Urinary Markers in Early Diagnosis of Renal Disorders in Dogs

S. Yogeshpriya*, Usha N. Pillai and Madhavan Unny
Department of Clinical Veterinary Medicine,
College of Veterinary and Animal Sciences,
Kerala Veterinary and Animal Sciences University,
Mannuthy, Thrissur, Kerala
**Email: dryogeshpriya@gmail.com*

Introduction

Renal diseases are very common in dogs and as in humans; mortality rates have remained essentially unchanged at approximately 50-70% (Brunker *et al.,* 2009) Traditional diagnostic markers such as blood urea nitrogen and serum creatinine will only be increased when already 75% of functional renal mass is lost (Clemo, 1999). Since there is a high risk of progression to irreversible renal damage in patients with chronic kidney disease, especially in a more advanced stage. But early diagnosis of decreased kidney function remains a challenge in veterinary medicine as diagnostic tests.

Accessible markers of kidney injury can be components of serum or urine or can be imaging studies or any other quantifiable parameter. The urine has yielded the most promising markers for the early detection of disorder and further characterization is anticipated, which will qualify these markers as useful tools for the earlier diagnosis, identification of mechanism of injury, and assessment of site and severity of injury. Therefore, novel urinary markers that have demonstrated their potential for early and site-specific detection of renal dysfunction in human studies are gaining interest in companion animal medicine.

Pathophysiology and mechanisms of kidney injury

A number of pathophysiological mechanisms (Fig.1) can contribute to kidney injury following an ischemic or toxic insult.

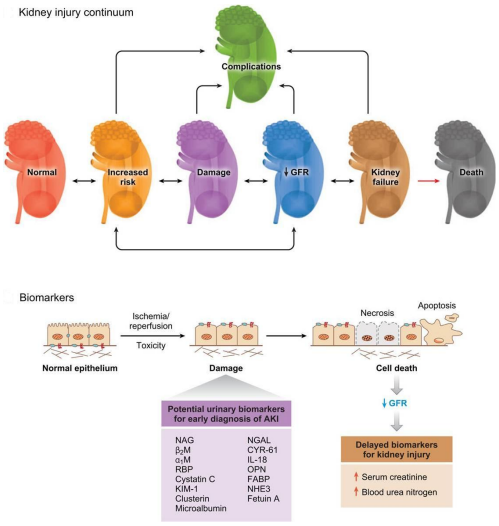

Fig.1. Kidney injury continuum and potential urinary biomarkers involved in early diagnosis of acute kidney injury

These include alterations in renal perfusion resulting from loss of autoregulation and increased renal vasoconstriction, tubular dysfunction and cell death by apoptosis and necrosis,

desquamation of viable and dead cells contributing to intratubular obstruction, metabolic alterations resulting in transport abnormalities that can lead to abnormalities of tubuloglomerular balance and Local production of inflammatory mediators resulting in interstitial inflammation and vascular congestion.

On cellular level, injury results in rapid loss of cytoskeletal integrity and cell polarity, with mislocalization of adhesion molecules and other membrane proteins such as the Na+K+ ATPase and β-integrins, shedding of the proximal tubule brush border, as well as apoptosis and necrosis. With severe injury, viable and nonviable cells are desquamated, leaving regions where the basement membrane remains as the only barrier between the filtrate and the peritubular interstitium. This allows for backleak of the filtrate, especially under circumstances where the pressure in the tubule is increased owing to intratubular obstruction resulting from cellular debris in the lumen interacting with proteins such as fibronectin that enter the lumen (Forterre *et al.,* 2004). The process of acute kidney injury can be divided into various reversible stages depending on the severity of insult, starting from increased risk to damage followed by decrease in glomerular filtration rate (GFR) further progressing to kidney failure and death. Diminished renal perfusion may result in decreased GFR in the absence of intrinsic damage, a condition termed prerenal azotemia (Fig.1). This injury to the epithelium results in the generation of inflammatory and vasoactive mediators, which can act on the vasculature to worsen the vasoconstriction and inflammation. Thus, inflammation contributes in a critical way to the pathophysiology of kidney injury (Fig.2).

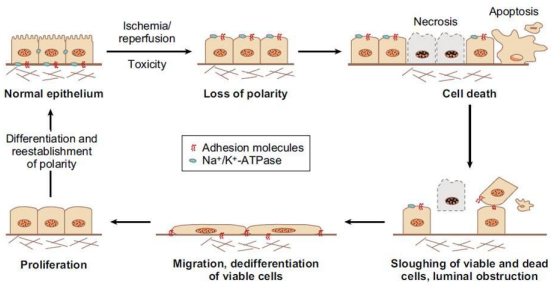

Fig.2. Cellular level injury

In contrast to the heart or brain, the kidney efficiently restores cells that were lost owing to an ischemic or toxic insult. Surviving cells that remain adherent contribute to repair. The kidney has the potential to recover a large amount of pre-insult renal function. A delay or inhibition of nephrogenic tissue repair appears to lead to progression of injury ultimately leading to chronic kidney disease, whereas timely tissue repair may arrest progression of injury, resulting in regression of injury and paving the way for recovery. Here need for early diagnosis is arises to safe the patient life.

Urinary Markers

Physiology of excretion

Tubular dysfunction is reflected by urinary loss of low molecular weight (LMW) proteins or urinary enzyme. Reversible tubular damage causes increase in enzymuria due to the leaking and shedding of enzymes from the affected tubular cells (Figure 3). So enzyme activity in urine increases before any alteration in kindey function. Similarly, a

minor damage to the glomerulus is responsible for an increase of proteinuria, starting with intermediate molecular weight (IMW) albumin and in more advanced stages to the presence of high molecular weight (HMW) proteins in ultrafiltrate (Gary *et al.,* 2004). According to Frances (1998) the contribution of serum enzymes to urine is negligible for most urinary enzymes because they are relatively large (>80,000 kDa) and unable to pass through the normal glomerulus.

The tubular histological lesions were graded as +, ++ and +++ on the basis of changes in the proximal renal tubules. The grading of tubular damage was based on a summarised evaluation of the following criteria: hyaline droplets and cell swelling, advanced cytoplasmic vacuolation and basement membrane thickening, pyknotic nuclei, tubular pigment accumulation, tubular atrophy, intraluminal protein casts and peritubular cellular infiltrates. Grade + specimens had only a few tubules affected one or two of the above-mentioned criteria present to a mild degree. Grade ++ specimens exhibited either mild generalised damage, or severe damage confined to isolated areas. Grade +++ specimens exhibited at least three of the above-mentioned criteria and at least one of them to a considerable degree.

List of urinary biomarkers

Alkaline phosphatase (AP)

Alkaline phosphatase was a sensitive indicator for assessing acute tubular dysfunction because there is no elevation of ALP reported in chronic renal disease. Urinary AP reported in normal dogs, 2•0 IU mmol–1 creatinine. The enzyme concentrations were considered low when AP is <10•0 IU mmol–1 creatinine, intermediate value when AP is 10•0–20•0. High enzyme values for AP >20•0 IU mmol–1 creatinine). Instability may limit clinical utility. High urinary enzyme values often reflected extensive lesions in renal proximal tubular cells and sometimes reduced GFR.

γ-glutamyl transpeptidase (GGT)

GGT also Proximal tubule brush border enzyme found low levels in normal urine (2.5 IU mmol–1 creatinine) and much higher levels in renal tubular injury (>20•0 IU mmol–1 creatinine). Among mammalian tissues highest activity of GGT reported in kidney. Low GGT is <10•0 IU mmol–1 creatinine and intermediate GGT value lies within 10•0–20•0 mmol–1 creatinine (Heiene *et al.,* 2001). Urinary GGT activity increases 78 fold after 90 minutes of complete renal ischemia. Instabilty requires samples to be analyzed quickly after collection, limiting clinical utility. In high urinary GGT cases, tubular lesions of grade +++ which includes hyaline droplet degeneration, pronounced vacuolation and basement membrane thickening observed.

N-acetyl-β-D-glucosaminidase (NAG)

NAG is a lysosomal enzyme found predominantly in the proximal renal tubular cells. Urinary NAG activity has been reported to increase under conditions of renal pathologic damage in both humans and other animals. Urinary NAG also proposed as a sensitive marker of the progression of renal diseases (Jacob *et al.,*

2005). Increased NAG observed in dogs with lower urinary tract infection with pyelonephritis. The enzyme concentrations is considered normal in 0.3 IU mmol–1 creatinine and low activity in the values of <1•0 IU mmol–1 creatinine. Intermediate and High enzyme values for NAG is 1•0–2•0 and >2•0 IU mmol–1 creatinine respectively (Heiene *et al.,* 2001).

C – reactive protein (CRP)

Only one study has been reported in urinary CRP (uCRP) concentrations in dogs with pyometra. Urinary CRP was not detected in healthy dogs, for CRP to appear in urine, its plasma concentrations must be increased and the glomerular barrier must be sufficiently damaged to allow high molecular weight (HMW) protein filtration. In humans, inflammation and oxidative stress stress start early in the process of failing kidney function and mild increases in CRP concentrations are present even in patients with moderate renal impairment. This might also have been the cause in one dog with increased urinary CRP/ creatinine, which was in an advanced staged of CKD (IRIS stage IV) (Lees, 2004). In young and older healthy dogs uCRP/ c values are 4.7mg/ g and 17.8 mg/g respectively where as in progressing stage of kidney injury as shown 84.3.

Retinol binding protein (RBP)

Retinol binding protein is low molecular weight (21kDa) protein circulates in plasma, where 90% of it bound to the 55 kDa protein transthyretin. The unbound fraction of RBP is freely filtered through the glomeruli and is catabolized after reabsorption in the proximal tubules. Normally reabsorption occurs through megalin-receptor dependent endocytosis mechanism in the proximal tubule. Because of this process only trace amounts should be excreted in the urine, where as urinary loss of RBP is increase in dogs with proximal tubule dysfunction (Maddens *et al.,* 2010). And RBP loss is greater in nephropathies with tubular lesions than in those with glomerular lesions because of failure of reabsorption and metabolism by proximal tubular epithelial cells.

Kidney injury molecule- 1 (kim-1)

The Kim-1 assay is a very sensitive and robust system with minimal interference from other components of the diseased urine and is not affected by variation in physicochemical properties of the urine. The assay has a dynamic range from 0 to 5,000 pg/ml with the lowest limit of detection being 38 pg/ml and an inter- and intra-assay variability 10% (Forterre *et al.,* 2004). Kim-1 is shed in the urine with mild insults, which result in minimal injury, thus giving sensitivity and early diagnostic ability of Kim-1 to serve as a biomarker because the concentrations of urinary Kim-1 were significantly higher on days 1 and 2 of experimentally induced renal injury when none of the conventionally used biomarkers of renal injury, including glycosuria, proteinuria, or urinary NAG levels.

Kim-1 is type-1 cell membrane glycoprotein in dedifferentiated proximal tubule epithelial cells and their ectodomain shed into urine in mild injury. Other name of this kim-1 includes Hepatitis A virus cellular receptor-1 (HAVCR-1), T cell

immunoglobulin like molecule-1 (TIM-1), and cochlear injury molecule-1 (CIM-1). So kim-1 is rapid, sensitive, reproducible and potentally high-throughput method to detect early kidney injury.

Microalbumin

Normally proteins were classified into 3 groups as HMW, MMW and LMW proteins. HMW proteins (High Molecular Weight) included bands as 98 kD and above 100 kD, and MMW proteins (Middle Molecular Weight) included bands as 76 and 66 kD. The presence of such urine proteins indicated a loss of glomerular function. LMW proteins included bands as 55, 45, 38, 30, 22, 16 and 14 kD and reflected tubular damage (Nabity *et al.,* 2007).

Microalbuminuria, defined as the pathologic excretion of urinary albumin at levels (30 to 300 mg/L) below the threshold of detection by conventional urinary dipstick, has long been established as a useful marker of the development and progression of renal disease (Raila *et al.,* 2010) that involves glomeruli (Maddens *et al.,* 2010). In normal dogs urine albumin level is less than 1.0mg/dl. If the urinary albumin lies beyond or equal to 30mg/ dl defines albuminuria and urine protein to creatinine (UPC) ratio greater than or equal to 0.5 indicates proteinuria (Reeko *et al.,* 2002). Microalbuminuria has been result from alterations in glomerular filtration secondary to changes in intraglomerular pressure and/or structural changes of the podocyte or glomerular basement membrane that leads to glomerular filter may leak albumin at higher levels and albuminuria may result from failure of the proximal tubule cell retrieval pathway (Smets *et al.,* 2010). And less than 15% of healthy dogs can be expected to develop microalbuminuria in response to mild to moderate exercise (Smets *et al.,* 2010). Whereas UPC ratio are <0.2,0.2-0.5, >0.5 as nonproteinuric, borderline proteinuric and frank proteinuric respectively observed (Vaidya *et al.,* 2008).

Clusterin

Clusterin is a multifaceted glycoprotein expressed on the dedifferentiated tubular cells, induced in the kidney and urine of rats, dogs, and primates after various forms of kidney injury such as ischemia/reperfusion injury, toxicant-induced kidney injury, unilateral ureteral obstruction, or subtotal nephrectomy. No clinical studies demonstrating its use.

Tamm-Horsfall protein (THP)

Tamm-Horsfall protein is a urinary glycoprotein exclusively synthesized by tubular cells in the distal part of the nephron and normally found in the urine. In the presence of chronic renal failure THP excretion was found to be reduced .The function of the distal tubule cells is likely to be reduced in chronic nephropathy, resulting in the urinary excretion rate of THP. So THP is the selective markers of distal tubular injury in dogs. Immunereactive THP was detected at molecular mass of 100Kd (Vaidya *et al.,* 2006).

Immunoglobulin G (IgG)

IgG plays an important role in the humoral response with a molecular weight

of 160kDa (HMW), that are unable to pass through an intact glomerular barrier unless glomerular damage. IgG in association with LMW protein such as RBP may correlate with the severity of histological lesions and outcome of the nephropathy (Brunker *et al.,* 2009).

Thromboxane B2 (TXB2)

Thromboxane A2 is a cyclooxygenase metabolite; it'll rapidly undergoes spontaneous hydrolysis to thromboxane B2 (TXB2). The main sites of its synthesis are glomerular mesangial cells and podocytes. Therefore, urinary TXB2 reflects renal synthesis and is as an approved marker for intra-renal hemodynamics alteration an ideal complementary marker urinary proteins (Brunker *et al.,* 2009) (Yalçin and Cetin, 2004).

Factors affecting urinary markers in urine

Methods for measuring urinary enzymes are generally similar to those used for assaying comparable serum enzymes; however, certain limitations and cautions should be considered because several in vivo and in vitro factors can affect enzyme activity in urine.

In vitro factors, including sample preparation such as centrifugation, dialysis, gel filtration and concentration and sample storage (time and temperature), contamination (feces, food and water), etc. affect enzyme activity and lead to erroneous results. Urine may also contain endogenous, low molecular weight enzyme inhibitors and activators so that preparation of urine by gel filtration or dialysis may be necessary prior to analysis (Zatelli *et al.,* 2010) Samples for analysis of uALB and uRBP can be stored at -20 ºC for up to 4 months, whereas -80ºC is preferred for storage up to 12 months. Urinary NAG enzyme activity is less stable at -20ºC as well as -80ºC (Yalçin and Cetin, 2004).

An in vivo factor such as kidney is involved in excretion of drugs, it also may be necessary to consider any potential interference of enzyme activity by drugs. Other tissues of urogenital tract, urine volume, time of collection and composition of urine such as pH, red blood cells, white blood cells, bacteria and enzyme inhibitors and activators also influence the concentration of urinary markers (Zatelli *et al.,* 2010).

Methods to quantitate biomarkers

The traditional method to quantitate urinary enzymes has been enzyme-substrate-based colorimetric assays followed by measurement using a spectrophotometer. Urinary markers measurements were related to urinary creatinine concentration in order to correct for variations in urine production (Heiene *et al.,* 2001) .Urinary creatinine was determined by the Modified Kinetic Jaffe Method using picric acid (Brunker *et al.,* 2009).

Conclusion

Glomerular and tubular markers in conjunction with traditional tests may provide more detailed information about the extent and location of renal damage. Hopefully, one or more of these biomarkers, either alone or in combination,

will prove to be useful in facilitating early diagnosis, guiding targeted intervention and monitoring disease progression and resolution. Unlike insensitive tests like BUN and creatinine, urinary markers are sensitive indicators of renal injury. Urinary biomarkers of kidney injury will facilitate earlier diagnosis and specific preventative and therapeutic strategies, ultimately resulting in fewer complications and improved outcomes.

Acknowledgements

The authors are grateful to The Department of Clinical Veterinary Medicine, College of Veterinary and Animal Sciences for the assistance in preparing this article.

Competing interests

The authors declare that they have no competing interests.

References

Brunker, J.D., Ponzio, M.N. and Payton, M.K. (2009). Indices of urine N-acetyl-β-D-glucosaminidase and γ-glutamyl transpeptidase activities in clinically normal adult dogs. *Am J Vet Res.,* **70:** 2

Clemo, F.S. (1998). Urinary Enzyme Evaluation of Nephrotoxicity in the Dog. *Toxicol pathol.,* **26:** 29-32

Forterre, S., Raila, J. and Schweigert, F.J. (2004). Protein profiling of urine from dogs with renal disease using ProteinChip analysis. *J Vet Diagn Invest.,* **16:** 271–277.

Gary, A.T., Cohn, L.A., Kerl, M.E. and Railaen. W.A. (2004). The Effects of Exercise on Urinary Albumin Excretion in Dogs. *J Vet Intern Med.,* **18:** 52–55.

Heiene, R., Moe, L. and Mølmen, G. (2001). Calculation of urinary enzyme excretion, with renal structure and function in dogs with pyometra. *Research in Veterinary Science.,* **70:** 129–137.

Jacob, F., Polzin, D.J., Osborne, C.A., Neaton, J.D., Kirk, C.A., Allen, T.A. and Swanson, L.L. (2005). Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. *J Am Vet Med Asso.,* **226:** 3

Lees, G.E. (2004). Early diagnosis of renal disease and renal failure. *Vet Clin Small Anim.,* **34:** 867-885

Maddens, B.E.J., Daminet, S., Demeyere, K., Demona, D., Smets, P. and Meyer, E. (2010). Validation of immunoassays for the candidate renal markers C-reactive protein, immunoglobulin G, thromboxane B2 and retinol binding protein in canine urine. *J vet imm.,* **134:** 259–264.

Nabity, M.B., Boggess, M.M., Kashtan, C.E. and Lees, G.E. (2007). Day-to-Day Variation of the Urine Protein: Creatinine Ratio in Female Dogs with Stable Glomerular Proteinuria Caused by X-Linked Hereditary Nephropathy. *J Vet Intern Med.* **21:** 425–430

Raila, J., Brunnberg, L., Schweigert, F.J. and Kohn, B. (2010). Influence of kidney function on urinary excretion of albumin and retinol-binding protein in dogs with naturally occurring renal disease. *Am J Vet Res.* **71:** 1387-1394

Reeko, S., Soeta, S., Miyazaki, S., Syuto, B., Sato, J., Miyake, Y., Yasuda, J., Okada, K. and Naito, Y. (2002). Clinical availability of urinary N-acetyl-ß-D-Glucosaminidase index in dogs with urinary diseases. *J. Vet. Med. Sci*., **64:** 361-365

Smets, P.M.Y., Meyer, E., Maddens, B., Duchateau, L. and Daminet, S. (2010). Effect of Sampling Method and Storage Conditions on Albumin, Retinol-Binding Protein, And N-Acetyl-b -D-Glucosaminidase Concentrations in Canine Urine Samples. *J Vet Diagn Invest.,* **22:** 896–902.

Smets, P.M.Y., Meyer, E., Maddens, B., Duchateau, L. and Daminet, S. (2010). Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. *J Vet Intern Med*., **24:** 65-72

Vaidya, V.S., Ferguson, M.A. and Bonventre, J.V. (2008). Biomarkers of Acute Kidney Injury. *Annu Rev Pharmacol Toxicol.,* **48:** 463–493.

Vaidya, V.S., Ramirez, V., Ichimura, T., Bobadilla, N.A. and Bonventre, J.V. (2006). Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. *Am J Physiol Renal Physiol.,* **290:** F517–F529

Yalçin, A. and Çetin, M. (2004). Electrophoretic Separation of Urine Proteins of Healthy Dogs and Dogs with Nephropathy and Detection of Some Urine Proteins of Dogs Using Immunoblotting. *Revue Méd. Vét.,* **155,2:** 104-112

Zatelli, A., Nizi, F., Roura, X. and Zini, E. (2010). Evaluation of urine dipstick test for confirmation or exclusion of proteinuria in dogs. *Am J Vet Res.,* **71:** 235-240.