

# Glomerular and Tubular Markers in Dogs with Chronic Kidney Failure and Healthy Control Dogs

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## Abstract

The study aimed to examine the changes in two glomerular markers (uALB, uCRP), as well three tubular markers (uGGT, uALP, and uLDH) in dogs with Chronic Kidney Failure (CKF) and to compare the changes to healthy control dogs. Fourteen dogs with CKF of different age, breed and sex were included in the study as well 10 healthy control dogs. The diagnosis was based on physical examination and laboratory findings. Urinary ALB/Cr was significantly higher in CKF dogs than in healthy control dogs. No significant increase in urinary LDH/Cr, uGGT/Cr and uAF/Cr was found in CKF dogs than in healthy control dogs. No significant difference was detected for uCRP, which was not detectable in control dogs and only in 4 of the CKF dogs. The studied glomerular marker (uALB) in dogs with CKF was significantly increased compared to healthy control dogs. Urinary CRP, uLDH, uGGT and uAF cannot be reliable indicators for CKF in dogs.

**Keywords:** *chronic kidney failure, dogs, glomerular markers, tubular markers*

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## INTRODUCTION

Chronic kidney failure (CKF) is a frequent disease in carnivores. Regardless of causes leading to loss of nephrons, CKF is characterised with irreversible kidney structure damage (Fine et al., 1998) and is a primary cause for high mortality (Smets et al., 2010). It is believed that the syndrome is more likely to occur in adult animals and that its incidence increases with age (Polzin et al., 1989). Routine diagnostic tests (serum creatinine - SCR and blood urea nitrogen - BUN) detect the disease at a rather late stage of development, when more than 75% of functioning nephrons are irreversibly damaged (Chew and DiBartola, 1989; Smets et al., 2010). Early diagnostics permits adopting a proper therapeutic approach to prevent the further progression of CRF.

Unlike conventional tests, in the recent years, more attention has been paid to the urinary proteins and enzymes as early markers of kidney injury in dogs. According to Clemon (1998), urinary markers have the potential to determine the localization and severity of renal lesions in different parts of

the nephron. Glomerular dysfunction leads to the appearance of intermediate molecular weight (IMW, albumin) protein in the filtrate and, at a more advanced stage - to high molecular weight (HMW, e.g. CRP) proteins (D'Amico and Bazzi, 2003; Maddens et al., 2010). Grauer et al. (2002) determine microalbuminuria (albumin from 1 to 30 mg/dl), as the earliest indicator of glomerular impairment in dogs.

Recently, two studies have assessed urinary CRP (uCRP) and CRP-to-creatinine ratio (uCRP/Cr) in dogs with pyometra and chronic renal failure, as indicators of glomerular damage (Maddens et al., 2010; Smets et al., 2010).

Tubular dysfunction is manifested by release of low molecular weight (LMW) proteins or urinary enzymes (Frances and Clemon, 1998; Sato et al., 2002; Raila et al., 2003), in urine as they are secreted by damaged tubular cells. Urinary enzymes have proved their diagnostic value in the detection of acute tubular damage (Rivers et al., 1996), and some chronic renal disorders in dogs (Smets et al., 2010). Microalbuminuria and urinary

CRP in dogs with CKF have been performed in previous studies, but changes in urinary GGT, ALP and LDH have not been researched so far, which motivated us to perform this particular study.

**Aims and objectives.** The present study aimed to investigate two glomerular (uALB, uCRP), and three tubular markers (uGGT, uALP, and uLDH) in healthy dogs and dogs with spontaneous CRF.

## MATERIALS AND METHODS

**Dogs.** The investigation was carried out between June 2011 and December 2013. Most investigations were performed in the Small Animal Clinic, Faculty of Veterinary Medicine at the Trakia University, Stara Zagora. The study cohort consisted of 14 dogs with CKF from both sexes, all ages and breeds represented. The diagnosis was posed on the basis of clinical signs specific for CRF (polydipsia/polyuria, weight loss, lethargy, vomiting etc.), laboratory test findings (anaemia, azotaemia, hyperparathyroidism, hyperphosphataemia, hypocalcaemia etc.), urine specific gravity (USG) < 1.030 and UPC > 0.5. Cases with co-morbidities of any type were excluded. Ten clinically healthy dogs were used as controls.

**Laboratory Methods. Routine Urinalysis.** Urine samples were collected from all animals (experimental and control) through ultrasound-guided cystocentesis for analysis of: creatinine (uCr), total protein (uUP), albumin (uALB), lactate dehydrogenase (uLDH), gamma glutamyl-transferase (uGGT), alkaline phosphatase (uALP) and for bacteriological examination. The following urinary ratios were calculated: urinary protein to creatinine (UPC), urinary albumin to creatinine (uALB/Cr), urinary lactate dehydrogenase to creatinine (uLDH/Cr), urinary gamma glutamyl-transferase to creatinine (uGGT/Cr) and urinary alkaline phosphatase to creatinine (uAF/Cr).

Urine specific gravity (USG) was determined by refractometry. Routine urinalysis with test strips, uroculture and sediment light microscopy were also performed. Urine was centrifuged (450 rpm for 3 min), aliquoted and frozen at -80 °C for uCRP assay.

The analysis was performed within 30 min after sampling on an automated biochemical analyzer and commercial kits for determination of urinary creatinine (uCr), urinary lactate dehydrogenase (uLDH), urinary gamma glutamyl transferase (uGGT), urinary alkaline phosphatase (uALP) and urinary albumin (uALB). The analysis of CRP was performed at the University of Milan, Italy, on a microplate spectrophotometric reader with species-specific commercial ELISA kits.

**Statistical Analysis.** All numerical results were statistically processed by one-way analysis of variance – ANOVA (Statistica v. 7.0 for Windows, Stat Soft Ins., USA 2004).

## RESULTS AND DISCUSSION

Relevant clinical signs, primary haematological and blood biochemical parameters in dogs with CKF are listed in Table 1.

Blood biochemical analysis in 14 CKF dogs demonstrated that serum creatinine (sCr) and blood urea nitrogen (BUN) increased proportionally to the severity of the syndrome (Table 2). The average ratio urinary protein/creatinine (UPC) in healthy controls was  $0.29 \pm 0.13$  (0.109-0.45). In dogs with CKF observed ratios were considerably elevated –  $2.3 \pm 0.945$  ( $p < 0.001$ ) vs control animals. Statistically significant differences were also observed for urine specific gravity (USG).

Urinary ratios of albumin, C-reactive protein, lactate dehydrogenase, gamma glutamyltransferase and alkaline phosphatase to creatinine in dogs with CRF and healthy dogs are presented in

**Tab. 1.** Clinical signs, haematological and blood biochemical results in dogs with CKF (mean  $\pm$  SD, n = 14)

Signalment and clinical signs		Haematological and biochemical results (mean $\pm$ SD)		Reference range
Polydipsia-polyuria	12/14	(Hb), (g/l)	$101.87 \pm 20.12$	120-180
Weight loss	11/14	(Er), ( $10^{12}$ /L)	$4.49 \pm 0.90$	5.5-8.5
Anorexia	12/14	Hct, (%)	$30.66 \pm 6.36$	37-55
Lethargy	9/14	TP, (g/L)	$66.11 \pm 6.35$	54-78
Hypothermia	5/14	Alb, (g/L)	$25.33 \pm 1.82$	25-37

**Tab. 2.** Routine parameters of kidney function. Results from the laboratory analysis in healthy dogs (H, n=10), dogs with chronic kidney failure (CKF, n=14) (sCr, BUN, UPC and UPC, mean  $\pm$  standard deviation).

Parameter	H (n=10)	CKF (n=14)	Reference range
sCr ( $\mu$ mol/l)	87.30 $\pm$ 18.0256	476.14 $\pm$ 330.17 <sup>c</sup>	< 125
BUN(mmol/l)	5.11 $\pm$ 1.353	27.96 $\pm$ 10.29 <sup>c</sup>	3.3 – 8.3
UPC	0.29 $\pm$ 0.13	2.3 $\pm$ 0.945 <sup>c</sup>	< 0.5
USG	1.035 $\pm$ 0.006	1.011 $\pm$ 0.003 <sup>c</sup>	1.015-1.045

Note: <sup>ns</sup>p>0.05, <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001, – compared to healthy dogs (H)

**Tab. 3.** Urinary concentrations of renal biomarkers in healthy dogs (H, n=10), dogs with chronic kidney failure (CKF, n=14) (uAlb/Cr, uCRP/Cr, uLDH/Cr, uGGT/Cr, uAF/Cr, mean  $\pm$  standard deviation).

Biomarker	H (n=10)	CKF (n=14)
uAlb/Cr (mg/g)	13.57 $\pm$ 83.829	1919.72 $\pm$ 1092.4 <sup>c</sup>
uCRP/Cr (mg/g)	BDL (n=12)	(4/10)
uLDH/Cr (IU/l/mg/dl)	0.119 $\pm$ 0.095	0.197 $\pm$ 0.077
uGGT/Cr (IU/l/mg/dl)	0.141 $\pm$ 0.071	0.207 $\pm$ 0.104
uALP/Cr (IU/l/mg/dl)	0.096 $\pm$ 0.055	0.155 $\pm$ 0.074

Note: <sup>ns</sup>p>0.05, <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001, – compared to healthy dogs (H)

Table 3. It could be seen that uAlb/Cr increased obviously in dogs with renal failure attaining 1919.72 $\pm$ 1092.4 mg/g (93.04 – 4232.6 mg/g) (p<0.001) as compared to control values – 13.57 $\pm$ 3.829 mg/g.

Urinary C-reactive protein (uCRP) was not found in any of healthy controls. Four out of the 14 CKF dogs had increased urinary uCRP/Cr ratios on the background of small amounts varying between 0.01 and 0.06 mg/g.

As the renal function exacerbated, uLDH/Cr underwent an insignificant alteration. In CRF dogs, the ratio uLDH/Cr changed slightly – 0.197  $\pm$  0.077 IU/l/mg/dl (p>0.05) vs values in healthy controls – 0.119 $\pm$ 0.095 IU/l/mg/dl.

The other two ratios (uGGT/Cr, uAF/Cr) were irrelevantly higher in CKF dogs attaining average values of 0.207  $\pm$  0.104 IU/l/mg/dl (p>0.05) and 0.155  $\pm$  0.074 IU/l/mg/dl (p>0.05) as compared to healthy dogs – 0.141  $\pm$  0.071 IU/l/mg/dl и 0.096  $\pm$  0.055 IU/l/mg/dl respectively.

In this study, tested glomerular markers were substantially increased in dogs with CRF than in healthy controls. Increased uAlb concentrations

and the detection of uCRP in some CKF dogs clearly indicated that the chronic renal disease had a negative effect on nephrons on a glomerular level. The detection of CRP in the urine of 4 out of 14 CKF dogs is an interesting finding pointing out at the fact that the glomerular membrane damage was so important that not only intermediate molecular weight proteins (uAlb), but also high molecular weight molecules such as CRP could penetrate through it (Smets et al., 2010). The advantage of glomerular markers compared to routine renal parameters is their ability to detect glomerular disease at an earlier stage (Maddens et al., 2010).

The inflammatory response resulting in increased plasma concentrations and transport of CRP through the damaged glomerular barrier was implicated by Smets et al. (2010) as a possible cause for the increased uCRP/Cr in some dogs with CKF. Maddens et al. (2010) established a considerable loss of Alb and CRP with urine in dogs with pyometra, suggesting uCRP as a glomerular parameter of unique diagnostic value.

According to IRIS proteinuria is a pathological process with UPC values over 0.5. In this study, the

levels of the studied parameter were considerably elevated as well as values of glomerular markers indicating significant differences between healthy and CRF animals. Furthermore, glomerular biomarkers (uAlb, uCRP) provide information about the origin of proteinuria, which is not possible with UPC.

In our study in CKF dogs, uLDH/Cr, uGGT/Cr and uALP/Cr ratios were insignificantly higher vs those in healthy control animals, supporting results from previous research (Heiene *et al.*, 1991; Rivers *et al.*, 1996; Frances and Clemo, 1998). In another study of experimental gentamicin nephrotoxicosis in dogs, uAF/Cr and uGGT/Cr increased rapidly attaining statistically significant higher values within 7 to 9 days after the beginning, with slow restoration of uGGT/Cr afterwards but not of uAF/Cr, which continued to increase until the 15<sup>th</sup> day (Maden and Aslan, 1999). Clemo (1998) established increase in levels of several urinary enzymes (uLDH, uGGT, uNAG and uAF), associated with acute proximal tubular necrosis, whereas the restoration of baseline levels was attributed to regeneration of kidney damage as also supported by microscopic findings showing proximal tubular regeneration. Similar was the thesis of Heiene *et al.* (1991), affirming that in dogs with spontaneous renal disease, normal uGGT or uAF values should be associated to chronic kidney disease whereas the increased uALP activity – as a sign of acute tubular damage.

## CONCLUSION

The established insignificant changes in the activities of urinary enzymes in dogs with chronic kidney disease confirmed existing information about relevant diagnostic value of enzymuria in acute tubular damage and its inconsistent value in cases of chronic renal damage. For detailed validation of diagnostic value of canine urinary enzymes, further research in experimental models of chronic nephrotoxicity is necessary.

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