Correlation of the Hematological and Urinary Profile of Adult Dogs With Different Degrees of Periodontal Disease

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Abstract

In view of the high incidence of periodontal disease in canine species and the possible local and systemic changes, the objective of the present study was to present the average of hematological and urinary parameters of adult patients and to analyze the correlation between the different degrees of periodontitis. For this, 60 dogs of different breeds, weights, ages and degrees of periodontal disease were used, from the Small Animal Dentistry Sector of the Veterinary Hospital of the University of Franca. Blood samples were obtained by external jugular venous puncture for analysis of red blood cells, hematocrit, total leukocytes, albumin, total protein, alanine aminotransferase, alkaline phosphatase, urea and creatinine and, urine samples by ultrasound-guided cystocentesis, to assess density, red blood cells/field large increase (FLI), leukocytes/FLI, cells, casts, proteins and urinary protein-creatinine ratio (PCR). Results were statistically evaluated by single analysis of variance (ANOVA). As for hematological parameters, alkaline phosphatase and urea showed a statistical increase in grade V compared to the other grades of periodontitis (p<0.05), despite being within the reference range for dogs. In relation to urinary exams, dogs affected by periodontal disease grade V showed a statistical increase in urinary proteins when compared with those of grades II and III (p<0.05), however, they remained within normal limits for adult canines. In view of the recommended methodology and the data obtained, it is possible to infer that periodontal disease in adult dogs did not change the hematological, biochemical and urinary parameters, regardless of breed and degree of involvement of the oral disease.

Keywords: Bacterial Plaque. Periodontitis. Systemic Diseases. Veterinary Dentistry.

1 Introduction

Among the conditions that compromise the oral cavity of dogs, periodontal disease stands out, considered the most common cause of local infection and tooth loss (FERNANDES et al., 2012; SILVA et al., 2017), characterized by inflammation and progressive destruction of periodontal tissues, which protect (gum) and support (cement, alveolar bone and periodontal ligament) the dental elements (FERNANDES et al., 2012). The etiological factor is the bacterial plaque formed by the association of bacteria, food remains, leukocytes, macrophages, mineral salts, metabolites and oral desquamation cells (GRUBBS et al., 2017).

With the organization of the bacterial plaque constituents, by-products are produced that damage periodontal and adjacent structures (ABUSLEME et al., 2013; DI BELLO et al., 2014) by triggering immune response (WHYTE et al., 2014), with consequent production of prostaglandins, activation of enzymes such as collagenases, proteases and stimulation of osteoclasts (SILVA et al., 2017).

According to Fernandes et al. (2012), periodontal disease is a progressive disease and is classified according to the degrees

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*Unidade de Odontologia de Pequenos Animais do Hospital Veterinário da Universidade de Franca. Amostras de sangue foram obtidas por punção venosa da jugular externa para análise de hemácias, hematócrito, leucócitos totais, albumina, proteína total, alamine aminotransferase, fosfatase alcalina, urea e creatinina e, amostras de urina por cistocentese guiada por ultrassom, para avaliação da densidade, hemácias/campo grande aumento (CGA), leucócitos/CGA, células, cílios, protéinas e relação proteína-creatinina urinária (RPC). Os resultados foram avaliados estatisticamente por análise de variância simples (ANOVA). Quanto aos parâmetros hematológicos, a fosfatase alcalina e a urea apresentaram aumento estatístico no grau V em relação aos demais graus de periodontite (p<0,05), apesar de estarem dentro da faixa de referência para cães. Em relação aos exames urinários, os cães acometidos pela doença periodontal grau V apresentaram aumento estatístico das proteínas urinárias quando comparados aos dos graus II e III (p<0,05), porém, permaneceram dentro dos limites normais para cães adultos. Diante da metodologia preconizada e dos dados obtidos, é possível inferir que a doença periodontal em cães adultos não alterou os parâmetros hematológicos, bioquímicos e urinários, independente da raça e grau de acometimento da doença oral.

of involvement of the periodontium: grade 0 (absence of oral symptoms); grade I (accumulation of bacterial plaque and mild gingivitis); grade II (accumulation of bacterial plaque, gingivitis and gingival edema); grade III (gingivitis, gingival edema and beginning of periodontal pocket formation); grade IV (deep periodontal pocket, onset of bone loss and tooth mobility) and grade V (bone loss, significant tooth mobility and predisposition to fracture of the mandible and/or maxilla).

Due to the rich vascularization of the periodontium and the movement of the tooth in the dental socket, bacteria and their metabolites can enter the lymphatic and blood vessels (bacteremia) during the patient’s mastication and, the secondary systemic immune response to microorganisms, predispose the production of immune complexes bloodstream (RAWLINSON et al., 2011). Immune complexes can adhere internally to the walls of the endothelium, causing local inflammation and endothelial lysis and causing functional damage to various organs such as the liver, nervous system, joints, heart and kidneys, especially in senile patients (ALMEIDA et al., 2017; KANG et al., 2017). In this way, complementary serum and urinary laboratory tests of animals with periodontal disease become indispensable in the diagnosis of systemic alterations in dogs, in an attempt to prevent the installation of diseases, delay the evolution and reduce possible complications, improving the quality of life and increasing the survival of those affected (FERNANDES et al., 2012; SILVA et al., 2017).

In view of the high incidence of periodontal disease and the possible local and systemic changes that it can cause in canine species, the objective of the present study was to present the average of hematological and urinary parameters of adult patients affected by this oral disease and, in addition, to analyze the correlation of these complementary exams between the different degrees of periodontal disease.

2 Material and Methods

The research was approved by the Ethics Committee on the Use of Animals (CEUA) of the University of Franca - UNIFRAN (process nº 4182260917). Sixty dogs from the Small Animal Dentistry Sector of the Veterinary Hospital of UNIFRAN were used, with different breeds, weights, ages and degrees of periodontal disease (classification made by clinical inspection by a dental professional).

Blood samples were obtained by puncture of the external jugular and, immediately, the aliquots were processed in the Clinical Laboratory of the Veterinary Hospital of UNIFRAN. The values of red blood cells (× 10⁶), hematocrit (%), total leukocytes (μl), albumin (g/dL), total protein (g/dL), alanine aminotransferase (ALT - U/L), alkaline phosphatase (ALP - U/L), urea (mg/dL) and creatinine (mg/dL) were analyzed. For the counting of red blood cells and hematocrit, the samples were processed in an automated hematology analyzer (Model pocH-100iv Diff - Sysmex do Brasil Indústria e Comércio Ltda, São José dos Pinhais - PR) and the leukocytes in a digital differential cell counter (Model CCS 02 - Phoenix Luferco - Industry and Commerce of Scientific Equipment, Araraquara - SP). The albumin, total protein, ALT, ALP, urea and creatinine in an automatic ChemWell analyzer (Labcrost Diagnóstica - Vista Alegre - Lagoa Santa - MG) by bromocresol green method, biuret, UV-IFCC kinetics, modified Bowers/Mc Comb, Enzymatic kit UV and picrate, respectively.

Urine samples were obtained by ultrasound-guided cystocentesis and processed in the same laboratory. Density, red blood cells/field large increase (FLI), leukocytes/FLI, cells, casts, quantitative protein biochemistry (mg/dL), in addition to the urinary protein-creatinine ratio (PCR) were evaluated. To measure urinary density, the samples were placed in refractometer (Refractometer UGI 1.000 - 1.050, Atago, Tokyo - Japan). Posteriorly, aliquots were centrifuged (FANEM®, Excelsa Cenfrifuge - model 206BL, Guarulhos - SP) and with the urinary sediment it was made slides were prepared for counting red blood cells, leukocytes, cells and cylinders under an optical microscope (Leica Microscope® DM 4000B, Wetzlar - Germany). The quantitative biochemistry of urinary protein measurements was performed using the pyrogallol red method (Sensiprot kit - Labtest Diagnóstica - Vista Alegre - Lagoa Santa - MG) in a semiautomatic device (Labtest Diagnóstica - Vista Alegre - Lagoa Santa - MG). To determine the PCR, measurements of urinary proteins were performed using pyrogallol red method (Sensiprot kit - Labtest Diagnóstica - Vista Alegre - Lagoa Santa - MG) and creatinine by picrate in alkaline medium (Labtest Diagnóstica - Vista Alegre - Lagoa Santa - MG), both in a semi-automatic device (Labtest Diagnóstica - Vista Alegre - Lagoa Santa - MG) in colorimetric mode; later, the calculation was obtained by the ratio between the concentration of these proteins over urinary creatinine. To allow statistical analysis of qualitative urinary parameters, scores (0: absent, 1: rare, 2: moderate and 3: severe) were assigned to the results of cells and casts.

The results were statistically evaluated by simple analysis of variance (ANOVA), being considered significant when p<0.05; in addition, Pearson correlations were performed to compare the different degrees of periodontal disease, with α<0.05, both using the Graphpad Prism 8® program.

3 Results and Discussion

Of the 60 dogs included in the study, eight were Beagle (13.4%), four Border Collie (6.7%), six Pinscher (10%), two Shintzu (3.3%), two Schnauzer (3%), twelve mongrels (20%), four Dachshunds (6.7%), four Blue Hillers (6.7%), four German Shepherds (6.7%), two Lhasa Apsos (3.3%), two Maltese (3.3%), six Spitz (10%), two Rottweilers (3.3%) and two Poodles (3.3%).

The average weight of the dogs was 14.1 kg and the average age was 6.1 years. Regarding the severity of periodontal disease, 18 animals were classified as grade II
(30%), 18 as grade III (30%), 10 as grade IV (16.6%) and 14 as grade V (23.4%).

Means and standard deviations of hematological parameters (blood count and biochemical) are shown in Table 1. As for red blood cells, hematocrit, total leukocytes, albumin, total protein, ALT and creatinine, there was no statistical correlation between the different degrees of periodontal disease and the values were within the normal range for the species. On the other hand, ALP and urea showed a statistical increase in grade V compared to the other grades of periodontal disease (p<0.05), despite being within the established for dogs (20 - 156 U/L and 15 - 59.5 mg/dL, respectively).

Table 1 - Mean and standard deviation of hematological parameters (blood count and biochemical) of 60 adult dogs with different degrees of periodontal disease (II, III, IV or V), treated at the Small Animal Dentistry Sector of the Veterinary Hospital of the University of Franca

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Grade of Periodontal Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
</tr>
<tr>
<td>Red cells (× 10⁶)</td>
<td>8.2±0.8</td>
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<tr>
<td>Hematocrit (%)</td>
<td>53.4±6.6</td>
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<tr>
<td>Total leukocytes (μl)</td>
<td>10.2±2.7</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7±0.8</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.4±0.9</td>
</tr>
<tr>
<td>ALT - U/L</td>
<td>50.6±25.3</td>
</tr>
<tr>
<td>ALP - U/L</td>
<td>57.1±30.5</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>33.3±14.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.83±0.16</td>
</tr>
</tbody>
</table>

*Significantly different from periodontal disease grade II, III and IV (p<0.05).

Source: Reaource data.

The means and standard deviations of the urinary parameters are shown in Table 2. There was no statistical correlation between the urinary parameters of density, erythrocytes, leukocytes, cells, casts and PCR with the different degrees of periodontal disease and the values were within the recommended parameters for the species. On the other hand, animals severely affected by periodontal disease (grade V) showed a statistical increase in urinary proteins when compared with those of grades II and III (p<0.05), however, they remained within the limits of normality for adult canines.

Table 2 - Mean and standard deviation of urinary parameters of 60 adult dogs with different degrees of periodontal disease (II, III, IV or V), treated at the Small Animal Dentistry Sector of the Veterinary Hospital of the University of Franca

<table>
<thead>
<tr>
<th>Urinary Parameters</th>
<th>Grade of Periodontal Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
</tr>
<tr>
<td>Density</td>
<td>1.036±0.01</td>
</tr>
<tr>
<td>Red cells/FLI</td>
<td>3.8±3.9</td>
</tr>
<tr>
<td>Leukocytes/FLI</td>
<td>1.4±0.7</td>
</tr>
<tr>
<td>Cells</td>
<td>0.5±0.6</td>
</tr>
<tr>
<td>Cylinders</td>
<td>0.16±0.4</td>
</tr>
<tr>
<td>Proteins (mg/dL)</td>
<td>12.8±7.8</td>
</tr>
<tr>
<td>PCR</td>
<td>161.8±87.9</td>
</tr>
</tbody>
</table>

*Significantly different from periodontal disease grade II and III (p<0.05).

Source: Reaource data.

Among the affections of the oral cavity of dogs, periodontal disease is the most frequent, affecting 85% of adults (ABDALLA et al., 2009), corroborating the results of the current study, which found that the average age of affected dogs was 6.1 years. Age is one of the predisposing factors of the periodontitis (SILVA et al., 2017), especially in those fed with homemade food and snacks, since due to the pasty consistency, it adheres more easily to the teeth, favoring the progression of oral disease (HARVEY, 2022). To date, there is no scientific evidence regarding the sexual predisposition of periodontal disease in dogs (FERNANDES et al., 2012).

In the present study, small-breed patients were the most affected by periodontal disease, coinciding with the descriptions by Di Bello et al. (2014), that these animals are more likely to accumulate plaque and, consequently, to odontoliths, due to the fact that the dental elements have little bone support, limited inter-dental space, dental crowding, in addition to malocclusion and dental anomalies, which makes it difficult to remove dirt by natural methods such as moving the lips and tongue, chewing and to gnaw objects. In addition, Harvey (2022) emphasized that toy dogs and small breeds tend to have greater survival compared to medium and large dogs, which consequently classify them as the most affected by periodontal disease.
Regarding the different degrees of periodontal disease, it was noted that no animal was classified as grade I, probably due to the rapid progression of the oral disease which, among other factors, can be attributed to the type of diet and the lack of regular tooth brushing due to the tutors’ unavailability of time associated with the lack of knowledge of this oral prevention method, in addition to the non-cooperation of the patients for not having been conditioned to the procedure since puppies, as described by Watanabe et al. (2015).

The results of the present study regarding the values of red blood cells and hematocrit validated the descriptions by Bastos et al. (2016), that periodontal disease, regardless of the degree of involvement, can trigger an antigenic response, but without a direct relationship with the erythropoiesis.

Although periodontal disease stimulates the systemic immune response as a result of oral infection (Rawlinson et al., 2011; Silva et al., 2017), no increase in the number of total leukocytes, the which remained within the normal range for the species in the different degrees of progression of the oral disease. On the other hand, Glickman et al. (2008) described positive relationship between the severity of periodontal disease and the increase in leukocyte count in dogs, while Lonsdale (1995) detected leukopenia.

Although liver enzymes were within the normal range for the species, ALP was higher in dogs with grade V periodontal disease, suggesting that this oral disease may predispose, in the long term, to liver impairment, as described by Kortegaard, Ericksen and Baelum (2014); however, serial hematological examinations would be indispensable to investigate this possibility.

Still on this topic, Debowes et al. (1996) and Pavlica et al. (2008) demonstrated that microscopic inflammatory or degenerative changes in distant organs of dogs, specifically liver, kidneys and heart, increased with the severity of periodontal infection. Furthermore, it is noteworthy that differential diagnoses are important for the therapeutic establishment, since the increase in AF associated with proteinuria may be related to excessive exogenous administration of corticosteroids, in addition to other systemic changes such as endocrinopathies, especially hyperadrenocorticism, which can cause hypertension and glomerulopathies (Grubbs et al., 2017).

Following recommendations from Chambrone et al. (2013) and Honkamp and Nabity (2016), in the present study, the serum levels of creatinine were evaluated together with those of urea, which is freely filtered by the glomeruli and passively reabsorbed in the renal tubules, and the filtration rate is influenced by of the flow of urine passing through the tubules (Nylund et al., 2017). Despite being within the normal range for the species studied, a considerable increase in serum urea levels was observed in dogs with periodontal disease grade V, when compared to the others; however, this isolated change (without concomitant increase in creatinine) did not characterize chronic kidney disease, as described by Freitas, Veado and Carregaro (2014). It should be considered that serum urea levels may change due to non-renal factors such as dehydration, protein-rich diet, gastrointestinal bleeding, liver failure and malnutrition (Arondon; Mittleman; Burger, 2004). Still in this context, it was decided to perform PCR, which helps in the investigation of the precocity of kidney injury, since the serum concentrations of creatinine and urea are considered renal biomarkers, however, of low sensitivity and late, remaining normal until more than 75% of nephrons become nonfunctional (Giraudiel; Pagès; Guelfi, 2012).

The quantitative biochemistry of urinary protein measurements was performed using the pyrogallol red method, which is more reliable compared to the reagent strip (Kortegaard; Ericksen; Baelum, 2014), since proteinuria is considered an early marker of kidney damage and, associated with to the measurement of glomerular filtration rate, the basis for assessing kidney disease (Waki et al., 2010).

Differently from what is described in the scientific literature (Glickman et al., 2011; Rawlinson et al., 2011; Brito et al., 2012), relevant glomerular changes were not detected in dogs with periodontal disease, because despite the fact that proteinuria was statistically higher in patients with more progressive degrees of oral disease (IV and V), it was within the normal range for the canine species. Still, no significant increase in serum creatinine, urinary density and urinary hyaline casts was detected, which, when associated with proteinuria, suggest glomerulonephritis (Mcgrotty, 2008). Thus, in these patients, it should be considered that proteinuria may result from other non-renal alterations such as pre-renal (hypertension and hyperproteinemias) and post-renal (urinary system infections that cause leukocyturia and hematuria) (Freitas; Veado; Carregaro, 2014).

Proteinuria is characterized by the loss of plasma proteins in the urine, specifically albumin, by glomerulonephritis and glomerular hypertension, as a result of changes in permeability and glomerular filtration, because under normal conditions, the proteins (with a molecular weight of less than 65 kDa) are filtered by the glomeruli and reabsorbed in the proximal tubules (Schaefter et al., 2011; Kortegaard; Ericksen; Baelum, 2014). Thus, the analysis of protein excretion requires the quantification of proteins eliminated in the urine during a complete day, requiring the use of metabolic cages, which are restricted to specialized centers (Mcgrotty, 2008; Schaefter et al., 2011). Thus, in the present study, proteinuria and its severity were reliably measured by PCR in a single random aliquot of urine, as indicated by Kang et al. (2017).

Even if the patients in the present study had presented a significant increase in serum urea associated with proteinuria, they could not be classified as renal patients; in this case, in order to define the diagnosis of nephropathies, it would be essential to carry out other complementary tests such as renal
ultrasound to investigate the cortico-medullary relationship, serial blood pressure measurement and the analysis of sensitive and reliable markers such as symmetrical dimethylarginine (SDMA) and cystatin C, considered an early indicator of progressive loss of renal function and which usually shows an increase before other parameters (ALMEIDA et al., 2017; SILVA et al., 2017).

Even though periodontal disease can cause inflammatory disorders, predisposing the occurrence of active urinary sediments, as reported by Grauer (2011), in the present study, red blood cells, leukocytes, cells and casts were within normal parameters, which probably did not cause changes in urinary density and, moreover, no difference was observed between the different degrees of oral disease.

In view of the data obtained in the current study and the complexity of the numerous factors involved in canine systemic diseases caused by periodontal disease, it is essential in all patients, the association of history, detailed anamnesis, complete physical examination and complementary exams (blood, urine, imaging and specific markers) to define the diagnosis and therapeutic institution. In this context, due to the high incidence of oral diseases in small animals, it is essential to disseminate and raise awareness among tutors about the methods of preventing periodontal disease, as well as early diagnosis and treatment, aiming at a better quality of life and survival, regardless of the age, race, sex, size and affected species (SILVA et al., 2017).

4 Conclusion

In view of the methodology applied and the results obtained, it is assumed that periodontal disease in adult dogs did not indicate impairment in hematological, biochemical and urinary parameters, regardless of breed and degree of involvement of the oral disease.

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References


