

Determination of renal blood flow with Doppler ultrasound and the hypertension prevalence and acid-base level in dogs with chronic renal failure.

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Research Article

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ABSTRACT

Chronic renal failure (CRF) is an important cause of morbidity and mortality in dogs. The aim of this study is to investigate the renal Doppler measurements, blood hypertension and acid-base levels in dogs with chronic renal failure. Twenty-six dogs previously diagnosed with chronic renal failure were compared with twenty healthy dogs. A complete blood cell, biochemical profile, urinalysis, blood gasses, blood pressure were analyzed and changes in renal blood flow were measured by renal Doppler ultrasonography. The dogs with CRF had significantly higher serum blood urea nitrogen, creatinine and phosphorus concentration, significantly lower packed cell volume, and urine specific gravity than control dogs. Positive correlation was determined between serum creatinine levels and renal Doppler indices. There were significant decreases in blood pH and bicarbonate. Indirect blood pressure measurements were slightly increased in CRF dogs. Renal Doppler measurement was observed as a helpful tool in diagnosing the CRF in dogs. Identification of acidosis and hypertension may help in developing treatments that slow the rate of progression of chronic renal failure.

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Introduction

Chronic renal failure (CRF) is defined as structural and/or functional impairment of one or both kidneys (Polzin, 2011; Bartges, 2012). Medical history and physical examination findings (eg, changes in kidney size or shape, changes in urine volume) are suggestive of kidney disease. The most commonly used serum urea and creatinine levels may be normal even when only 25% of the nephrons have normal filtration capacity. Therefore these methods may remain incapable in the early stages of renal insufficiencies. Duplex Doppler ultrasonography, provides a real time

information about the anatomy and dynamics of kidney. No absolute contraindication related to Doppler USG is reported/known (Drelich-Zbroja et al., 2018). The first study on the detection of normal values in dogs was published by Nyland et al. in 1993 (Nyland et al., 2002). When renal failure occurred, normal balance between vasoconstrictive and vasodilator factors deteriorates in time and intrarenal vasoconstriction occurs. Renal resistive index (RI) and pulsatility index (PI) measurements are used for the calculation of the resistance to tissue perfusion that

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occurs in that area (Bragato et al., 2017; Mitchell et al., 1998). They are also used for the determination of the changes in blood flow due to congestion, diuretic effects, acute and chronic renal failure and congenital dysplasia (Novellas et al., 2007). Although these alterations have been reported to be proportional to the severity and progression of chronic renal disease, there are few studies focusing on this subject in animals (Morrow et al., 1996).

Hypertension is one of the most common complication of renal failure. In recent years the prevalence of hypertension in CRF was studied by many researchers (Acierno et al., 2018; McMurphy et al., 2006). Although the direct measurement with the use of electronic transducer within the artery is accepted as the golden standard, indirect measurement with Doppler and oscillometric devices are also commonly used (McMurphy et al., 2006). Standards for the validation of indirect measurement devices in human medicine are well established. However no device has met these criteria in dogs or cats still (Acierno et al., 2018).

Metabolic acidosis is the other important complication of renal failure. It is the result of decreased renal sulfate and phosphate production, reduction of H⁺ ion excretion and increased urinary bicarbonate (HCO₃) loss (Polzin et al., 2005).

The aim of this study was to investigate the effects of the disease in dogs with CRF with the help of renal Doppler measurements and blood pressure and blood gas. Thus, one of the most important causes of death in dogs, chronic renal diseases and their complications will be examined in detail.

Materials and methods

Animals: Twenty-six dogs previously diagnosed CRF and twenty healthy dogs from several breeds and ages were included into the study. All animals were privately owned and presented to our clinics. CRF was diagnosed for each patient according to the history, clinical signs, and laboratory examinations accepted typical for the disease.

Physical examination, routine blood tests (hematological and biochemical), B-mode and Doppler ultrasonography examinations were performed on all dogs on the presentation day. Total cell count (Mindray BC 2800 vet, Chine), plasma concentrations of glucose, urea, creatinine, aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total proteins, albumin, calcium, phosphorus were assayed by the autoanalyzer (Tokyo Boeki TMS 1024, Tokyo, Japan). Urine specimens were obtained from dogs either by voluntary voiding or catheterization. Routine dipstick analysis were

performed and all samples were underwent microscopic examinations. Urinary protein/creatinine (UPC) ratio was measured. UPC ratio was determined using routine methods.

Blood pressure: Blood pressure was measured non-invasively with the use of an ultrasonic Doppler flow monitor (Parks Medical Electronics, INC. Aloha, Oregon, USA) before all procedures. All measurements were taken from cranial tibial artery. The cuff width was approximately 40% of the limb circumference. After the hair over the artery was clipped, the cuff was inflated till the blood flow no longer was heard. The cuff was then gradually deflated. The point where the blood flow could be first detected was systolic pressure. (Acierno and Labato, 2005; Brown et al. 2007; Henik et al., 2005).

Blood gas analysis: 1 ml of blood were collected from femoral artery into a blood-gas specific syringes that included lithium heparin as anticoagulant. The samples were analyzed (Irma Trupoint blood gas analyzers, ITC, USA) immediately after the collection (Batemen, 2008).

Renal Doppler Ultrasonography: Ultrasonography and Doppler measurements (Terason 2000, Samsung, China) were performed by the same person before the treatment. A multiconvex prob with 5 or 7 MHz was used. Renal Doppler measurements were obtained from the renal, interlobar, or arcuate arteries. After hair clipping, an acoustic gel was applied to the skin. Different transducers and frequencies were used depending on animal weight. A morphometric examination was performed and renal length and width were measured on the longitudinal axis.

Color Doppler was used to visualize the intrarenal vasculature. Sample volume was positioned in the middle of the renal vessels and the insonation angle did not exceed 60° after correction (Figure 1). The Doppler examination required more than 10 minutes in most of the dogs because of the movement or tachypnea.

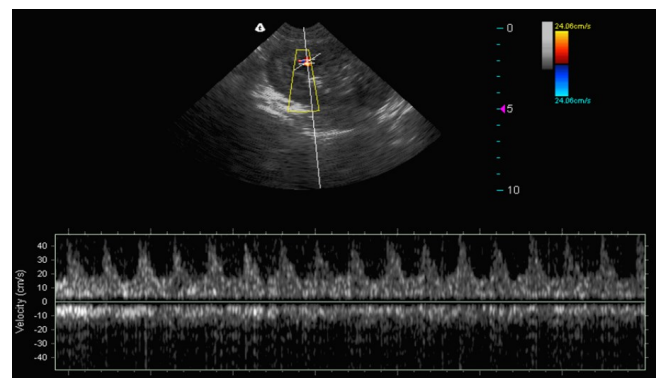


Figure 1: Doppler ultrasonography of a dog with chronic renal failure

Parameters of blood flow velocity such as systolic peak velocity (PS), end diastolic peak (ED), and time average maximum velocity (TMV), as well as hemodynamic parameters such as resistive index (RI) and systolic-diastolic ratio (S/D) were calculated automatically by the ultrasound device.

Pulsatility index (PI) was calculated manually according to on the formula below (Novellas et al., 2007). $PI = (\text{peak systolic velocity}) - (\text{end diastolic velocity}) / (\text{time average maximum velocity})$.

Statistical Analysis: A commercial software package (SPSS10.0) was used to analyze data. Independent samples t-test was applied to compare the changes in dogs with CRF and healthy dogs. Pearson correlation analyses were made to determine a significant correlation between UPC and renal RI and PI values. Level of significance was set at $P < 0.05$.

Results

A total of twenty-six dogs with previously diagnosed with chronic renal failure and twenty healthy dogs were included in our study. The majority of healthy dogs were under the age of 5, while the majority of dogs with CRF were over 10 years old. Vomiting (22), anorexia (16), polydipsia (12), polyuria (11), weakness (10), weight loss (8), diarrhea (6), reduction in the amount of urine (5), reduction in the amount of water drinking (5), wounds in the mouth (2) and halitosis (2) were revealed symptoms in dogs with CRF.

The mean RI and PI values of renal arteries were detected as 0.54 ± 0.01 and 1.3 ± 0.04 in healthy dogs, respectively. The same indices were found as 0.68 ± 0.02 and 3.4 ± 0.4 in patients with CRF, respectively (Table 1).

Among these dogs suffering from CRF, 10 of them

Table 1: Comparison of intrarenal Doppler measurements

Parameters	Healthy dogs	Dogs with CRF
PS	13.3 ± 1.5	$21.7 \pm 2.2^{**}$
ED	6.2 ± 0.9	6.7 ± 1.0
SID	2.3 ± 0.2	5.2 ± 1.5
RI	0.54 ± 0.01	$0.68 \pm 0.02^{***}$
FV	7.2 ± 3.6	4.6 ± 1.6
TMV	5.6 ± 0.6	5.2 ± 0.8
PI	1.3 ± 0.04	$3.4 \pm 0.4^{***}$

** $p < 0.01$, *** $p < 0.001$, PS = Peak systolic velocity, ED = End diastolic velocity, SID = Systolic/diastolic velocity, RI = Resistive index, FV = Follow volume, TMV = Time average maximum velocity, PI = Pulsatility index.

showed increased intrarenal RI values. Additionally, 21 dogs which also include the patients with high RI values displayed PI values higher than reference limits. The mean values of intrarenal RI and PI in dogs with CRF found significantly higher than control group ($P < 0.001$). The sensitivity and the specificity of RI were found 35% and 99%, respectively.

When the dogs with CRF evaluated in terms of blood pressure; 15 of our cases had minimal risk, 2 had low risk, 8 had moderate risk and 1 had high grade risk. Three low-grade dogs and one dog with high-grade blood pressure had grade 4 renal failure. 14% of the intermediate dogs and 40% of the minimal -grade dogs exhibited were diagnosed with third-grade renal failure. In the present study, there was no statistical significance between the systolic blood pressures of healthy dogs and dogs with CRF. When diastolic blood pressure measurements were compared between groups, no statistical significance

Table 2: Comparison of diastolic blood pressure in healthy dogs and dogs with Chronic renal failure

Healthy dogs	Dogs with CRF
92.3 ± 3.4	89.7 ± 1

CRF = Chronic renal failure

was found (Table 2). However, when blood pressure measurement methods were compared with GLM analysis, it was determined that Doppler method was better than oscillometric method (Table 3).

Table 3: Comparison of Doppler and oscillometric blood pressure measurement methods between healthy and chronic renal failure dogs

	Blood pressure
Method	
Doppler	141.6 ± 2.87
Oscillometric	129.7 ± 2.87
	*
Health status	
Healthy	131.7 ± 2.96
Dogs with CRF	139.7 ± 2.79
	NS
Overall average	135.7 ± 2.03

*: $p < 0,01$, NS = Non significant, CRF = Chronic renal failure.

In dogs with CRF, pH and total carbon dioxide (TCO₂) values were decreased, while base excess (Beb) values were statistically significant at P<0.001 level. Although serum iCa level was within normal limits, a significant decrease was observed when compared to healthy group (P<0.001). Although the serum K value was found to be within normal limits in dogs with CRF, it was statistically significant increased in dogs with CRF (P<0.01). Decrease in bicarbonate (HCO₃) and increase in partial pressure of oxygen (PO₂) were statistically significant compared to P<0.01 level. Also, a statistically significant decrease in partial pressure of carbon dioxide (PCO₂) and total haemoglobin (tHb) values was determined (P<0.05) (Table 4).

Table 4: Comparison of blood gases in healthy dogs and dogs with chronic renal failure

	Healthy Dogs	Dogs with CRF	Reference values
pCO ₂	33 ± 1.3	28.4 ± 1.7*	30.8 – 42.8
PO ₂	100.7 ± 6.6	129.6 ± 6**	80.9 – 103.3
Hct	41.5 ± 3.2	38.7 ± 1.3	40.3– 60.3
Na	146.9 ± 0.7	146.5 ± 2.9	150 – 165
K	4.1 ± 0.1	4.9 ± 0.2**	3.5 – 5.8
iCa	1.4 ± 0.02	1.2 ± 0.05***	1.2-1.5
HCO ₃	21.1 ± 0.7	15.7 ± 1.4**	18.8 – 25.6
TCO ₂	22.2 ± 0.7	15.5 ± 1.3***	22 ± 2
Beb	2.7 ± 0.4	-9.3 ± 1.3***	-2 – +2
O ₂ sat	96.4 ± 1.2	97.3 ± 0.7	93 – 100
tHb	15.5 ± 0.8	13.7 ± 0.4*	8 – 15

*p <0.05, **p<0.01, ***p<0.001, pCO₂ = Partial pressure of carbon dioxide , PO₂ = Partial pressure of oxygen, Hct = Hematocrit, Na= Sodium, K= Potassium, iCa = Ionized calcium TCO₂ = Total Carbon dioxide , Beb = Base excess, O₂sat= Oxygen saturation tHb = Total haemoglobin concentration.

Red Blood cell (RBC) and hemoglobin (HGB) values are known to be important in dogs with chronic renal failure (P <0.001) and normocytic-normochromic type anemia developed in 20 dogs in our study. In the animals of the CRF group, when compared with the healthy group, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values remained within normal range, however mean corpuscular hemoglobin concentration (MCHC) were significantly decreased in dogs with CRF (Table 5).

Blood urea and creatinine levels increased in all dogs with CRF (P<0.001). The calcium (Ca) level was found to be elevated in 3 patients however decreased

Table 5: Complete blood count in healthy dogs and dogs with chronic renal failure

Parameters	Healthy Dogs	Dogs with CRF	Reference Values
RBC (X 10 ⁶ / μL)	6.4 ± 0.2	5.4 ± 0.2**	5.5-8.5
HGB (g/dL)	15.5 ± 0.6	12.3 ± 0.7**	12-18
HCT (%)	39.9 ± 1.4	35.3 ± 2	37-55
WBC (X 10 ³ /μL)	13.2 ± 0.7	15.8 ± 1.5	6-17
PLT (X 10 ³ /μL)	282.4 ± 17.3	300.5 ± 34.8	200-500
MCV (fL)	61.8 ± 0.6	65.1 ± 1*	60-77
MCH (pg)	21.4 ± 1.4	23.2 ± 0.5	19.5-26
MCHC (%)	37.1 ± 0.7	35 ± 0.6*	32-36

** p<0.01, *** p<0.001, CRF = Chronic renal failure, RBC = Red blood cell, HGB, = hemoglobin, HCT = Hematocrit, WBC = White blood cell, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration.

Table 6: Serum biochemistry in healthy dogs and dogs with chronic renal failure

Parameters	Healthy Dogs	Dogs with CRF	Reference Values
Glucose (mg/dl)	129 ± 2.3	129.2 ± 5.4	60-125
Urea (mg/dl)	43.9 ± 2	310.8 ± 31*	7-27
Creatinine (mg/dl)	1.2 ± 0.03	7.7 ± 0.8*	0.4-1.8
AST (IU/L)	34.4 ± 2.5	47.3 ± 6	5-55
ALT (IU/L)	34.8 ± 5.3	50 ± 6	5-60
T. Protein (mg/dl)	6.5 ± 0.2	7.5 ± 0.6	5.1-7.8
Albumin (g/dl)	3.5 ± 0.1	2.6 ± 0.1*	2.6-4.3
Ca (mg/dl)	8.9 ± 0.2	8.6 ± 0.4	7.5-11.3
P (mg/dl)	3.8 ± 0.06	10.9 ± 1.3*	2.1-6.3

* p<0.001, CRF = Chronic renal failure, ALT = Alanine aminotransferase, AST = aspartate aminotransferase, Ca = Calcium, P = Phosphorus.

Table 7: Comparison of urea, creatinine, Resistive index and Pulsatility index values

	Creatinine	RI	PI
Urea	0.787**	0.613**	0.457**
Creatinine	-	0.432*	0.399*
RI	-	-	0.787**

*: p<0,01, **: p<0,001, RI = Resistive index, PI = Pulsatility index.

Discussion

in 6 patients. The blood phosphorus (P) level was found to be statistically higher at the level of $P < 0.001$ compared to the control group. Albumin levels were significantly decreased at $P < 0.001$, but this decrease was within normal limits (Table 6). CRF is one of the most important causes of morbidity and mortality in dogs (McGrotty, 2008). Clinical evaluation of renal blood flow gives important information about diagnosis, treatment and prognosis of the disease (Morrow et al., 1996). Many diagnostic methods such as physical examination, CBC and biochemical blood examination, urinalysis, radiography and ultrasonography are used in the detection of renal damage. While there are many studies in humans about renal Doppler, blood pressure and blood gases, which are helpful diagnostic methods for the diagnosis, and better prognosis of the disease, we found that there are few studies including all of them in dogs.

As the first changes in renal diseases begin with the change in blood flow, renal Doppler ultrasonography, which is rarely used in veterinary practice is one of the most important diagnostic tools (Bragato et al., 2017). Previous studies have shown many normal RI values. Although different RI intervals are specified one by one, the RI value is considered abnormal when it exceeds 0.70 (Novellas et al., 2008; Mitchell et al., 1998; Morrow et al., 1996). RI and PI above the upper limit indicate the presence of renal disease (Bragato et al., 2017). The mean intrarenal RI value of healthy dogs was 0.54 ± 0.01 in our study. This value is within the normal limits reported by the researchers. In dogs with CRF, the mean RI value was 0.68 ± 0.02 . This value is lower than the normal value reported by Morrow et al. (1996) and Novellas et al. (2008). However, the RI value was found statistically increased at $P < 0.001$ when compared with healthy dogs. On the other hand, in 10 cases, the RI value was above normal limits in dogs with CRF. The changes in RI values may be affected by many external factors such as pressure, hypotension, digestion, and heart rate (Choi et al., 2003; Morrow et al., 1996; Szatmari et al., 2001). However, not all forms of renal failure may change the vessel resistance to the same extent. It has been observed that increased RI in glomerular diseases is rare in both human and dog studies. In addition, the elevation of intrarenal RI may occur in both tubulointerstitial and glomerular diseases, however it is not useful in differential diagnosis of these situations (Morrow et al., 1996; Rivers et al., 1997). In a study comparing RI value to histopathological findings in dogs with glomerular disease, increased RI levels were obtained in patients

with interstitial nephritis or tubular degeneration (Morrow et al., 1996).

Another important parameter used in renal Doppler measurements is PI value. PI value above 1.52 is reported to be abnormal (Novellas et al., 2008, Morrow et al., 1996). In our study, we determined that the measurements in healthy dogs were within normal limits. This value was increased in dogs with CRF. In addition, compared to healthy dogs, the increase in dogs with CRF was statistically significant at $P < 0.001$ level. Despite the more frequent use of RI value, PI is more sensitive in determination of abnormalities. Hence PI value is not affected by external factors and it is taken into account at the average rate when determining PI value (Mitchell et al., 1998, Novellas et al., 2008). Similar to the report by Novellas et al. (2008), we found that PI is more sensitive than RI. Renal PI was found increased in 21 of the dogs with CRF, whereas only 10 of them had increased renal RI in our study. High positive correlation was found between these two parameters ($r = 0.787$).

A positive correlation between creatinine levels and RI and PI was reported in a study on patients with CRF in human medicine (Peterson et al., 1997). In addition, Baltazar et al. (2016) and Torroja (2007), found a positive correlation between these parameters in cats and dogs. In the study of dogs managed in 1996, renal diseases were compared with the RI value and it was reported that there was no correlation between RI value and urea and creatinine (Morrow et al., 1996). Rivers et al. (1997), supported this finding in their study. In this study, a positive correlation between the RI value ($r = 0.432$) and PI value ($r = 0.399$) with creatinine was found. Similarly RI and PI positively correlated with serum urea level (respectively, $r = 0.613$ and $r = 0.457$) (Table 7).

In order to determine the availability of intrarenal RI values in CRF dogs, sensitivity and specificity were also calculated in this study. For this reason, 0.70 was selected as normal upper limit similar to the study of Morrow et al. (1996), where the specificity and the sensitivity were 36% and 96% respectively. Our results were similar to the study of Morrow et al. (1996).

Hypertension is the common sequela of renal failure (McMurphy et al., 2006; Henik et al., 2005; Uzlu and Kalınbacak, 2005). It is concluded from previous studies that 50% to 93% of canine patients with renal disease are affected from hypertension (Acierno and Labato, 2005). Similarly, 42% of dogs with CRF had high blood pressure in our study.

Kidneys, have an important role in acid-base balance regulation. Normally, daily H⁺ charge is excreted in the urine by NH₃ or NH₄ or by the conversion of the phosphate into H₂PO₄. When CRF is formed, total H⁺ excretion is impaired. Thus, less H⁺ is excreted in the kidneys and HCO₃ is not reabsorbed enough, acidosis occurs in the animal (Morais et al., 2008). In our cases, statistically significant decrease in pH value at P<0.001 level was found; which was similar to previous studies. The characteristic laboratory finding of metabolic acidosis are the reduction of the plasma bicarbonate level and the reduction of the base. In our study, HCO₃ was determined as P<0.01 and BE value was statistically lower than P<0.001. In this case, all of these show parallelism with previous studies (Bartges, 2012).

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