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A Transversal Study of Biochemical Profile, Urinalysis, UPC, Electrolytes and Blood Pressure in Dogs with Chronic Kidney Disease

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ABSTRACT

***Background*:** Chronic kidney disease (CKD) affects both dogs and cats, mainly elderly animals, due to tubulointerstitial inflammation associated with the increase of fibrosis through the excess deposition of extracellular matrix (ECM) which leads to decrease glomerular filtration. Many different underlying renal diseases can affect the kidneys of dogs such as congenital or acquired in origin. Therefore, the main objective of this transversal study was to evaluate the epidemiology through clinical and laboratory evaluation of 225 client-owned dogs with CKD.

***Materials, Methods & Results*:** Complete blood count (CBC), urinalysis, and biochemical profile were retrospectively selected and evaluated from 225 client-owned dogs with CKD of both sexes, different ages, and breeds from the patient population of the Nephrology and Urology Small Animal Service of the Teaching Hospital of the School of Veterinary Medicine and Animal Science - São Paulo State University from 2011 to 2017. All dogs were divided in groups according to the International Renal Interest Society (IRIS) CKD grading and statistical analysis was performed according to Kruskal-Wallis non-parametric test complemented with Dunn's multiple comparisons test, and analysis of variance for the model with a factor complemented with the test of multiple comparisons of Tukey. In this retrospective study, we observed that most dogs in all groups were elderly (≥ 9 years old). CBC demonstrated lower RBC's ($P < 0.005$), hemoglobin ($P < 0.001$), hematocrit (Ht%) [$P < 0.001$] at the highest stage of the disease. However, urinary specific gravity (USG) did not demonstrate significant differences between the disease stages, but urinary protein: creatinine ratio (UPC) was statistically different ($P < 0.01$) between IRIS-CKD stages 1 and 4. Furthermore, serum phosphate concentrations demonstrated significantly higher levels in dogs at IRI-CKD stage 4 compared with IRIS-CKD stage 3 ($P < 0.001$).

***Discussion*:** The analysis of 225 dogs with CKD showed that 130 animals were elderly, older than 9 years, and according to previous studies, 15% of dogs over 10 years of age are diagnosed of CKD, presenting significant morbidity and mortality. Laboratory findings such as the presence of non-regenerative anemia is expected in dogs with CKD. In our study, the degree of anemia corresponded with the stage of the disease, similarly to serum creatinine concentrations. Another important laboratory finding in diagnosing CKD is the early detection of the kidney's abilities in concentrating its tubular filtrate. In this retrospective study, isosthenuria was not significant due to all dogs presented CKD as criteria of inclusion, especially those without azotemia, although, proteinuria was reported in 90% of the population investigated. Electrolyte imbalances are also expected in CKD. However, despite serum sodium, potassium, and total calcium did not demonstrate significant results, serum phosphate had its significance between IRIS-CKD stage 3 and stage 4. Hence, despite the age factor of most dogs in all groups, with the association of laboratory results such as serum creatinine, serum phosphorus, ionized calcium, erythrogram, isosthenuria, SBP, and the degree of proteinuria, it was possible to perform early diagnosis of CKD even in dogs with IRIS-CKD Stage 1 in a stable and hydrated patient. With the proper diagnostic, staging and substaging according to IRIS guidelines, these parameters can be monitored, predicting longevity and good quality of life, or progression of the disease with a more reserved prognosis.

Keywords: chronic kidney disease, kidney, canine, azotemia.

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INTRODUCTION

Chronic Kidney Disease (CKD) is a global disease for humans, as well as for dogs and cats [4,20,42]. Each species has its own nephropathy particularities, although the progressive and irreversible loss of kidney function is a common ending point [4,20,42]. Furthermore, chronic tubulointerstitial nephritis and kidney fibrosis are the main responsible factors for loss of renal function, and thus leading to a progressive renal dysfunction [27].

This nephropathy usually presents a long progression time (2 to 3 months) until the onset of clinical signs associated with azotemia [4,38,41], which can delay the diagnosis and, thus, reducing the survival rate of these animals [4,41,43]. Despite clinical signs are mostly non-specific, such as polyuria and polydipsia [4,12,26,32,43,45], laboratory findings and imaging exams, particularly the ultrasonography, improves the accuracy for its diagnosis because its etiology is very difficult to determine, and it still relies on the biopsy as a gold standard diagnostic [4,36].

According to the International Renal Interest Society (IRIS), dogs with CKD are classified into 4 stages [22]. Its classification is based on the serum concentrations of creatinine, and most recently, in serum symmetric dimethylarginine (IDEXX SDMA)[1] concentration. Additionally, its sub-classification is based on proteinuria and blood pressure, contributing to proper diagnosis, prognosis, and treatments [22,41].

The aim of this retrospective study was to perform a comparative clinical, etiological and laboratory analysis of dogs with chronic kidney disease treated at the Teaching Veterinary Hospital of School of Veterinary Medicine and Animal Science - São Paulo State University (UNESP) from 2011 to 2017.

MATERIALS AND METHODS

Animal data collection

Data from 225 client-owned dogs from both sexes, of different ages, and breeds were retrospectively selected from the patient population of the Nephrology and Urology Small Animal Service of the Teaching Hospital of the School of Veterinary Medicine and Animal Science - São Paulo State University from 2011 to 2017.

Selection criteria

The selection criteria for inclusion of the animals were based on the complete history, compatible clinical signs to the disease, laboratory exams such as CBC (PocH-100iv Diff, Sysmex)[2], urinalysis, and biochemical profile (Cobas Mira Plus, Roche)[3], Systolic Blood Pressure (SBP) was monitored by non-invasive Doppler (Doppler Ultrasound device)[4], and abdominal ultrasonographic evaluation (Mylab AlphaVET, Esaote)[5]. Dogs diagnosed with concurrent AKI, sepsis, pancreatitis, coagulation disorder, infectious disease, and nephrolithiasis or had a previous history of familial or congenital renal disease, congestive heart failure, autoimmune disease, and neoplasia were automatically excluded from the study.

After selection, all dogs were diagnosed and substage according to the IRIS guidelines [22] and divided into four groups to simplify the comparative aspects of the study and to grant better results. All data were present with the median and interquartile ratio of each category in each group.

Statistical analysis

Statistical analysis was performed according to Kruskal-Wallis non-parametric test complemented with Dunn's multiple comparisons test [57]. Also, the technique of analysis of variance for the model with a factor complemented with the test of multiple comparisons of Tukey [57]. All discussions of the results of the statistical tests were performed at the 5% level of significance.

RESULTS

The analysis of 225 dogs with CKD demonstrated that 130 animals were elderly and older than 9 years. There was a significant difference between the mean ages between IRIS-CKD stage 1 and 2 with IRIS-CKD stage 3 and 4 ($P < 0.005$), but the mean was lower in IRIS CKD stage 3 and 4 (9,2 and 8,9 years respectively) [Table 1]. Although, sex distribution was homogeneous with no statistical significance between dogs with different stages of CKD, according to Goodman's Homogeneity Test [Table 1].

The worst exam results were at the highest stage of CKD, especially for RBC's ($P < 0.005$), hemoglobin ($P < 0.001$), and Ht% ($P < 0.001$) [Table 2]. In the present study, all dogs had a damaged kidney function with high values of serum urea and creatinine concentrations and, as expected, it was significant according to the stage of the disease ($P < 0.001$) [Table 3].

According to the urinalysis values despite USG did not demonstrated significant differences between stages, urinary pH had small significant difference

between stages with an increase of its pH ($P < 0.05$) [Table 4]. Although urine culture was not performed in this study, urinary protein detected in the dipstick did increase as well along stages, thus revealing a significant difference between stage 1 and 4 ($P < 0.01$). Additionally, in this study, approximately 90% of dogs had proteinuria. The urinary protein: creatinine ratio (UPC) of dogs on IRIS-CKD stages 1 and 4 were statistically different ($P < 0.01$); furthermore, we observed that the higher the protein loss, the higher stage of the disease was, seen as a significant difference especially between stages 3 and 4 ($P < 0.05$) [Table 5].

In respect of electrolytes concentrations, phosphate concentrations were significantly higher in dogs at IRIS stage 4 compared with IRIS stage 3 ($P < 0.001$) [Table 3]. Notwithstanding, some data did not present significant differences such as USG, SBP, serum total calcium, sodium and potassium concentrations, requiring a longer follow up evaluation during the progression of the disease ($P > 0.05$).

DISCUSSION

As mentioned before, breed and age may represent relevant risk factors which contribute to the onset of the disease by either genetic factors and the presence of concomitant disease [20,38,41]. The analysis of 225 dogs with CKD showed 130 animals were elderly, older than 9 years. This result corroborates with the literature and shows the chronicity of the disease. According to studies, 15% of dogs over 10 years of age suffer from CKD, presenting significant morbidity and mortality [53].

The age of the patients is one of the main variables to be analyzed because the disease occurs mainly in elderly animals, although, in our study younger animals were in an advanced stage of the disease (Table 1).

The progression of the disease was also accessed by comparing the ages of the dogs with all IRIS stages as shown in Table 1. This finding shows that CKD contributes with the decrease of life expectancy. However, other authors showed that age of animals with CKD may not differ among different etiologies [28] and in elderly animals it is more severe [31].

Some risk factors as breed and sex may vary between the studies; Breed, for instance, may represent a confounding variable, since it may vary according to the location of the study [21,29,38,50,58]. In this study, most of the animals were mixed breed. The authors have attributed it of some country bias, since it is one of the most common breeds in Brazil [5,8,14,24,30,48].

In respect of CBC, non-regenerative anemia is one of the common findings in dogs with CKD [3,16,46]. In accordance with literature, the present data demonstrated that all groups presented a normocytic and normochromic anemia (Table 2) [25]. Notwithstanding, and according to other authors, there was no difference regarding the predictors of regeneration among the stages of the disease [9]. The presence of inflammation and the increase of oxidative stress plays an important role during the progression of CKD [7,35,49]; furthermore, uremia contributes negatively on the lifespan of RBCs and to iron deficiency; Also, if uremia is not treated, uremic thrombocytopathia can also intensify the degree of anemia [44].

The most common worldwide and available biomarker to evaluate kidney function is serum creatinine. Additionally, concentrations of serum urea is another biomarker responsible in monitoring the retention of nitrogen metabolites that should be excreted by the nephrons, both biomarkers when associated reflects the renal function [33,38,43]. In the present study, all dogs had a compromised kidney function with an elevation of creatinine concentration as expected (Table 3).

The renin-angiotensin system is correlated with creatinine increasing and decrease of glomerular filtration which contribute to the progression of the disease [34]. Another parameter to be considered to the progression of the disease is the urea concentration at the diagnosis, it may be a predictor of survival time during the CKD progression (Table 3) [22].

During the past years, new studies are focusing on earlier renal disease biomarkers such as SDMA [47,56]. A small amino acid that is mainly secreted by the kidneys, making it sensitive to evaluate GFR and allows early diagnosis of CKD when compared to serum creatinine [10,18,37]. Due to the start date of this study and the standardization of all animals evaluated, it was not possible to perform the SDMA evaluation. Unfortunately, it was added to the IRIS CKD guidelines after the beginning of this project which made SDMA dosage impossible.

Hyperphosphatemia in dogs with CKD is associated with an imbalance in its intestinal absorption, renal excretion and bone absorption [39,47,48]. Phosphate evaluation and management is important at the

Table 1. Distribution of the mean, standard deviation of age and absolute sex frequency within each CKD staging of canines attended at TH-FMVZ-UNESP in the period 2011-2017.

Parâmetro	IRIS-CKD Stages				
	1	2	3	4	Value *P*
Age (years)	11.94 ± 3, 01	11.42 ± 3.46	9.23 ± 4.08	8.97 ± 3.73	$P < 0.005$
Sex					Total
Female	7	14	37	51	109
Male	10	12	54	40	116
Total	17	26	91	91	225

Table 2. Distribution of mean and standard deviation (with lowest and highest value, respectively) of the analysis of the complete blood count of each IRIS-CKD stage of the canines attended at the TH-FMVZ-UNESP in the period of 2011-2017.

Parâmetro	IRIS-CKD Stages				
	1	2	3	4	*P* value
Red Blood Cells (x10^6/µL)	4.60 ± 1.60	4.52 ± 1.48	3.76 ± 1.25	3.56 ± 1.13	$P < 0.005$
Hemoglobin (g/dL)	11.15 ± 3.54	10.68 ± 2.96	9.13 ± 2.84	8.86 ± 3.05	$P < 0.001$
Ht (%)	32.94 ± 10.67	30.35 ± 8.06	26.26 ± 7 , 71	25.53 ± 8.14	$P < 0.001$
MCV (fL)	70.93 ± 8.72	68.39 ± 9.99	70.70 ± 7.28	70.47 ± 6.14	$P > 0.05$
MCHC (%)	33.85 ± 2.56	35.05 ± 3.58	34.76 ± 2.91	34.97 ± 3.27	$P > 0.05$
RDW	12.40 ± 3.96	14.16 ± 4.11	13.69 ± 2.93	13.69 ± 2.96	$P > 0.05$
PT (Plasma) (g/dL)	7.14 ± 0.97	7.85 ± 1.19	7.54 ± 1.51	7.94 ± 1.23	$P > 0.05$
Platelets (x10^3/µL)	327.6 ± 168.0	319.8 ± 193.8	305.3 ± 190.3	251.2 ± 149.8	$P > 0$ 05
WBC (x10^3/uL)	10.2 (6.2; 25.0)	9.1 (5.6; 359.0)	10.3 (3.2; 54.1)	10.8 (2.1; 30.4)	$P > 0.05$

Table 3. Distribution of the mean and standard deviation (with lowest and highest values, respectively) of the complete biochemical analysis of each IRIS-CKD stage of the canines attended at TH-FMVZ-UNESP in the period 2011-2017.

Parâmetro	IRIS-CKD Stages				
	1	2	3	4	*P* value
Urea (mg/dL)	83.09 ± 47.17	97.43 ± 39.29	188.02 ± 117.77	312.60 ± 160, 79	$P < 0.001$
Creatinine (mg/dL)	1.09 ± 0.23	1.77 ± 0.17	3.43 ± 0.89	9.78 ± 6.91	$P < 0.001$
Albumin (g/dL)	2.65 (1.80; 9.70)	2.55 (1.30; 5.90)	2.40 (0.90; 11.3)	2.50 (0.10; 13.50)	$P > 0.05$
Total Calcium (mg/dL)	8.51 ± 2.25	10. 82 ± 1.73	10.19 ± 2.94	9.68 ± 2.68	$P > 0.05$
Phosphorus (mg/dL)	4.47 ± 1.27	6.30 ± 4.78	9.48 ± 7.04	19.19 ± 15.13	$P < 0.001$
Sodium (mEq/L)	147.64 ± 12.83	143.07 ± 30.64	21.98	143.70 ± 145.22 ± 11.18	$P > 0.05$
Potassium (mEq/L)	4.20 ± 0.85	4.69 ± 1.10	4.75 ± 1.41	4.80 ± 1.55	$P > 0.05$

Table 4. Distribution of the mean and standard deviation (with lowest and highest value, respectively) of the urinalysis of each IRIS-CKD stages of the canines attended at TH-FMVZ-UNESP in the period 2011-2017.

Parâmetro	IRIS-CKD Stages				
	1	2	3	4	Value *P*
pH	6.50 ± 1.12	6.12 ± 1.09	5.91 ± 0.78	5.87 ± 0.85	$P < 0.05$
Specific Gravity	1.013 (0.005)	1.014 ± 0.004	1.014 ± 0.004	1.014 ± 0.010	$P > 0.05$
Protein	0.91 ± 0.62	1.19 ± 0.87	1.70 ± 0.86	1.69 ± 0.86	$P < 0.01$
Glucose	0.0 (0.0; 0.0)	0 0 (0.0; 3.0)	0.0 (0.0; 1.0)	0.0 (0.0; 2.0)	$P > 0.05$
Ketone Bodies	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	$P > 0.05$
Urobilinogen	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	$P > 0.05$

Bilirubin	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	$P > 0.05$
Hemoglobin	0.5 (0.0; 4.0)	1.0 (0.0; 4.0)	1.0 (0.0; 4.0)	2.0 (0.0; 0)	$P > 0.05$
Leukocytes	0.5 (0.0; 1.0)	0.0 (0.0; 1.0)	0.0 (0.0; 1.0)	0.0(0.0; 1.0)	$P > 0.05$

Table 5. Distribution of the mean and standard deviation (lowest and highest values, respectively) of the urinary Protein:Creatinine Ratio (UPC) and Systolic Blood Pressure (SBP) of each IRIS-CKD stages of the canines attended at TH-FMVZ-UNESP in the period of 2011-2017.

	IRIS-CKD Stages				
	1	2	3	4	*P* value
UPC	1.76(0.80; 6.22)	1.59(0.28, 9.48)	2.54(0.22; 21.20)	2.30(0.05; 11.09)	$P < 0.05$
SBP	158.64 ± 24.91	157.50± 37.23	34 157.32 ± 85	163.62 ± 34.00	$P > 0.05$

diagnosis of CKD, and it is one of the main triggering factors for secondary renal hyperparathyroidism contributing to bone demineralization and mineralization of other tissues [11,19,40].

Furthermore, as kidney function decreases, PTH and the renal excretion of phosphate increases, leading to the activation of calcitriol from calcidiol even in earlier stages of the disease [11,13,19,40]. The significant hyperphosphatemia in dogs at IRIS-Stage 4 represents a higher risk to develop mineral and bone disorders as the diseases progresses (Table 3). Thus, it would be interesting to carry out statistical comparisons between the phosphate and ionized calcium concentrations among the same groups, proving that ionized hypocalcemia is linked to hyperphosphatemia.

Ionized hypocalcemia is another laboratorial finding in dogs with CKD [13]. Its occurrence can be associated with hyperphosphatemia, low vitamin D production by the kidneys, calcium deposition in other organs, and low absorption of calcium via gastrointestinal tract [15,39]. In the present study, most dogs presented normal serum total calcium (tCa) concentrations (Table 3); However, tCa does not support as a correct diagnosis as well as for monitoring bone and mineral disorders. Total calcium is composed of ionized calcium (iCa), complexed calcium, and protein-bound calcium; Hence, ionized hypocalcemia should be considered in dogs with advanced CKD [13].

In addition to the hematological, biochemical exams and clinical signs, the reduction in renal function was also saw by the lower urinary specific gravity (USG) through the urinalysis. In our retrospective study, USG presented mean of 1.013-1.014 in all stages (Table 4). Isosthenuria is one of the findings in animals with CKD due to the loss of kidney function in concentrating urine. Tubular water loss contributes to dehydration, which also worsens when associated with anorexia, vomiting, and diarrhea lead by CKD [38,44].

Proteinuria in patients with CKD is due to an impaired glomerular filtration related to its damaged barrier [17]. It is considered a useful marker of the disease progression once it is an indirect measurement of renal function loss [21,55]. One of the most used methods to quantify non-specific proteinuria is through (UPC) which is also used in combination with SBP to classify CKD into substages [57].

In the present study, the urine test strip revealed approximately 90% of the animals had proteinuria, also in agreement with its UPC concentrations, thus corroborating with literature data [21]. The degree of proteinuria is one of the main hallmarks of CKD [21,38], thus in the present study, dogs with higher proteinuria also presented a higher stage of kidney disease, therefore, supporting a direct correlation between the degree of proteinuria and loss of renal function, leading to a reserved prognosis (Table 5). One of the reasons might be derived from the occurrence of epithelial to mesenchymal transition on tubular epithelial cells which contributes with tubulointerstitial damage and fibrosis of the kidney [6].

Patients with CKD have the Renin-Angiotensin-Aldosterone System (RAAS) activated as a tempt to restore the hemodynamic status and thus GFR. This activation has a secondary effect on the increase of SBP of the animal due to vasoconstriction and inflammation. Thus, SBP correlates with the presence of proteinuria [2,4,32,52]. With the new consensus of hypertension [1], and consequently with the update of IRIS guidelines for blood pressure substages [22], despite there were no significant differences of SBP among the groups (Table 5), in our retrospective study, dogs with IRIS-CKD Stage 1 to 3 are now substage

as prehypertensive patients (SBP between 140 to 159 mmHg) and dogs at the end-stage were substage as hypertensive (SBP between 160 to 179 mmHg). Failure in managing hypertension may contribute to the intensification of inflammation, and consequently, to kidney fibrosis. Hence, the management of systemic hypertension is essential to prevent the progression of CKD [1,22,23].

This study had some limitations, firstly, GFR evaluation was solely measured by serum creatinine concentrations, and since SDMA was validated by IRIS as a novel kidney biomarker in diagnosing and monitoring CKD in 2016, we suggest that SDMA would increase the number of non-azotemic dogs (IRIS-CKD Stage 1) that were not included in this study. Secondly, iCa and FGF-23 analysis suggest a better evaluation of bone and mineral disorder of dogs with CKD. Unfortunately, our chemistry machine does not perform iCa as a routine exam. Therefore, a blood gas analyzer would be more suitable for such. Moreover, the evaluation of FGF-23 is costly and only available for research purposes. At last, the evaluation of reticulocyte and erythropoietin concentrations could better demonstrate the progression of anemia and blood cell regeneration of these animals.

CONCLUSION

In conclusion, CKD diagnosis, staging, and monitoring can be achieved with the sum of clinical evaluations, history and imaging findings, SBP measurement, and laboratory results such as serum creatinine, serum phosphorus, iCa, CBC, USG and UPC in a stable and hydrated patient. With the proper diagnostic, staging and substaging, these parameters can be monitored, predicting longevity and good quality of life, or progression of the disease with a more reserved prognosis.

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