in the gums; 1=Film of plaque adhering to the free gingival margin. The plaque can only be detected by passing a probe over the tooth surface at the entrance of the gingival sulcus; 2=Moderate formation of soft deposits within the gingival sulcus or pocket, on the gingival margin and/or adjacent tooth surface that can be detected by inspection; 3=Profuse soft matter in the gingival sulcus or pocket, and/or on the adjacent tooth surface that can be detected by inspection (Hennet, 2002). To the avoidance of inter-examiner differences, all the measurements were done by a skilled veterinarian.

Laboratory parameters: To confirm the health of the animals that were studied, CBC and biochemical analysis (e.g., measurement of glucose, urea nitrogen, creatinine, alanine aminotransferase, alkaline phosphatase and total protein) were performed using an automated blood cell counter and standard serum analyzer after a night without food. A portion of the extracted serum was kept at −20°C for later analysis of studied parameters. Ascorbic acid levels were analyzed spectrophotometrically by the method of Kyaw (Kyaw, 1978). Calcidiol levels were analyzed by enzyme-linked immunosorbent assay (Monokit Vitamin D, Monobind Inc., Iran) according to the manufacturer's instructions (Rodriguez-Cortes et al., 2017).

Statistical analysis: All statistical tests were carried out by SPSS software version 19.0 (SPSS Inc., Chicago, USA). The normality of data was analyzed with the Shapiro-Wilk test. The differences in serum ascorbic acid and calcidiol levels between the groups were analyzed using one-way analysis of variance followed by the LSD test as a post hoc. The difference between sexes was analyzed by independent-sample T-test. Kruskal-Wallis test was used for non-parametric data. The association between gingivitis index, plaque index, BSC, and age with serum levels of ascorbic acid and calcidiol was tested using bivariate correlation. A P-value less than 0.05 was considered statistically significant.

RESULTS

The studied population (n=60) included 36 male and 24 female dogs of medium to large breeds with an age range between 24 and 120 months (average age of 62.56 months) and body condition scores of 2-5/5 (median 3/5). The mean ± standard deviation of serum ascorbic acid in the periodontally healthy and moderate periodontitis groups was significantly higher than the severe periodontitis group (3.93±0.84, 3.52±0.66 and 1.86±0.40, respectively) as shown in figure 1. Serum calcidiol in groups 2 and 3 (18.08±10.05 and 13.81±6.87) were significantly lower compared to group 1 (33.49±18.43) (Figure 1). There was no statistical difference between male and female dogs in terms of serum ascorbic acid and calcidiol status (p>0.05).

The grade of periodontitis and the gingivitis and plaque indices increased with age (Table 1). Bivariate correlation analysis showed a significant correlation between gingivitis index, plaque index, and age with serum levels of ascorbic acid (rs=-0.421, p<0.01; rs=-0.513, p<0.001; r=-0.416, p<0.01) and calcidiol (rs=-0.331, p<0.05; rs=-0.270, p<0.05; r=-0.282, p<0.05). There were no significant results between BCS and

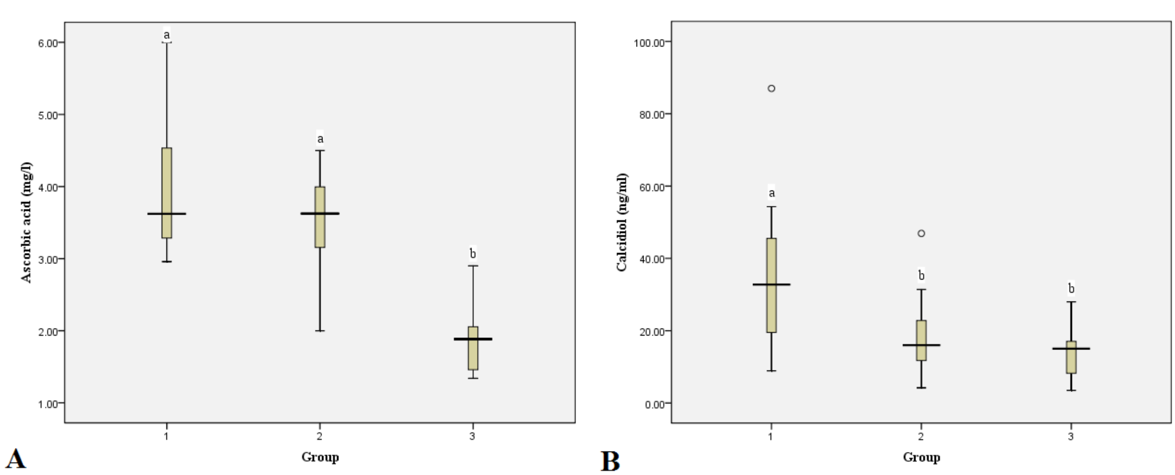

Figure 1. Box and whisker plot showing serum ascorbic acid (A) and calcidiol (B) status in the studied groups. The outliers were marked as hollow circles. Different letters indicate a statistically significant (p<0.05).

Table 1: Median and interquartile range (IQR) of clinical parameters in the studied groups

Group	Gingivitis index	Plaque index	Body condition score	Age (months)
1	1 (0-1)[a]	1 (1-2)[a]	3 (3-3.75)	39.00 (29.25-50.50)[a]
2	1 (1-2)[a]	2 (1-2)[a]	3 (3-4)	66.00 (50.00-91.00)[b]
3	2.5 (2-3)[b]	3 (2-3)[b]	3 (3-4)	79.50 (57.00-98.50)[b]
Asymp. Sig.	< 0.001	< 0.001	0.226	< 0.001

Different letters of each column indicate a statistically significant (p<0.05).

serum ascorbic acid and calcidiol levels in Spearman's correlation test (p>0.05).

DISCUSSION

Our results showed that dogs with higher periodontitis grade, gingivitis and plaque indices, as well as older age, have lower serum levels of ascorbic acid and calcidiol. In humans, clinical and in vitro studies also demonstrated the positive effect of vitamins C and D on periodontal health (Woelber et al., 2016).

Ascorbic acid can play effective roles in periodontal health by the following mechanisms: a) Low levels of ascorbic acid affect the collagen metabolism of the periodontium and thus the tissue regeneration ability; b) Deficiency of ascorbic acid disrupts bone formation, causing periodontal bone loss; c) Optimal levels of ascorbic acid maintain the barrier function of oral mucosa against bacterial products; d) Sufficient level of ascorbic acid is needed to maintain the periodontal microvasculature integrity, vascular response to bacterial invasion, and wound healing; e) Deficiency of ascorbic acid can impair the ecologic equilibrium of plaque bacteria, hence enhancing its pathogenicity (Pavithra et al., 2017).

Although dogs do not need much exogenous vitamin C, the rate of synthesis of vitamin C in the liver of dogs is lower than that of other animals, and in some conditions need for this vitamin can exceed the capacity of liver synthesis (Hesta et al., 2009). In our study, low levels of ascorbic acid in dogs with periodontitis could be related to increased vitamin C metabolism. A significant elevation in the vitamin C level following non-surgical treatment of people with periodontitis can confirm this hypothesis (Velden, 2020). Furthermore, it's possible that periodontopathic pathogens through biodegradation, decreased the blood level of vitamin C (Tada and Miura, 2019). The decreased absorption capacity of vitamin C can also play a role in periodontitis, although this hypothesis should be further investigated in veterinary patients. Recently, it was shown that some people with periodontitis have a lower capacity to absorb vitamin C than healthy people, and the reason for this inter-individual difference can be genetic variations in the vitamin C transporter protein, which affects the blood vitamin C concentration (Velden, 2020). The grade of gingivitis, in humans, is directly associated with blood vitamin C status (Velden, 2020). Vitamin C has strong anti-oxidative effects, especially at the intracellular level, and reduces the oxidative stress produced in gingivitis (Tada and Miura, 2019). A clinical study showed that injecting vitamin C, as an anti-inflammatory agent, into the gum tissue in patients suffering from gingivitis, significantly improves the inflammation at the injection site (Li et al., 2022).

Some of the vitamin D benefits in periodontitis can be associated with its immunomodulatory and anti-inflammatory actions through inhibiting T-cell proliferation, interleukin-17 (IL-17), and interferon-gamma, as well as activation of IL-4. Furthermore, salivary levels of IL-8, IL-6, IL-1, and TNF-a, which degrade collagen, connective tissue, and bone in patients with periodontitis, may be reduced by vitamin D supplementation (Rawlinson et al., 2011) (Khan and Ahad, 2021). Interestingly, the gingival epithelium has special vitamin D receptors which augment host defense against microorganism insult through the improvement of the epithelial barrier function (Khan and Ahad, 2021). Also, vitamin D boosts the release of antimicrobial peptides (e.g., cathelicidins) from the epithelial cells of the gingiva (Khan and Ahad, 2021). Bayirli et al. (2020), demonstrated that in low vitamin D status, the ability to respond to bacterial challenges via antimicrobial peptides in periodontal tissues can be impaired, resulting in inadequate immune response and disease progression.

The possible causes that can be considered in animals with low levels of calcidiol include reduced food intake, dietary vitamin D deficiency, intestinal malabsorption, and increased utilization of vitamin D metabolites for the immune system (Corbee, 2020). Dogs rely on their diet for most of their vitamin D needs (O'Brien et al., 2018). In the present study, the significant decrease in calcidiol levels in the second and third groups can probably be attributed to painful gums and hyporexia associated with periodontitis. In addition, the studied dogs consumed commercial diets, however, it cannot be assumed that the vitamin D content was sufficient. So that significantly different serum levels of calcidiol were documented among dogs that consumed diets from various manufacturers (Weidner and Verbrugghe, 2017). Also, it should be kept in mind that periodontitis or gingivitis are inflammatory conditions and inflammation can act to reduce calcidiol levels (Parker et al., 2017). In this regard, Waldron et al. (2013), claimed that serum calcidiol is a negative acute phase reactant, and hypovitaminosis D can be the consequence rather than the cause of human inflammatory diseases. Another hypothesis to explain the low levels of calcidiol in the studied dogs could be the excessive consumption of the vitamins in the inflammatory conditions, similar to the pattern previously demonstrated for vitamin A in children with chickenpox infection (Rodriguez-Cortes et al., 2017). Sufficient levels of vitamin D intensify the anti-bacterial resistance of gingival epithelial cells and decrease gingivitis through its anti-inflammatory properties (Lee et al., 2021) (Alzahrani et al., 2021). In gingivitis, insufficient levels of calcidiol can compromise the expression of antimicrobial peptides in gingival crevicular fluid and gingival tissues (Bayirli et al., 2020).

Aging is known as one of the risk factors for periodontal disease in dogs (Wallis and Holcombe, 2020). The increase in the grade of periodontitis and gingivitis and plaque indices related to the aging of the studied dogs can be explained by the increase in accumulation of bacterial plaque, the decrease in the function of the immune system, as well as the occurrence of predisposing conditions for periodontal diseases, such as cardiac disease, renal failure, neoplasia or diabetes mellitus (Carreira et al., 2015). Moreover, the following hypotheses are presented to explain the possible reason for the decrease in the levels of vitamins C and D with age in the studied dogs. The decline of vitamin C with aging could be due to increased turnover (e.g., due to increased oxidant load), decreased availability (e.g., reduced dietary intake or absorption), or impaired transport of ascorbic acid (Lykkesfeldt and Moos, 2005). Age-related changes that affect vitamin D status can include decreased vitamin D receptors, decreased renal production of 1,25$(OH)_2$D, and substrate deficiency of vitamin D (Gallagher, 2013). Also, it has been suggested that vitamin D deficiency may be caused by inflammatory processes related to aging (Rodriguez-Cortes et al., 2017). It is worth mentioning that the lack of significant correlation between BCS and serum levels of ascorbic acid and calcidiol can be caused by the lack of variation in BCS so most of the studied dogs were almost in the ideal score (BCS≈3).

In healthy dogs, the reported reference range for blood ascorbic acid is 3.2 to 8.9 mg/l (Hishiyama et al., 2006); accordingly, serum ascorbic acid levels in the studied dogs with severe periodontitis (group 3) were significantly lower than the reference range. Regarding serum calcidiol levels, there was no consensus on the reference range for healthy dogs (variation range 9.5 to 249.2 ng/ml (Corbee, 2020)). Although differences in methodology, breed, genetic, age, sex or neutering status can affect the results, recently, a median of 52.50 ng/ml was determined as the reference value for serum calcidiol levels in healthy dogs (Alizadeh et al., 2022). According to this value, periodontally healthy dogs had almost sufficient levels of serum calcidiol, but dogs with moderate and severe periodontitis (groups 2 and 3) probably suffered from calcidiol deficiency. Our results are consistent with human periodontology findings that periodontitis is significantly associated with insufficient levels of vitamin C and D (Bayirli et al., 2020) (Velden, 2020) (Alzahrani et al., 2021) (Li et al., 2022). Therefore, administration of ascorbic acid and calcidiol supplements for dogs with periodontitis can be reasonable.

This study had two main limitations that should be addressed. First, intraoral radiography was not possible in this study, which could have helped in confirming alveolar bone loss. However, the periodontal probe provides the foundation for the clinical examination of the periodontium and helps establish the periodontal disease diagnosis as well as assess the treatment results. Also, the measurement of clinical periodontal attachment level (CAL) is well known as the “gold standard” for the recognition of progressive periodontitis, which can be done directly with a periodontal probe (Fitzgerald et al., 2022). Second, it was not possible to analyze the vitamin D content of the diet provided to dogs, and the studied animals used different commercial diets. However, in evaluating the studied animals’ dietary history, all of the consumed foods were complete and balanced as claimed by the manufacturer.

CONCLUSIONS

The findings of this study for the first time highlighted the significance of ascorbic acid and calcidiol as essential vitamins in the health of the periodontal tissues in dogs. It can be considered that maintaining sufficient levels of serum ascorbic acid and calcidiol at a young age can be useful to prevent the progression of gingivitis, as a reversible condition, to irreversible periodontitis and at an older age to manage periodontitis. However, it is necessary to investigate the potential benefits of ascorbic acid and calcidiol supplementation on the outcome of periodontitis in dogs in the future.

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CONFLICT OF INTEREST

None declared

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