Review Article

Novel Horizon of Biomarkers for Detection of Acute Kidney Injury in Animals

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Abstract

Prevalence of kidney diseases is widespread in animals especially in dogs and cats. Insensitive diagnostic approach and use of nephrotoxic drugs may worsen the situation. Acute kidney injury (AKI) is associated with a sudden damage to the renal parenchyma and generally occurs due to various causes. Conventionally, serum creatinine and blood urea nitrogen (BUN) are used as gold standard for detection of kidney diseases, but its late existence may delay the diagnosis. Next generation of biomarkers, like proteins, enzymes and small molecules are over expressed during the tubular or glomerular injuries in urine and serum and are explored as novel biomarkers of AKI. These biomarkers are specific, non-invasive and sensitive enough to detect even the minute disturbances in the kidney. Although, these novel biomarkers outperformed over the traditional biomarkers and are well correlated with histopathological changes in kidney but they are still not widely used.

Key words: AKI, NGAL, KIM-1, NAG, Microprotein, Cystatin C.

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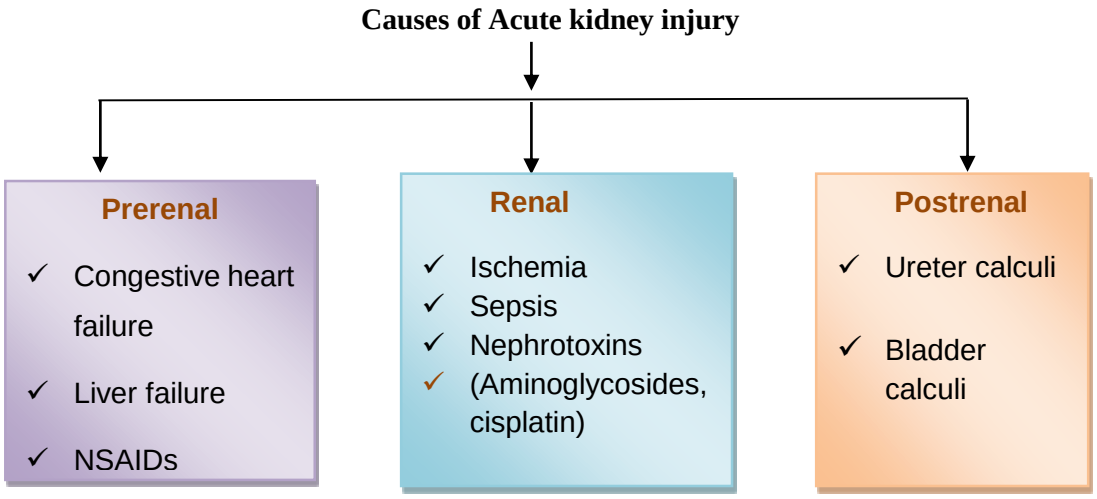
Introduction

Kidney diseases are obvious in animals especially in dogs and cats, and are often associated with poor prognosis in later stages. Animals suffered from various kidney dysfunctions like acute kidney damage, chronic kidney damage, nephritis, glomerulonephritis, nephrosis etc. Acute kidney injury (AKI) is a sudden onset of potentially life-threatening kidney dysfunction, related with a sudden damage to the renal parenchyma and characterized by increase in serum creatinine with oligouria or anuria (Vaidya *et al.*, 2008). High morbidity and mortality has also been reported in dogs upto 60% and in cats upto 50% usually associated with delayed detection due to insensitive diagnostic tests (Clarkson *et al*., 2008, Eatroff *et al*., 2012). Various nephrotoxic drugs produce AKI in animal and human. High doses of intravenous and

intramuscular administration of oxytetracycline or gentamicin caused AKI in a dehydrated cow (Vaala *et al*., 1987). In bovine and caprine, infection of common inhabitant *Streptococci* and *Corynebacterium* has been reported to cause AKI. AKI in equine is usually prerenal or renal in origin and is most often caused by hemodynamic or nephrotoxic insults (Geor, 2007). Sepsis in equine is considered as the leading cause of AKI. AKI may progress chronic kidney injury which is another commonly diagnosed condition in the adult and aged population. The prevalence increases up to 15% in dogs over 10 years of age and to up to 31% in cats over 15 years of age (O'Neill *et al*., 2013; Haytham *et al*., 2017).

The diagnosis of renal dysfunction at an early stage represents a challenge in veterinary medicine (Polzin, 2011). There is an urgent need for the biomarkers which can detect the kidney damage at an early stage for the therapeutic intervention, so that further damage to kidney can be avoided and mortalities can be checked. Several new biomarkers have been explored in this direction and some of them found to be very promising are explored in this review.



Pathogenesis of AKI

Renal injury leads to disruption of renal perfusion, auto-regulation and vasoconstriction in kidney. This causes loss of cytoskeletal integrity, cell polarity and displacement of adhesion molecule such as Na+K+ATPase and beta integrin in renal tubular cells. With continuing damage, cells are desquamated leaving space of basal membrane, which may results in back leak of the filtrate, this causes intratubular obstruction due to interaction of cellular debris with protein in the lumen. Epithelial damage may induce secretion of inflammatory and vasoactive mediators, which may worsen the vasoconstriction and inflammation (Vaidya *et al*., 2008; Price *et al*., 2010).

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Current Biomarkers and their Limitations

Traditionally, serum creatinine and blood urea nitrogen (BUN) concentration are used as gold standard for the diagnosis of kidney diseases. But serum creatinine and BUN are increased when approximately 75% of nephrons are damaged. They are also influenced by age, gender, muscle mass, muscle metabolism, diet, medications, and hydration status (Polzin, 2011).

Novel Biomarkers

An ideal marker should be specific, non-invasive and sensitive enough to detect even the minute disturbances in the renal tubules. Various proteins, enzymes and small molecules which are up or down regulated during the tubular or glomerular injuries are targeted by researcher, which can be the component of serum or urine. However, urine biomarkers are quite promising to detect early AKI, as minor tubular dysfunction can be translated in the urine earlier than any other sample. This can help in identification of disorders, determination of location and severity of dysfunction (Vaidya *et al.*, 2008; FDA 2009). These classified as mentioned in below table.

Up regulated protein	Functional	Tubular enzymes
Neutrophill gelatinase associated lipocalin (NGAL)	Microalbumin	N-acetyl-beta-glucosaminidase (NAG)
Kidney injury Molecule – 1 (KIM-	Beta 2 microglobulin	Gamma-glutamyl transpeptidase
Interleukin – 18 (IL -18)	Alpha 1 microglobulin	Alkanine phosphatase (AP)
Renal papillary antigen 1 (RPA-1)	Cystatin C	
Clusterin	Retinol binding protein	

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL), also called as lipocalin 2 / siderocalin, is a glycoprotein with a molecular weight of 25 kDa and a member of the lipocalin family (Xu *et al*., 1994). NGAL is expressed by the variety of epithelial cells predominantly the renal tubular cells and to some extent by neutrophils. Normally, plasma NGAL filtered through glomerulus and reabsorbed by proximal tubular cells by receptor mediated endocytosis. When there is proximal tubular damage NGAL excrete in urine due to disruption of NGAL reabsorption or increase NGAL synthesis. NGAL level in steady state is ~20 ng/mL in serum and urine of human being. Various workers have reported the up regulation of NGAL genes and protein in kidney tissues during various clinical conditions of kidneys. Mishra *et al.* (2004) in genetic expression studies showed rapid and 1000 fold increase of NGAL mRNA in ascending tubules of loop of henle and collecting tubular epithelium. A frank increase of 130-141 fold in urinary NGAL levels were reported in rats, dogs and monkeys with gentamicin or cisplatin induced AKI. NGAL levels were detected as early as 2 h after AKI, much earlier than the increase in serum creatinine (Uchino *et al.*, 2017).

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A tenfold or more increase in the urine and plasma NGAL, within 2–6 hours of the AKI noted in human beings. Elevated NGAL found after the cardiac surgery, contrast nephropathy and kidney transplant cases (Xu *et al*., 1994). Increased NGAL levels were well correlated with serum creatinine, GFR and proteinuria. Increased urine NGAL noticed in chronic injury in animals with IgA nephropathy and lupus nephritis and in urinary tract infections (Ding *et al.*, 2007; Nickolas *et al.*, 2008; Suzuki *et al.*, 2008). A canine-specific NGAL ELISA has been validated for dogs to quantify NGAL activity in urine (Nabity *et al*., 2012). Zhou *et al*. (2014) has recommended urinary NGAL as a sensitive and specific biomarker for the detection of AKI and prediction of nephrotoxicity in animals. Plasma NGAL level may also get influenced by atherosclerotic plaques and aortic aneurysms.

NAG (***N*-acetyl-β-d-glucosaminidase**)

N-acetyl-β-d-glucosaminidase (NAG) is a high molecular weight (150 kDa) lysosomal enzyme abundantly present in brush border of proximal tubular cells in two isoenzymes- NAG-A and NAG-B (Bourbouze *et al.*, 1984). Normally, urinary NAG activity is low in healthy human and animals. NAG-A is located in the soluble intra lysosomal compartment and NAG-B on membrane of lysosomes of proximal tubules. During the damage of renal tubular brush border epithelium, NAG is released from the lysosomes to the urine (Sato *et al.*, 2002; Skalova, 2005). Elevated NAG in urine is noticed during nephrotoxic effects of aminoglycosides and heavy metals or in the diagnosis of diabetic nephropathy. Higher urinary NAG was found in patients with nephrotic syndrome or developmental kidney abnormalities before the increase in serum creatinine level or urinary protein (Skalova, 2005). For animals an enzymatic colorimetric NAG assay has been validated in dogs and cats. Lapointe *et al.* (2008) noticed NAG within the range of 0.28 and 2.76 U/g in healthy cats. The increase in urinary NAG was significantly higher in AKI in dogs with pyelonephritis and urinary tract infection (UTI) (Borges *et al*., 2013).

In experimental studies urinary NAG found to be quite stable, it did not show any circadian variations, age difference and sex difference in dogs and cats. Isoenzymes of NAG were quite stable in a wide range of pH. Urinary NAG concentrations were found to be similar in natural voided and cystocentesis samples. Excretion of NAG into urine during mid to late-stage renal disease is reported to be relatively constant by various workers (Nabity *et al*., 2012; Smets *et al.*, 2012). NAG was found to be sensitive, persistent and robust indicator of brush border injury in proximal convoluted tubules in rats, dogs, cats and monkeys (Vlasakova *et al.*, 2014; Uchino *et al.*, 2017). However, NAG is inhibited by endogenous urea and increased urinary NAG level also seen in rheumatoid arthritis

KIM – 1/TIM-1 (Kidney Injury Marker/ Tubular Injury Marker)

KIM -1 is a cell membrane glycoprotein type 1 which contains 6-cystein domain and a mucin domain in its extracellular region. There are two variants, Kim-1a, major form expressed in the liver and Kim-1b,

predominant form in the kidneys (Bailly *et al*., 2002). KIM-1 is expressed by proximal tubule cells and promotes the apoptotic and necrotic cell clearance. Up on injury, KIM-1 is up regulated and shed into the urine and extracellular space. It also activates the injury-induced immune response (Ichimura *et al*., 2012). Increased expression of KIM-1 mRNA reported in proximal tubular cells within 24-48 hours after ischemic events in mice, whereas it was not detected in normal kidney. KIM-1 mRNA levels increase more than any other known gene after ischemia/reperfusion (I/R) injury. Extensive analysis of studies in patients with AKI conducted between 2002 and 2009 demonstrated that Kim-1 was an early biomarker of AKI and found elevated within 24 h after a kidney insult (Vaidya *et al*., 2005). In situ hybridization and immunohistochemistry demonstrated that Kim-1 was expressed by proximal tubules (Ichimura *et al*., 2008; Ichimura *et al*., 2012).

Interleukin 18 (IL- 18)

Interleukin 18 (IL-18), member of the IL-1 cytokine super family and known as interferon-γ (IFN-γ)-inducing factor, is produced by mononuclear cells, macrophages and non-immune cells including proximal tubule cells (Gracie *et al*., 2003). Faubel *et al.* (2005) found increased IL-18 in AKI and thought the proximal tubules are the reason; not macrophages, neutrophils or CD4 T cells. It has been found that IL-18 urine level increases two days before the increase in serum creatinine in non-sepsis patients with AKI. The IL-18 urine level has sensitivity and specificity >90% in diagnosing AKI (Vlasakova *et al*., 2014). Though, IL-18 produced by number of cells and also influenced by some non-renal conditions.

Retinol Binding Protein

Retinol binding protein (RBP) is 21 kDa protein synthesized in liver and has important role in transporting vitamin A from liver to tissue. The unbound fraction of RBP is freely filtered through the glomeruli and is catabolized after reabsorption in the proximal tubules. Healthy dogs excrete small amounts of RBP in urine. Increased levels of urinary RBP are reported in dogs and cats with proximal tubule disorders. Increase RBP in urine of cats with chronic renal failure and hyperthyroidism has also been observed by earlier workers (Sato *et al*., 2002; Forterre *et al*., 2004; Hoek *et al*., 2008). Urinary RBP may be measured using validated ELISA assays. It is not affected by the sampling method and is stable after storage at –20 °C and –80 °C. Urinary RBP may increase in hematuria samples. Serum RBP level decreases in patients with vitamin A deficiency (Vaidya *et al*., 2008).

Cystatin C

Cystatin C, a 13-kDa protein, arises in all nucleated cells and is not bound to plasma proteins. Therefore, it is freely filtered by the glomerulus, and subsequently reabsorbed and degraded in the renal proximal tubule by the endocytic receptor megalin, but not secreted by the tubules. Cystatin C is used to detect early GFR

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and development of AKI at one or two stages earlier than serum creatinine. It increases rapidly in contrast induced nephropathy (Biesen *et al*., 2006). In animals exposed to cisplatin or gentamicin, urinary cystatin C rose before evident proximal tubular damage, supporting its value as an early biomarker. Cystatin C level is affected by diabetes, corticosteroids administration, hyperthyroidism and hyper bilirubinemia (Royakkers *et al*., 2011; Sasaki *et al.*, 2011; Nejat *et al*., 2012).

Microproteins

These are useful marker of the development and progression of renal disease, particularly diabetic nephropathy. Alpha1-microglobulin is a 27 kDa immunomodulatory glycoprotein produced by the liver. Free α1-microglobulin passes through the glomerulus and is reabsorbed by the proximal tubules. Normal urine contains very small amounts of α1-microglobulin. In disturbed tubular function, reabsorption of α1-microglobulin is reduced and increased amounts are found in urine. Alpha1-microglobulin is stable at different urine pH values and at room temperature for seven days (Penders and Delanghe, 2004). Beta 2-microglobulin is a low molecular weight protein (11.8 kDa) present on the surface of all nucleated cells and is unstable in acidic urine. Increased levels of urinary β2-microglobulin were observed in dogs with X-linked hereditary nephropathy, where it increased during the early stage of the disease (Nabity *et al*., 2012). Microalbuminuria is a nonspecific, may also be increased by vigorous exercise, hematuria and dehydration.

Renal Papillary Antigen1- (RPA-1)

It is a high molecular weight membrane bound glycoprotein, which is present in collecting duct of renal papilla. It is increased 20-60 folds in renal papillary necrosis caused by 2-bromo methanamine in rats (Price *et al.*, 2010).

Clusterin

Clusterin is a glycoprotein (70–80 kDa) synthesized in many tissues. In dogs, clusterin was recently measured as a potential marker of drug-induced acute kidney injury. Together with NGAL, clusterin was found to be the most sensitive biomarker for detection of gentamicin-induced renal proximal tubular toxicity (Zhou *et al*., 2014).

Trefoil Factor-3

It is a peptide present in collecting duct in normal kidney, which is decreased in response to injury (FDA, 2009).

Current Status of the Biomarkers

At present, these biomarkers are evaluated by the various regulatory bodies of different countries. Food and Drug Administration (FDA) formed a kidney injury biomarkers qualification committee (Critical-Path

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Predictive Safety Testing Consortium), which evaluated a total of 22 biomarkers and found some of the biomarkers outperform or added valuable information over the traditional biomarkers like serum creatinine and BUN. These markers are found to be well correlated with the histomorphological and immunohistochemical changes in renal tubules. FDA has approved the seven biomarkers for nonclinical evaluation of AKI in research namely, NGAL, KIM-1, clusterin, albumin, trefoil factor 3, β2 microglobulin, cystatin C (FDA, 2009).

Though number of hurdles still remains, before these biomarkers can be implemented in routine diagnostic practice. These biomarkers require standardization and validation for different renal pathology and to various causes of AKI. Impact of species, gender, age and cut off values of each biomarker in different conditions need to be studied. Once these obstacles are removed, in near future, these biomarkers can definitely be playing role for quick and rapid diagnosis of kidney injury.

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