Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.100

RESEARCH ARTICLE

Alteration of Serum Cystatin-C Levels after Hemodialysis in Dogs with Kidney Disease

Woonchan Ahn[1,2], Tae Hyeong Kim[3], Taeho Lee[2], Jin-Ok Ahn[1], Jung Hoon Choi[3] and Jin-Young Chung[1,*]

[1]Department of Veterinary Internal Medicine and Institute of Veterinary Science, College of Veterinary Medicine, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon-si, 24341, Gangwon-do, South Korea
[2]Smart animal hospital Sinsa, Seoul, 06026, Korea; [3]Department of Veterinary Anatomy, College of Veterinary Medicine, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon-si, 24341, Gangwon-do, South Korea
*Corresponding author: jychung77@gmail.com

ARTICLE HISTORY (20-306)

Received:	June 13, 2020
Revised:	November 16, 2020
Accepted:	November 24, 2020
Published online:	December 10, 2020

Key words:
Cystatin-C
Dog
Hemodialysis
Kidney disease
Middle-molecular-weight proteins

ABSTRACT

Kidney diseases are common life-threatening diseases in small animals. To evaluate the efficiency of hemodialysis in kidney disease treatment, several low-molecular-weight biochemical markers can be assessed. Recently, middle-molecular-weight proteins such as cystatin-C have been investigated for use as alternative biomarkers to evaluate renal function and predict mortality, especially in human hemodialysis patients. The goal of this study is to assess changes in cystatin-C levels when performing low-flux hemodialysis with an ultraflux dialyzer in dogs with kidney disease. This was a retrospective study. A total of twenty hemodialysis sessions were included in the study. The concentrations of cystatin-C level before and after hemodialysis were determined by examining ELISA results. The cystatin-C concentration was significantly lower after hemodialysis than before hemodialysis (P=0.0016). Cystatin-C can be removed by using low-flux hemodialysis. Thus, cystatin-C can be considered a potential kidney function biomarker in dogs with kidney disease being treated with low-flux intermittent hemodialysis.

To Cite This Article: Ahn W, Kim TH, Lee T, Ahn JO, Choi JH and Chung JY, 2020. Alteration of serum cystatin-c levels after hemodialysis in dogs with kidney disease. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2020.100

INTRODUCTION

Kidney diseases such as acute kidney injury (AKI) and chronic kidney disease (CKD) are common life-threatening diseases in small animals (Ettinger and Feldman, 2010). In recent decades, extracorporeal renal replacement therapy (ERRT) has been considered an effective treatment choice for patients with kidney diseases that fail to respond to medical management (Ettinger and Feldman, 2010; Cowgill, 2011). ERRT may take the form of peritoneal dialysis, intermittent hemodialysis (IHD), or continuous renal replacement therapy (CRRT). IHD has been the most commonly used ERRT in veterinary medicine, but currently, CRRT is preferred because of its low blood flow rate (Diehl and Seshadri, 2008). Hemodialysis (HD) is considered an effective way to remove urea, toxins, especially low-molecular-weight solutes and overhydrated fluids (Bloom and Labato, 2011).

To evaluate the efficiency of HD in renal function, several low-molecular-weight biochemical markers may be measured, including blood urea nitrogen (BUN, molecular weight 60 Da) and creatinine (Crea, molecular weight 113 Da) (Polzin, 2013). However, BUN and Crea are influenced by extra-renal factors including subject muscle mass, diet, and nutritional status. Crea measurement is not suitable for small animals, especially toy breed dogs and cats that weigh under 10 kg, because Crea levels are affected by muscle mass, which in small animals is similar to that of pediatric human patients. Crea levels increase with body weight and may decrease in cachectic dogs (Braun *et al.*, 2003). Therefore, the Crea level can be underestimated in small breed dogs and cats. Moreover, Crea levels do not increase until the presence of approximately 75% nephron function damage.

Several years ago, middle-molecular-weight proteins (MMWP), such as beta-2-microglobulin (β2m) and cystatin-C (CysC), were investigated as alternative biomarkers for the evaluation of renal function and the prediction of mortality, especially in human HD patients (Okuno *et al.*, 2007; Shahjahan *et al.*, 2011; Shafi *et al.*, 2016; Wong *et al.*, 2016; Barton *et al.*, 2018). In veterinary fields, CysC has been studied as a renal prognostic biomarker to assess of renal function and

glomerulus filtration rate (GFR) (Almy *et al.*, 2002; Ghys *et al.*, 2016; Iwasa *et al.*, 2018). But there is no study of CysC during HD session in dogs or cats.

The CysC protein is a middle-molecular-weight (molecular weight 13.4 kDa) protease inhibitor within the cystatin family. Its major function is to control inflammation by inhibiting lysosomal protease activity (Filler *et al.*, 2005). CysC is considered a better indicator of the GFR than Crea. Moreover, CysC has been reported to be unaffected by non–renal factors (e.g., sex, age, body weight, or muscle mass) (Vilar *et al.*, 2015). The CysC protein has characteristic features that include production at a constant rate by nucleated cells, reabsorption and metabolism by proximal tubular cells, and removal from the body by glomerular filtration (Filler *et al.*, 2005). Based on those traits, it is suggested that CysC may act as a good marker of the removal of MMWP toxins.

Removing and monitoring MMWPs such as CysC is very important because the serum MMWP level is an important predictor of mortality in HD patients. Middle-molecular-weight solutes, including CysC, are more effectively removed by high-flux dialysis modalities that perform both convective and diffusive therapies, such as on-line hemodiafiltration (O-HDF) and hemofiltration (HF), than by low-flux dialysis modalities, such as HD, that only perform diffusive therapies (Palmer *et al.*, 2012; Roumelioti *et al.*, 2018). High-flux dialysis is a more suitable way to remove these types of uremic toxins; however, high-flux dialysis requires a blood flow rate that is too fast for use in pediatric human patients or small breed dogs or cats that weigh under 10 kg. Thus, other than low-flux dialysis, there are no suitable therapy options for small breed dogs and cats.

Recently, several studies reported on CysC kinetics in human patients receiving hemodialysis (Krishnamurthy *et al.*, 2010; Vilar *et al.*, 2014; Fadel *et al.*, 2017). Some studies revealed that CysC was effectively removed by high-flux HD or HF (Vilar *et al.*, 2014; Fadel *et al.*, 2017). Conversely, other studies have reported that CysC levels were higher after low-flux HD than before the treatment (Krishnamurthy *et al.*, 2010; Marsenic *et al.*, 2013). To date, alteration of CysC levels during HD has not been reported in veterinary medicine.

The goal of this study is to describe the alternation of CysC in HD performed in small dogs using a CRRT system with an ultraflux dialyzer. The results should help determine the suitability of using CysC as a renal biomarker when monitoring HD efficacy.

MATERIALS AND METHODS

This study was a retrospective pilot study based on small dogs with kidney diseases. Informed client consent was obtained prior to enrollment in the study. A total of twenty HD sessions were included in the study. The inclusion criteria required that the dogs had a kidney disease such as AKI or CKD. Dogs that had AKI caused by toxicosis were excluded to eliminate influences of nephrotoxin on kidney. Diagnostic work-up data, obtained from hematological examinations using a VETTEST 8008 reader (IDEXX Laboratories Inc., Westbrook, Maine, USA), included BUN and creatinine measurements.

The HD sessions were performed on Fresenius multifiltrate systems (Fresenius Medical Care AG & Co. KGaA, Bad Homburg, Germany) using polysulfone dialyzers (Ultraflux® AV paed, Fresenius Medial Care AG & Co. KgaA, Bad Homburg, Germany) and the pediatric continuous venovenous hemodialysis (CVVHD) mode. Dialysis access via the right or left jugular vein was obtained by using 7 Fr- or 8 Fr-sized two-lumen central venous catheter sets with blue catheters (Blue FlexTip® ARROWgard® Blue catheter, Arrow international, Inc. PA, USA). Blood flow was maintained at 2–3 mL/kg/min in the first 30 min, then increased to 3–5 mL/kg/min (minimum 14 mL/kg/min) for the remainder of the HD session. MultiBic® dialysate (Fresenius Medical Care Deutschland GmbH, Wendel, Germany) was used and its flow rate (300–1200 mL/h) was determined by considering the urea reduction rate and the treatment time. The selected ultrafiltration rate was decided after assessing the patient's hydration state, urine output, and concurrent disease status. The HD treatment durations ranged from 180–300 min. A 5 IU/mL dose of heparinized (HEPARIN inj. JW pharmaceutical, Seoul, Korea) saline was used as the anticoagulant agent for priming the HD circuit. A 150 IU/kg dose of Dalteparin (Fragmin inj. Pfizer Ltd. NY, USA) was injected subcutaneously before treatment and three times a day during hospitalization.

From the 20 HD sessions, 40 blood samples were collected. Blood samples were collected before (pre-HD $n = 20$) and after the end (post-HD $n = 20$) of the sessions. All blood samples were taken from peripheral veins to reduce the risk of underestimating solute concentrations due to vascular access recirculation during the HD session. Blood samples were collected in plain tubes and left at room temperature for 30 min before centrifuging for 5 min at 2500 r/min to obtain serum. All samples underwent serum BUN and serum Crea measurements using a VETTEST 8008 reader.

To determine the concentration of CysC, each sample underwent ELISA analysis using the Cystatin C Canine ELISA kit (RD491009100R, BioVendor, Laboratorni medicina a.s., Brno, Czech) in accordance with the manufacturer's recommendation. Microplates were read by SpectraMax ABS plus microplate readers (Molecular Devices, LLC., CA, USA) and the plates were scanned at 450 nm and 630 nm (reference wavelengths). SoftMax Pro Data Acquisition and Analysis Software (Molecular Devices, LLC., CA, USA) was used to determine analyte concentrations. The concentrations of the samples were calculated based on a standard curve using a four-parameter algorithm.

Calculation of each value's reduction rate was calculated as a percentage based on the pre- and post-HD levels using the equation: Reduction rate = [(pre-HD level – post-HD level) / pre-HD level] × 100.

Statistical analyses of all data were performed using the GraphPad Prism (GraphPad software, CA, USA) statistical analysis software. A Wilcoxon signed-rank test was used to assess changes in BUN, Crea, and CysC levels pre- and post-HD. The level of significance was set at $P<0.05$. Correlation analysis was performed and Spearman's correlation coefficients and corresponding *p* values were determined.

RESULTS

Patient characteristics are presented in Table 1. All dogs in the study had kidney disease and had undergone several IHD sessions. Total 20 HD modalities and 40 blood samples (20 samples pre-HD and 20 samples post-HD) were examined. The BUN, Crea, and CysC levels were significantly lower post-HD than pre-HD (P<0.05, Table 2 and Fig. 1); BUN (pre-HD 86.75±52.71 mg/dL and post-HD 41.15±28.29 mg/dL, P<0.0001), Crea (pre-HD 3.86±2.53 mg/dL and post-HD 2.06±1.11 mg/dL, P<0.0001), and CysC (pre-HD 5.82±3.27 ng/mL and post-HD 3.35±1.99 ng/mL, P=0.0016).

The reduction rates for each parameter are as follows: BUN 52.02±9.61%, Crea 43.58±12.22%, CysC 36.98±23.82% (Fig. 2). Comparison of the reduction rates revealed that there was a significant correlation between the BUN and Crea reductions (r = 0.6215, p = 0.0034), but there were no correlations between the Crea and CysC reductions (r = –0.03612, p = 0.8798) or between the BUN and CysC reductions (r = 0.1218, p = 0.6090) (Table 3).

DISCUSSION

There have been several studies conducted to search out alternative biomarkers that would be useful in evaluating renal function and predicting mortalities, especially in human HD patients, because of the limitations of using low-molecular-weight proteins (e.g., BUN and Crea) as biomarkers (Barton *et al.*, 2018; Shafi *et al.*, 2016; Wong *et al.*, 2016; Shahjahan *et al.*, 2011; Okuno *et al.*, 2007).

Numerous studies have indicated that CysC is suitable for evaluating GFR and residual kidney function in human HD patients (Vilar *et al.*, 2014; Shahjahan *et al.*, 2011; Filler *et al.*, 2005). In addition, CysC level has been correlated with the GFR in dogs, and that correlation was not influenced by non-kidney disease status (Wehner *et al.*, 2008; Almy *et al.*, 2002). However, some authors have reported that CysC levels are higher after low-flux HD (Marsenic *et al.*, 2013; Krishnamurthy *et al.*, 2010) and the reduction rate in high-flux HD was higher than that in low-flux HD (Fadel *et al.*, 2017). Thus, in high-flux HD sessions, CysC may be good marker for assessing the efficacy of the HD session and has potential for use as an index of middle-molecule toxin clearance (Fadel *et al.*, 2017).

However, there is no other option than low-flux HD for small dogs. Due to their low body weight, similar to that of human children patients, they cannot endure the high blood flow rate and large extracorporeal volume necessary when using high-flux HD. For that reason, CRRT systems, which can be run at a low blood flow rate and with a small extracorporeal volume, are used for low-flux IHD in veterinary medicine.

There are no previous reports on CysC alternation during HD, neither from high-flux nor low-flux studies, in veterinary medicine. Our study is the first to evaluate the alternation of CysC during low-flux HD in dogs. We observed that CysC can be removed during low-flux HD when using an ultraflux dialyzer. The removal of CysC was not correlated with that of the smaller molecular weight proteins BUN and Crea. Also, the CysC reduction rate was lower than those for BUN and Crea.

Table 1: Characteristics of each hemodialysis session (n = 20).

Blood flow rate	30±8.26 ml/min
Dialysate flow rate	1002±324.16 ml/hr
Ultrafiltration rate	14±10.21 ml/hr
Dialysis time	227±44.97 min
Patient Age	11.44±3.00 yr
Patient Body Weight	4.58±2.21 kg
AKI/CKD stage	3.45±0.73

Mean±standard deviation.

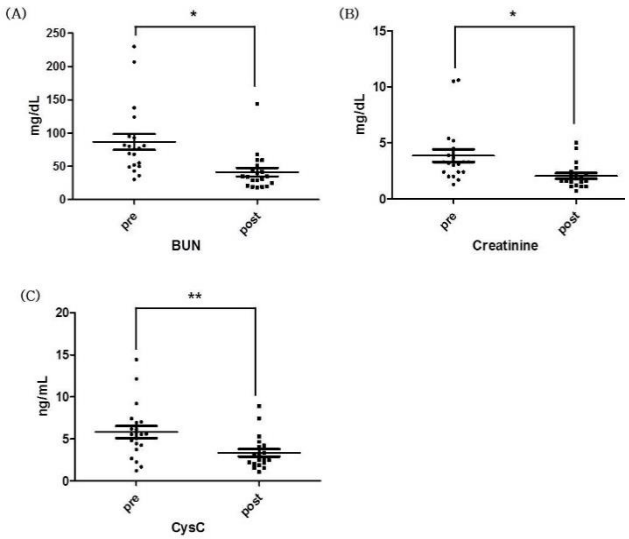

Fig. 1: Serum levels of (A) BUN, (B) Crea, (C) CysC at before (pre) and after (post) hemodialysis (HD). The post-HD level of each parameter was significantly lower than the pre-HD level (P<0.05). *P<0.0001, **P=0.0016.

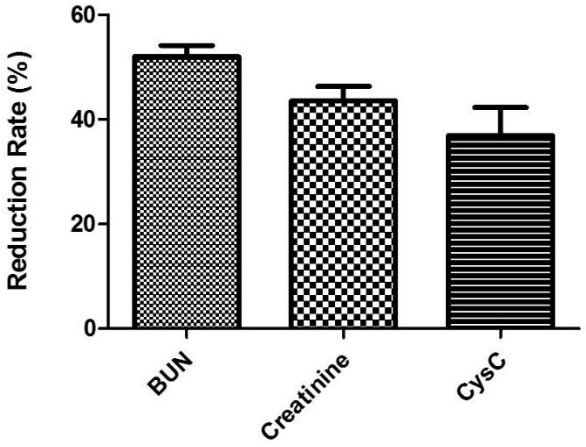

Fig. 2: Comparison of the reduction rates of BUN, Crea, and CysC. The reduction rate of CysC is slightly lower than the others.

As mentioned, in some studies the level of, CysC increased after low-flux HD. In contrast, CysC had a tendency to decrease after low-flux HD in our study. Differences in species studied or differences in dialyzers, systems, and modalities might have affected the results of these studies. In our study, we used a polysufone-based ultraflux dialyzer that can remove relatively large molecules (up to approximately 30 kDa) and provide a high sieving coefficient, resulting in a high filtration rate (Tattersall, 2007). Also, in this study, we used CRRT systems for our veterinary patients due to their lower blood flow rate than those of common IHD machines.

Table 2: Comparison of BUN, Crea, and CysC results before and after HD

	BUN			Crea			Cystatin-C		
	Pre	Post	*p*	Pre	Post	*p*	Pre	Post	*p*
Mean	86.75± 52.71	41.15±28.29	<0.0001	3.86±2.53	2.06±1.11	<0.0001	5.82±3.27	3.35±1.99	0.0016
RR	52.02%±9.61			43.58%±12.22			36.98%±23.82		

Mean±standard deviation, RR = Reduction rate.

Table 3: Correlations of reduction rates among BUN, Crea, and CysC

	BUN vs Crea		Crea vs CysC		BUN vs CysC	
	r value	*p* value	*r* value	*p* value	*r* value	*p* value
Value	0.6215	0.0034	-0.03612	0.8798	0.1218	0.6090
Correlate	Yes		No		No	

When using the CVVHD modality, a CRRT system can provide a small amount of ultrafiltration via convection, which can remove fluids and larger molecules. The results showing that an MMWP such as CysC can be removed effectively by using low-flux HD indicate that middle-molecule toxins could also be removed by such an approach. Thus, low-flux HD can be effective in small dogs, for whom charcoal hemoperfusion, hemofiltration, and high-flux HD cannot be used because of their small weight (Tauk and Foster, 2016).

The results show that CysC levels can be removed by using low-flux HD; therefore, CysC has potential as a kidney function biomarker in small dogs undergoing HD.

Acknowledgement: This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science (NRF-2020R1F1A1073007) and by ICT & Future Planning Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01395602), Rural Development Administration, Republic of Korea.

Authors contribution: WA wrote the manuscript. TL, JA and JHC analyzed and interpreted the date. WA and THK executed the experiment and analyzed data. JYC analyzed and interpreted the data and submitted the manuscript.

REFERENCES

Almy FS, Christopher MM, King DP *et al*., 2002. Evaluation of cystatin C as an endogenous marker of glomerular filtration rate in dogs. J Vet Intern Med 16: 45-51.

Barton KT, Kakajiwala A, Dietzen DJ *et al*., 2018. Using the newer Kidney Disease: Improving Global Outcomes criteria, beta-2-microglobulin levels associate with severity of acute kidney injury. Clin Kidney J 11:797-802.

Bloom CA and Labato MA, 2011. Intermittent Hemodialysis for Small Animals. Vet Clin North Am-Small Anim Prac 41:115-33.

Braun JP, Lefebvre HP and Watson AD, 2003. Creatinine in the dog: a review. Vet Clin Pathol 32:162-79.

Cowgill LD, 2011. Urea kinetics and intermittent dialysis prescription in small animals. Vet Clin North Am Small Anim Pract 41:193-225.

Diehl SH and Seshadri R, 2008. Use of continuous renal replacement therapy for treatment of dogs and cats with acute or acute-on-chronic renal failure: 33 cases (2002-2006). J Vet Emerg Crit Care 18:370-82.

Ettinger SJ and Feldman EC, 2010. Textbook of veterinary internal medicine : diseases of the dog and the cat, 7th ed. Elsevier Saunders, St. Louis, Mo.

Fadel FI, Mourad AA, Abdel Rahman AMO *et al*., 2017. Levels of cystatin C in low- and high-flux hemodialysis in children with end-stage renal disease. Pediatr Nephrol 32:1603-9.

Filler G, Bokenkamp A, Hofmann W, *et al*., 2005. Cystatin C as a marker of GFR-history, indications and future research. Clin Biochem 38:1-8.

Ghys LF, Paepe D, Lefebvre HP, *et al*., 2016. Evaluation of cystatin C for the detection of chronic kidney disease in cats. J Vet Intern Med 30:1074-82.

Iwasa N, Takashima S, Iwasa T, *et al*., 2018. Serum cystatin C concentration measured routinely is a prognostic marker for renal disease in dogs. Res Vet Sci 119:122-6.

Krishnamurthy N, Arumugasamy K, Anand U, *et al*., 2010. Effect of hemodialysis on circulating cystatin c levels in patients with end stage renal disease. Indian J Clin Biochem 25:43-6.

Marsenic O, Wierenga A, Wilson DR, *et al*., 2013. Comparison of cystatin C and Beta-2-microglobulin kinetics in children on maintenance hemodialysis. Hemodial Int 17 Suppl 1: S11-16.

Okuno S, Ishimura E, Maeno Y, *et al*., 2007. Serum beta2-microglobulin level is a significant predictor of mortality in Hemodialysis patients. Blood Purif 25:339-9.

Palmer SC, Rabindranath KS, Craig JC, *et al*., 2012. High-flux versus low-flux membranes for end-stage kidney disease. Cochrane Database Syst Rev CD005016.

Polzin DJ, 2013. Evidence-based step-wise approach to managing chronic kidney disease in dogs and cats. J Vet Emerg Crit Care 23:205-15.

Roumelioti ME, Trietley G, Nolin TD, *et al*., 2018. Beta-2 microglobulin clearance in high-flux dialysis and convective dialysis modalities: a meta-analysis of published studies. Nephrol Dial Transp 33:542.

Shafi T, Michels WM, Levey AS, *et al*., 2016. Estimating residual kidney function in dialysis patients without urine collection. Kidney Int 89:1099-110.

Shahjahan, Yasmin R, Mahsud MAJ, *et al*., 2011. Correlation of beta 2 microglobulin with serum creatinine and creatinine clearance in patients with different levels of renal function. Gomal J Med Sci 9:178-82.

Tattersall J, 2007. Clearance of beta-2-microglobulin and middle molecules in haemodiafiltration. Contrib Nephrol 158: 201-9.

Tauk BS and Foster JD, 2016. Treatment of ibuprofen toxicity with serial charcoal hemoperfusion and hemodialysis in a dog. J Vet Emerg Crit Care (San Antonio) 26:787-92.

Vilar E, Boltiador C, Viljoen A, *et al*., 2014. Removal and rebound kinetics of cystatin C in high-flux hemodialysis and hemodiafiltration. Clin J Am Soc Nephrol 9:1240-7.

Vilar E, Boltiador C, Wong J, *et al*., 2015. Plasma levels of middle molecules to estimate residual kidney function in haemodialysis without urine collection. PLoS One 10:e0143813.

Wehner A, Hartmann K and Hirschberger J, 2008. Utility of serum cystatin C as a clinical measure of renal function in dogs. J Am Anim Hosp Assoc 44:131-8.

Wong J, Sridharan S, Berdeprado J, *et al*., 2016. Predicting residual kidney function in hemodialysis patients using serum beta-trace protein and beta 2-microglobulin. Kidney Int 89:1090-8.