# **Topical Review**

# Relationship Between Gender, Age, and Weight and the Serum Ionized Calcium Variations in Dog Periodontal Disease Evolution



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Keywords: dog periodontal disease serum ionized calcium gender age weight

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### Introduction

Inflammatory conditions induced by bacterial plaque that affect the gingiva and other periodontal tissues are known as periodontal disease (PD). PD can be present in 2 forms: gingivitis (i.e., the initial and reversible stage of the disease, which may or may not progress) and periodontitis (i.e., an advanced stage of the disease in which inflammatory reactions damage the soft tissues and destroy the bone that supports teeth).<sup>1-6</sup> Approximately 80%-85% of dogs older than 2 years exhibit one or more signs of PD,<sup>7</sup> with the disease affecting nearly all animals older than 5 years. Therefore, PD is one of the most common diseases in dogs.<sup>8-10</sup> An increased prevalence is observed in miniature and small dog breeds and in dogs with problems caused by malocclusion and dental crowding.<sup>11-13</sup> Because of the negative influence of PD on an animal's health and welfare, which is often devalued in veterinary clinical practice, it is important to invest in the prevention and treatment of PD.<sup>1</sup> The etiology of PD is multifactorial, and it includes microbiological, behavioral, environmental, genetic, and systemic factors contributing to individual susceptibility and clinical expression of the disease.<sup>6-9,14-23</sup> Alveolar bone resorption occurs with PD evolution, and 2 types of patterns can be considered (i.e., horizontal and vertical).<sup>18,23</sup>

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To analyze the relationships between gender, age, weight, and variations in the levels of serum ionized calcium ( $[iCa^{2+}]$ ) during periodontal disease (PD) evolution. In this study, dogs (n = 50) were divided into 5 groups according to the stage of PD: G0 (no PD), G1 (gingivitis), G2 (initial periodontitis), G3 (moderate periodontitis), and G4 (severe periodontitis). Statistically significant correlations were observed between age,  $[iCa^{2+}]$  levels, and PD stage. Older dogs had lower  $[iCa^{2+}]$  levels and more advanced PD stages (high positive correlation), and their body weight decreased as PD developed (negative correlation). Lower  $[iCa^{2+}]$  values were associated with more severe PD.

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Mineralized plaque develops in the dental calculus; this plaque is also known as tartar and due to crystallized carbonate and phosphate calcium salts on the tooth surface, with carbonate being the principal salt present in dogs.<sup>1,13,18,19,24-27</sup> The presence of calcium salts is therefore an essential condition for tartar development, and the ionized form ([iCa<sup>2+</sup>]) is the most important in biochemical reactions.<sup>28-32</sup>

Calcium levels in saliva are positively related to the blood calcium concentration; consequently, diseases leading to hypercalcemia may increase salivary calcium and consequently predispose dogs to the development of dental calculus, which is a risk factor for the development and progression of PD.<sup>9,28,33-36</sup> This study was conducted using 50 dogs of the *Canis familiaris* species (n = 50) and had the following aim: (1) to analyze the relationships between gender, age, weight, and variations in levels of serum ionized calcium ([iCa<sup>2+</sup>]) during PD evolution.

#### **Material and Methods**

The present study used a sample of 50 dogs (n = 50) between 1 and 16 years of age. The sample included dogs of both genders and all breeds. The dogs were presented at the Anjos of Assis

Veterinary Medicine Centre (CMVAA), Barreiro, and at the Association for the Abandoned Dogs (APCA), Sintra, for routine examination. Before each dog's physical and oral cavity evaluation, the gender, age, body weight, type of nutrition, and clinical history were recorded to eliminate specimens presenting with cachexia, obesity, or clinical signs of any underlying disease that could interfere with calcium metabolism or PD evolution. A blood sample was collected through a peripheral venipuncture in an anaerobic environment using a BD vacutainer device for routine hematologic tests. The owners signed a written consent form allowing the use of 0.5 mL of the blood sample to quantify the  $[iCa^{2+}]$  level. Next, 5 groups of 10 subjects each were formed based on the stage of PD as follows: GO (no PD), G1 (gingivitis), G2 (initial periodontitis), G3 (moderate periodontitis), and G4 (severe periodontitis). All the blood samples used for [iCa<sup>2+</sup>] determination were centrifuged to obtain serum for [iCa<sup>2+</sup>] quantification after analysis with the CG8+ Abbott analytical system using the i-STAT device. The results were adjusted to a pH of 7.4 with normal reference values between 1.12 and 1.32 mmol/L. For the statistical analysis, we used the R Commander software version 1.6-4. and the results were validated for a P < .05. The Shapiro-Wilk test was used to test normality, the Spearman test was used to evaluate correlations between variables, and the Kruskal-Wallis test was used to assess the significance of differences between quantitative variables. The Wilcoxon test was used to compare differences in the [iCa<sup>2+</sup>] values observed between PD groups.

## Results

The sample results are listed in the Table. In the sample, normocalcemia was recorded in 42.5% of the dogs, hypocalcemia was recorded in 40% of the dogs, and hypercalcemia was recorded in 17.5% of the dogs.

#### Table

Mean and Dispersion Measures Linked to the Serum Ionized Calcium ( $[Ca_i]$ ) in the Total Sample (n = 50) and Considering the 5 Groups (n = 10 Each) of Periodontal Disease (PD) Studied: G0 (no PD), G1 (gingivitis), G2 (initial periodontitis), G3 (moderate periodontitis), and G4 (severe periodontitis)

Parameter	Group		п	$\overline{X}$	Minimum	Maximum
Age (y)	G0 G1		50 50	$2.63 \pm 1.19$ 3 00 + 1 60	1	4
	G2		50	$675 \pm 149$	5	9
	G3		50	9.00 + 3.39	5	15
	G4		50	$11.63 \pm 3.23$	7	16
Body weight (Kg)	G0		50	$14.84~\pm~1.57$	12.50	17.10
	G1		50	$13.52~\pm~2.18$	10.90	16.80
	G2		50	$14.12~\pm~2.07$	11.00	16.80
	G3		50	$13.56~\pm~1.92$	10.50	16.10
	G4		50	$12.84~\pm~1.92$	10.50	15.10
[Ca <sub>i</sub> ] (mmol/L)	TS		50	$1.15~\pm~0.15$	0.77	1.62
[Ca <sub>i</sub> ] and Gender	F	EF	50	$1.14~\pm~0.22$	0.77	1.62
		SF	50	$1.17~\pm~0.12$	0.94	1.30
	Μ	EM	50	$1.19~\pm~0.15$	0.97	1.41
		SM	50	$1.13~\pm~0.12$	0.90	1.29
[Ca <sub>i</sub> ] and PD stage	G0		50	$1.27~\pm~0.17$	1.08	1.62
	G1		50	$1.21~\pm~0.08$	1.02	1.30
	G2		50	$1.15~\pm~0.15$	0.90	1.30
	G3		50	$1.06~\pm~0.08$	1.02	1.29
	G4		50	$1.08~\pm~0.12$	0.94	1.24

Abbreviations: EF, entire female; EM, entire male; F, female; M, male, SF, spayed female; SM, spayed male; Total sample (TS);  $\bar{x}$ , mean.

#### Discussion

No published studies have investigated the influence of gender on PD development in dogs. According to our results, no genderbased differences in PD prevalence exist (P = .24) in dogs in contrast to humans. For females, intact animals (43.7%) typically presented with a PD stage of G0 (30%), and spayed (56.3%) animals typically presented with PD stages of G1 and G2 (33.3% each); no significant differences in PD prevalence were observed between spayed and unspayed dogs (P = .40). This finding contrasts with observations in females, who exhibit increased susceptibility to developing the disease during the postmenopausal period. This increase occurs because of an increased propensity to develop osteoporosis associated with reduced estrogen levels and reduced mandible and maxilla bone tissue, which potentiate PD. Because female dogs are diestric, with consistently low levels of estrogen except at the time of ovulation, and do not undergo menopause, a less marked influence of estrogen on the bone tissue<sup>37</sup> may explain why ovariohysterectomy is not associated with an increased prevalence of osteopenia-potentiated PD in this species as observed in females.<sup>38</sup> For males, the tendency was the same as in females, with no differences in the PD prevalence between castrated (43.7%) and uncastrated dogs (56.3%). This finding reflects the male physiology in which the andropause phenomenon occurs and is associated with reduced testosterone, which exerts less bone effects than estrogen dose and therefore acts as a minor risk factor for osteopenia in the mandible and the maxilla. Consequently, this factor does not increase the risk of PD development.<sup>39,40</sup> The results demonstrated that for intact males, the main PD stages were G0 and G4 (28.0% each) and that for castrated males, the main PD stage was G2 (26.3%). In this study, PD manifested in dogs ranging from 1-16 years old, with older dogs exhibiting stages of the disease that were more advanced. Statistically significant differences in PD prevalence were observed between different age groups (P = .00), with a high positive correlation (r = 0.86). These results are consistent with those reported by other authors, <sup>12,26,41,42</sup> who concluded that PD prevalence increases with age (similar to what is observed in humans) and that the incidence of the disease is already high in 1-year-old patients, with 85% of the patients exhibiting at least one clinical sign of PD (19). Furthermore, the PD stage becomes worse as the patient grows older. The observed increase in the prevalence and severity of PD associated with aging is understandable because during aging, increased bacterial plaque accumulation occurs, the general and oral cavity immune response is reduced, and the incidence of PD-predisposing diseases, such as neoplasias, renal failure, diabetes mellitus, or cardiac diseases, increases.43-46 Various studies performed in humans and dogs concluded that increased PD severity promotes lower body weights,<sup>26,47,48</sup> which can be explained by the observation that as PD develops, clinical signs are usually observed, such as swollen, tender, or bleeding gums; painful chewing; sensitivity; tooth mobility; and loose teeth.<sup>49</sup> Consequently, food intake becomes painful and an anorexic stage occurs, leading to a reduction of body weight. On the contrary, Khader et al.<sup>50</sup> demonstrated in their study on humans that overweight and obese dogs exhibit double and triple the incidences of PD, respectively. This difference results from the different cytokines and hormones produced by fat cells, which may increase overall inflammation, leading to decreased immunity. Reduced immunity increases susceptibility to PD. In addition, this inflammatory reaction reduces gum blood flow, promoting PD progression. The obtained results revealed a negative correlation between body weight and PD (i.e., as PD develops, body weight decreases); however, no statistically significant differences were observed (P = .06). If the sample were bigger, the correlation may have reached statistical significance. The  $[iCa^{2+}]$  values are

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