

Renal morphometric and doppler studies in different stages of canine chronic kidney disease

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Abstract

Doppler ultrasound is a useful diagnostic tool to evaluate renal blood flow in canine chronic kidney disease (CKD). The aim of this study was to describe and compare renal blood flow in different stages of CKD dogs based on the ultrasonographic appearance of kidneys. One hundred and three dogs with CKD confirmed clinically, hematologically, biochemically and ultrasonographically were included in the study. Ten healthy dogs with no haematological and ultrasonographic abnormalities of kidneys were also included in the study as control group. Based on SDMA levels, the dogs with CKD were divided into four groups viz., Stage I (n=17), Stage II (n=15), Stage III (n=25) and Stage IV (n=46). The Renal volume was measured with two-dimensional ultrasonography. All the dogs were subjected to pulsed-wave doppler to obtain hemodynamic parameters such as resistance index (RI) and pulsatility index (PI) which were significantly ($P < 0.05$) higher in CKD stage III and IV dogs than control. However, no significant difference was reported in RI and PI values in dogs with CKD Stage I and II ($P > 0.05$). It could be concluded that hemodynamic parameters appear as useful markers to understand the pathological process and progression in dogs with CKD.

Keywords: Canine, Chronic kidney disease, Doppler studies, Morphometry, Renal artery.

Introduction

Chronic kidney disease is defined as the presence of structural or functional abnormalities of one or both kidneys that have been presented for an extended period, usually 3 months or longer and is commonly diagnosed in the geriatric population (Nabi *et al.*, 2018). There are many potential causes for initial kidney damage which included trauma, infection, immunological diseases, neoplasms, renal ischemia, genetic anomalies and exposure to toxins. Unfortunately, the compensatory mechanisms that respond to nephron loss (glomerular hypertension, hyperfiltration) facilitate progression of chronic kidney disease, potentially contributing more than the original injury (Polzin, 2011). Early diagnosis is key in stabilising renal function and in ameliorating the disease progression.

Trans abdominal B-mode ultrasonography could be instrumental in differentiating renal diseases into diffuse (nephritis and end stage kidney), regional (hydronephrosis and nephrolithiasis) and focal or multifocal (polycystic kidney) and the dogs in stage I and II did not show any structural abnormalities associated with chronic renal disease. (Kavitha *et al.*, 2013; Nyland *et al.*, 2015). Renal volume could be an important parameter in detecting severity of disease and it was calculated using the formula as the volume of an ellipsoid which gives a good indication of the true renal volume (Barrera *et al.*, 2009)

However, ultrasonography was poor in detecting early changes in renal diseases that begins with altered blood flow. Renal doppler ultrasonography, provides a realtime information about the anatomy and haemodynamics during various stages of chronic kidney disease (Bragato *et al.*, 2017). Renal resistive index (RI) and pulsatility index (PI) measurements are

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used for calculation of the resistance to tissue perfusion in chronic kidney disease and these alterations were directly proportional to severity and progression of disease (Morrow *et al.*, 1996). Limited studies were available that demonstrate the hemodynamic changes in the vascular supply during pathology of different stages of chronic kidney disease. The aim of this study was to determine the morphometric and doppler measurements in different stages of chronic kidney disease according to the IRIS classification (2019) and comparison with healthy dogs.

Materials and Methods

The study was performed on one hundred and three client owned dogs diagnosed with different stages of CKD based on SDMA. Dogs were evaluated for clinical signs of systemic illness, haematological and biochemical changes. Haematological parameters such as hemoglobin (g/dL), packed cell volume (%), total erythrocyte count ($\times 10^6/\mu\text{l}$), total leukocyte count ($\times 10^3/\mu\text{l}$) and differential leukocyte count (%) were estimated as per the standard procedures described by Jain (2000).

Ultrasonography and doppler measurements were recorded before the treatment. Two standard planes of sections were imaged in each kidney to measure renal parameters. Coronal or sagittal section was taken along the long axis of kidney to measure the maximum bipolar length (L) and the width at the renal pelvis (W) were measured. Then the Head of transducer was rotated through 90° to achieve a transverse section. Fine adjustments were made until the section was as round or oval as possible and renal pelvis could be clearly seen and the depth (D) was measured. Renal volume was calculated from the measurements by using the formula

of ellipsoid $V = (L \times W \times D) 0.523$ in both left and right kidneys in chronic kidney diseases compared to healthy animals. The renal measurements were also compared in dogs based on their body weight (Jeyaraja *et al.*, 2015). The dogs above 15kg and below 15kg bodyweight were grouped separately.

Color Doppler was used to visualize the intrarenal vasculature. Renal Doppler measurements were obtained from the renal, interlobar or arcuate arteries. Sample volume was positioned in the middle of the renal vessels and the angle of correction did not exceed 60° . Doppler examination required more than 10 minutes in most of the dogs because of the panting or tachypnea. Colour mapping signals were visualized on both transverse and longitudinal ultrasound scan sections around the renal arteries (Koenhems and Gonul, 2019). Hemodynamic indices *viz.*, resistive index (RI), pulsatility index (PI) were calculated and recorded with the help of software provided in the machine.

Serum biochemical parameters like creatinine, blood urea nitrogen, total protein, albumin, serum alanine amino transferase and serum alkaline phosphatase were estimated by using a Thermo Scientific Instrument (Multiskan GO Analyser) using standard kits manufactured by Transasia Biomedicals Ltd., Erba-Mannheim. All the dogs under study were subjected to trans abdominal ultrasonography by following the standard procedure using ALOKA PROSOUND α 6 machine equipped with 3.5 to 7.5 MHz trans-abdominal curvilinear and linear probes. Ten apparent healthy dogs with no haematological and biochemical abnormalities were also included in the study as control group. Based on the SDMA levels, the affected dogs were categorized into four stages which was adopted by IRIS (2019).

	CKD Stage			
SDMA concentration ($\mu\text{g/dL}$)	I	II	III	IV
	14-18	18-35	36-54	>54

The ultrasonographic measurements were analyzed by One-way Analysis of Variance (ANOVA) using SPSS (SPSS 20.0, Chicago, IL, USA). Tukey's multiple comparison post hoc test was also used to find the differences between groups.

Results and Discussion

In the present study, the dogs with chronic kidney disease were categorized into 4 stages based on SDMA values as 16.50 per cent of dogs in stage I

(17/103), 14.56 per cent in stage II (15/103), 24.27 per cent in stage III (25/103) and 44.67 per cent in stage IV (46/103). The mean $\pm$ SE of SDMA in stage I, II, III and IV were $16.59 \pm 0.53 \mu\text{g/dL}$, $25.07 \pm 1.30 \mu\text{g/dL}$, $46.89 \pm 1.09 \mu\text{g/dL}$ and $87.51 \pm 3.11 \mu\text{g/dL}$ respectively. The SDMA values in the affected dogs ranged from 14 to $152 \mu\text{g/dL}$ in this study. The mean hematological and biochemical parameters observed in healthy and dogs with different stages of chronic kidney disease were presented in Table 1 and Table 2 respectively

Table 1: Mean $\pm$ SE of Haematological Parameters in healthy control and dogs with different stages of chronic kidney disease

S.No	Parameter	Healthy Control	CKD(n=103)			
			CKD (I) (n=17)	CKD(II) (n=15)	CKD(III) (n=25)	CKD(IV) (n=46)
1.	Haemoglobin (g/dL)	14.55 ^c $\pm$ 0.47	14.54 ^c $\pm$ 0.06	11.48 ^b $\pm$ 0.86	11.38 ^a $\pm$ 0.83	9.09 ^d $\pm$ 0.32
2.	Packed cell volume(%)	46.61 ^c $\pm$ 1.39	43.61 ^{bc} $\pm$ 0.19	34.24 ^b $\pm$ 2.68	33.34 ^a $\pm$ 2.17	22.91 ^a $\pm$ 1.44
3.	Total Erythrocyte count (10 ⁶ /cumm)	6.51 ^c $\pm$ 0.14	7.27 ^b $\pm$ 0.03	5.51 ^b $\pm$ 0.45	5.59 ^a $\pm$ 0.37	4.74 ^e $\pm$ 0.18
4.	Total Leukocyte count(10 ³ /cumm)	12.21 ^a $\pm$ 0.88	13.64 ^a $\pm$ 0.31	15.11 ^b $\pm$ 2.02	17.54 ^c $\pm$ 1.77	13.35 ^b $\pm$ 0.98
5.	Differential Leukocyte count					
	Neutrophils(%)	74.80 ^a $\pm$ 0.18	74.94 ^a $\pm$ 0.47	79.73 ^b $\pm$ 3.11	79.93 ^b $\pm$ 2.55	84.20 ^c $\pm$ 1.37
	Lymphocytes(%)	22.92 ^a $\pm$ 0.92	24.88 ^a $\pm$ 0.48	18.73 ^b $\pm$ 2.86	18.55 ^b $\pm$ 2.62	14.24 ^c $\pm$ 0.97
	Monocytes(%)	1.02 $\pm$ 0.46	0.18 $\pm$ 0.09	0.14 $\pm$ 0.13	0.40 $\pm$ 0.32	1.07 $\pm$ 0.26
	Eosinophils(%)	1.00 $\pm$ 0.01	0 $\pm$ 0.00	1.40 $\pm$ 0.43	1.12 $\pm$ 0.36	0.49 $\pm$ 0.2
	Basophils(%)	0.26 $\pm$ 0.34	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00	0.00 $\pm$ 0.00

Means with similar superscripts did not vary significantly in a row (P>0.05)

Table 2 : Mean $\pm$ SE of serum biochemical parameters in healthy dogs and dogs with different stages of chronic kidney disease kidney disease.

S.No	Parameter	Healthy	CKD (n = 10 ³)			
			I (n = 17)	II (n = 15)	III (n = 20)	IV (n = 46)
1.	Creatinine (mg/dL)	0.69 ^a $\pm$ 0.10	0.84 ^a $\pm$ 0.07	1.83 ^b $\pm$ 0.09	2.85 ^c $\pm$ 0.15	8.93 ^c $\pm$ 0.51
2.	Blood Urea Nitrogen (mg/dL)	16.36 ^a $\pm$ 1.07	14.90 ^a $\pm$ 0.39	37.76 ^{ab} $\pm$ 3.75	55.87 ^b $\pm$ 6.01	127.25 ^d $\pm$ 16.41
3.	Total Protein (g/dL)	7.83 ^d $\pm$ 0.66	6.49 ^d $\pm$ 0.29	6.93 ^{cd} $\pm$ 0.33	5.60 ^a $\pm$ 0.28	5.54 ^a $\pm$ 0.23
4.	Albumin (g/dL)	3.25 ^c $\pm$ 0.07	2.86 ^b $\pm$ 0.04	2.85 ^b $\pm$ 0.30	2.54 ^a $\pm$ 0.24	3.11 ^c $\pm$ 0.12
5.	Globulin(g/dL)	3.71 ^d $\pm$ 0.68	3.73 ^d $\pm$ 0.31	4.08 ^d $\pm$ 0.28	3.06 ^b $\pm$ 0.19	2.43 ^a $\pm$ 0.21
6.	A/G ratio	0.87 ^a $\pm$ 0.23	1.32 ^a $\pm$ 0.07	0.79 ^a $\pm$ 0.13	0.97 ^a $\pm$ 0.12	1.27 ^b $\pm$ 0.87
7.	Alanine aminotransferase(U/L)	17.81 ^a $\pm$ 3.92	19.24 ^a $\pm$ 0.28	22.63 ^a $\pm$ 3.69	30.18 ^b $\pm$ 3.35	27.98 ^b $\pm$ 1.49
8.	Alkaline phosphatase(U/L)	48.37 ^a $\pm$ 5.77	51.88 ^a $\pm$ 0.77	61.28 ^{ab} $\pm$ 8.38	87.22 ^b $\pm$ 7.91	145.52 ^c $\pm$ 20.30

Means with similar superscripts did not vary significantly in a row (p>0.05)

Ultrasound investigation was a useful and reliable method to detect renal pathology. The mean nephrosonographic measurements in healthy and dogs with kidney diseases were depicted in Table 3. There was significant (P>0.05) reduction in the length, width

and depth of both the kidneys between healthy and dogs with CKD. Similarly a significant reduction (P<0.05) in both kidneys was also seen in dogs with CKD with that of healthy control. The mean $\pm$ SE of renal volume in CKD was significantly less (P<0.05) than control

Table 3: Mean $\pm$ SE values of nephrosonographic measurements in healthy and dogs with chronic kidney disease.

Parameter		Dogs with body weight <15KG		Dogs with body weight >15KG	
		Control	CKD	Control	CKD
Length (cm)	Left	4.6 ^a $\pm$ 0.32	3.43 ^b $\pm$ 0.14	6.8 ¹ $\pm$ 0.12	5.8 ² $\pm$ 0.32
	Right	4.5 ^a $\pm$ 0.36	3.58 ^b $\pm$ 0.13	6.7 ¹ $\pm$ 0.15	6.2 ² $\pm$ 0.24
Width (cm)	Left	2.7 ^a $\pm$ 0.16	2.49 ^b $\pm$ 0.08	3.8 ¹ $\pm$ 0.07	3.5 ² $\pm$ 0.15
	Right	2.7 ^a $\pm$ 0.14	2.39 ^b $\pm$ 0.02	3.7 ¹ $\pm$ 0.22	3.4 ² $\pm$ 0.14
Depth(cm)	Left	2.8 ^a $\pm$ 0.12	2.48 ^b $\pm$ 0.09	3.7 ¹ $\pm$ 0.13	3.5 ¹ $\pm$ 0.12
	Right	2.8 ^a $\pm$ 0.13	2.50 ^b $\pm$ 0.09	3.6 ¹ $\pm$ 0.14	3.4 ¹ $\pm$ 0.14
Volume(cm ³)	Left	17.98 ^a $\pm$ 3.18	11.01 ^c $\pm$ 0.57	50.02 ¹ $\pm$ 2.49	39.16 ³ $\pm$ 3.32
	Right	17.79 ^a $\pm$ 2.18	11.18 ^c $\pm$ 0.64	46.67 ¹ $\pm$ 3.20	41.12 ³ $\pm$ 3.28

Means bearing different superscripts differed significantly with in a row (P<0.05)

The wave form of renal artery was recorded in healthy control and dogs with CKD using pulsed wave doppler. The mean resistive index (RI) and pulsatility

index(PI) values recorded in healthy and dogs with kidney diseases were presented in Table 4.

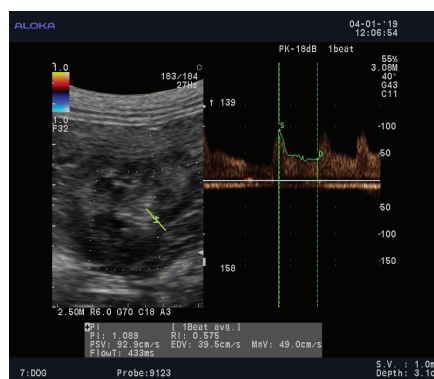
Table 4:Mean $\pm$ SE values of renal resistive and pulsatility indices in different stages of CKD dogs and healthy dogs

S. No	Parameter	Control	CKD			
			I (n=17)	II (n=15)	III (n=25)	IV (n=46)
1.	Resistive Index	0.59 ^a $\pm$ 0.12	0.57 ^a $\pm$ 0.14	0.61 ^a $\pm$ 0.12	0.64 ^b $\pm$ 0.14	0.73 ^c $\pm$ 0.11
2	Pulsatility Index	1.02 ^a $\pm$ 0.32	1.01 ^a $\pm$ 0.38	1.38 ^a $\pm$ 0.24	1.46 ^b $\pm$ 0.36	1.59 ^b $\pm$ 0.42

Means with similar superscripts did not vary significantly in a row (p>0.05)

Non-significant difference (P<0.05) was noticed in mean values of RI and PI in chronic kidney disease stage I and II with the healthy control while the

same in chronic kidney disease stage III and IV varied significantly (P<0.05).

**Figure1. Pulsed wave doppler of renal artery in Mongrel with Stage I CKD**

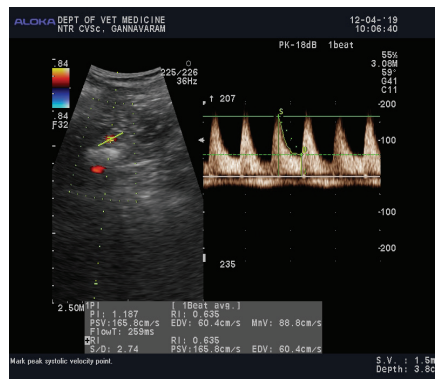


Figure 2. Pulsed wave doppler of renal artery in German Shepherd dog with Stage II CKD

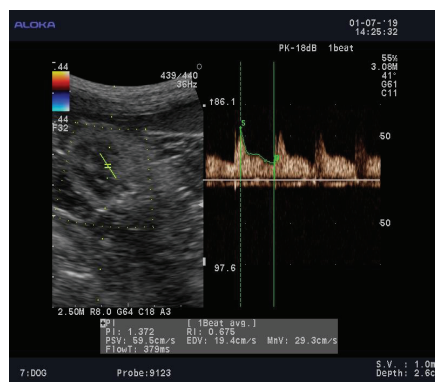


Figure 3. Pulsed wave doppler of renal artery in Labrador dog with Stage III CKD

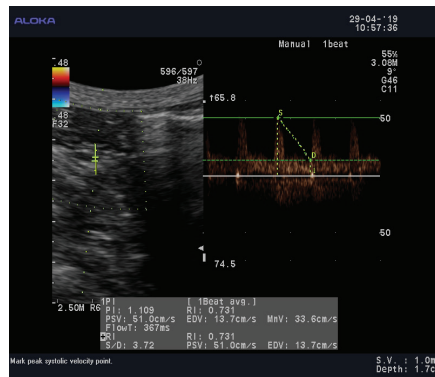


Figure 4. Pulsed wave doppler of renal artery in a Pomeranian dog with Stage IV CKD

In the present study, the renal measurements related to body weight of dog obtained ultrasonographically was in accordance with that of Jeyaraja *et al.* (2015) who concluded that renal volume was the accurate reflection of renal mass. The renal dimensions in the present study revealed bilateral reduction in the renal volume in CKD dogs which indicated severe renal impairment. The length, width and depth significantly varied only in dogs affected with chronic kidney disease. These findings commensurate

with Hecht and Henry (2011) and Jeyaraja *et al.* (2015), who observed small sized kidney in chronic kidney disease. Further it was documented that a normal renal size does not preclude renal disease, but departure from normality provide extra information which may contribute towards diagnosis (Kaya, 2012).

In Veterinary Medicine, despite many diagnostic methods such as physical examination, CBC and biochemical blood examination, urinalysis, radiography

and ultrasonography was well established, little attention was given to the evaluation of renal vascular supply (Koenhems and Gonul, 2019). The RI and PI values as measured by doppler ultrasonography in the present study(0.64 ± 0.14 , 1.46 ± 0.36 in stage III and 0.73 ± 0.11 , 1.59 ± 0.42 in stage IV) were in concurrence with that of Rivers *et al.* (1997); Novellas *et al.* (2007) and Koenhems and Gonul (2019). Increase in RI and PI were suggestive of increased intrarenal vascular resistance associated with reduced renal function in the advanced stages of renal disease (Saraiva, 2010 and Winther *et al.*, 2012). According to Morrow *et al.* (1996) and Parolini *et al.*, (2009), elevated RI with normal nephrosound at the time of initial presentation indicates interstitial nephritis or tubular degeneration which lead to rapid progression of CKD with poor prognosis. Bragato *et al.* (2017) also documented that the early changes in renal diseases begins with change in blood flow and renal doppler ultrasonography is one of the important diagnostic tool in determining CKD progression.

In conclusion, our study demonstrated that Doppler ultrasound is an accurate diagnostic tool to reflect hemodynamic changes in renal pathology in different stages of CKD and also helps in monitoring the prognosis of CKD which in turn helps in designing an effective therapeutic protocol and keep the disease under long-term control.

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