Limitations of Conventional Parameters and Role of Urinary Protein Biomarkers in the Determination of Drug Induced Acute Kidney Injury in Very Early Stage

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Authors’ contributions

Authors RSS, BRA and GK designed the review article, protocol, and wrote the first draft of the manuscript. Authors LG, MS, TT, SP and GKP managed the collection of relevant literatures from various sources. This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Drug-induced Acute Kidney Injury (AKI) constitutes an important cause of acute renal failure and chronic kidney disease in present day clinical practice. Drug-induced acute
renal failure (ARF) accounted for 20% of all ARF in an Indian study. The incidence and prevalence of chronic kidney disease (CKD) has dramatically increasing worldwide. Progression of AKI from mild or moderate to end stage may be prevented by selecting potentially effective therapies, if it is detected in very early stage. But early detection of AKI is often difficult due to paucity of early predictive noninvasive biomarkers. Development of omics technology has led to the identification of several urinary protein biomarkers and transcriptional biomarkers, which enable earlier detection of kidney injury. Urinary protein biomarkers have great benefit due to the easy or non-invasive availability of urine and many showing good predictive power. Several urinary protein biomarkers have been identified and have demonstrated superiority in detecting kidney injury in comparison to conventional parameters like serum creatinine (SCr), blood urea nitrogen (BUN) etc. These promising experimental biomarker of kidney damage require further confirmation of its use in routine clinical use.

Keywords: Kidney injury biomarkers; acute renal failure; SCr; BUN; omics.

1. INTRODUCTION

Kidney is one of the major organs evoking drug-related toxic responses as it plays a crucial role in performing several important functions including detoxification and excretion of drug and its toxic metabolites [1]. Therefore, it is an important target for toxicological studies. In the pharmaceutical industry, kidney is one of the routinely assessed organs during preclinical safety evaluations.

Acute Kidney Injury (AKI) remains a common and serious clinical problem which represents an acute decline in renal function finally leading to structural changes and is associated with increased morbidity, mortality, length of hospital stay and costs in both surgical and medical patients. Ischemia, sepsis and toxins are the most common etiologies in hospitalized patients. Drug-induced kidney disease constitutes an important cause of acute renal failure (ARF) and chronic kidney disease (CKD) in present day clinical practice. Drug-induced ARF accounted for 20% of all ARF in an Indian study [2]. Recently, the incidence of end-stage renal disease (ESRD) has dramatically increasing worldwide [3]. The incidence and prevalence of CKD in the world is increasing particularly in developing countries [4], because compared to 30 years ago, patients today have a higher incidence of diabetes and cardiovascular disease, taking multiple medications and are exposed to more diagnostic or therapeutic procedures with the potential to harm kidney functions [5]. Many drugs have nephrotoxic potential and some of them can cause more than one pattern of injury [6,7]. The incidence of drug-induced nephrotoxicity has been increasing with an ever increasing number of drugs. Chemotherapy with anti-cancer drugs has limited use due to its serious nephrotoxicity [8]. NSAIDs, antibiotics, ACE-inhibitors, contrast agents, and heavy metals are other major culprit drugs contributory to kidney damage [2].

This review has made an attempt to focus on the limitation of conventional parameters and the importance of further confirmation of most promising novel biomarkers for its use in routine clinical use.

1.1 Conventional Parameters

Standard parameters employed in preclinical and clinical studies as well as in routine clinical care for the detection and monitoring of renal function are the serum creatinine (SCr) level
and blood urea nitrogen (BUN) level [9]. Some other tests that measure abnormal kidney functions include lowered glomerular filtration rate (GFR) (<60%), proteinuria, hematuria, detection of urinary components like electrolytes, enzymes and other waste products. The signs and symptoms often associated with AKI are high blood pressure, swelling of hands or in feet, puffiness around the eyes and frequent or painful urination due to kidney stone formation that later leads to AKI.

Serum level of creatinine (a break down product of muscle tissue) depends on age, gender, food intake, ethnicity, muscle mass, muscle metabolism, medication, hydration status and weight. Congestive heart failure (CHF), dehydration [10], eclampsia [11], preeclampsia [12], rhabdomyolysis [13] etc., are various non-renal-related causes of alteration in serum creatinine level without producing any negative impact on kidney. BUN level in serum also increases in other pathological processes like enhanced protein catabolism or urea production, congestive heart failure [14], excessive protein levels in the gastrointestinal tract, gastrointestinal bleeding [15], hypovolemia [16], shock [17] and dehydration [10].

Creatinine clearance rate is traditionally used to estimate glomerular filtration rate. GFR is best measured by injecting compounds such as inulin, radio isotopes or radio contrast agents such asiohexol. But these techniques are complicated, costly, time consuming and have potential side effects. Good results have been produced by using cystatin C clearance instead of creatinine clearance in selected patient groups, such as patients with reduced muscle mass [18].

Reabsorption capability of the kidney for endogenous components like small proteins, sugars or metabolites is highly reduced in acute or chronic kidney damage mainly due to exposure to nephrotoxic substances. Some urinary proteins with enzymatic activity can be employed as nephrotoxic biomarkers, which include alanine aminopeptidase, alkaline phosphatase, α-glutathione-S-transferase, γ-glutamyl transeptidase, π-glutathione-S-transferase and lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG). High molecular weight proteins like albumin [19], transferrin and immunoglobulin G (IgG) can be detected in urine due to improper function of selective penetration through glomerulus [20]. β2-microglobulin, α1-macroglobulin and retinol binding protein are low molecular weight proteins representing tubular damage [21]. Cast and fractional excretion of sodium have been insensitive and non-specific for the early recognition.

Glutathione-S-transferase (GSTs) are proteins found in high concentrations in the luminal cells of proximal and distal tubules. Different isoforms are found in different parts of nephron, α- GST is localized exclusively in the proximal tubular cells, whereas GSTYb1 is detectable in distal tubular cells and glomerular podocyte in Bowman’s capsule [22]. Renal tubule injury can thus be precisely localized. GSTs play an important role in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress by conjugation with glutathione. These proteins are not released in healthy rats and act as a very sensitive indicator of site specific injury. In urine, these enzymes are normally not present. Urinary excretion of GST might be reflection of the site of tubular injury when tubular cell wall integrity is damaged [23]. Walsh et al. [24] reported that both enzymes were bad predictors with in patients with developing AKI and sepsis admitted to general ICU, suggesting sepsis might be the confounder triggering the production of these enzymes [24]. Urinary αGST level was found to be increased compared to serum creatinine, BUN and NAG in proximal tubular toxicity but decreased in collecting duct injury [25]. However, early detection of acute kidney injury with these enzymes and proteins are difficult due to their instability and highly variable levels in urine.
Drug induced renal toxicity on animal system can be directly observed and characterized through histopathological observation, currently remaining as ‘gold standard’ in preclinical studies. However, it is difficult to identify the time at which kidney damage occurs. To generate this information, it would be necessary to use many more animals for histopathological observation at different time points that leads to increase in animals, cost and time of drug development process. It does not give detailed information of non-histopathology associated type of kidney disturbances. This method also requires sacrificing animals which is not optimal to study kidney injury in live animals and use of histopathology for kidney injury in human is impractical.

Therefore, sensitivity or diagnostic values of all traditionally used parameters are poor, unreliable and their delayed response often delivered false-positive or false-negative results which finally lead to a late detection of renal damage that only dialysis for the patient is possible. Moreover, these parameters are not specific for kidney damage but are also reported for other organ damage or diseases. A reduction of renal functionality occurs only after two third of renal biomass has injured [26].

Ideal features of biomarkers are those that identify kidney injury at a very early stage using standardized assay procedures, specific for AKI, reflecting the degree of toxicity to characterize dose dependencies, displaying similar reliability across multiple species, localizing site of kidney injury, tracking progression of injury and recovery from damage, predictive of clinical outcomes such as need of dialysis, length of hospital stay and mortality, able to guide initiation of therapies at the beginning stage, preferably noninvasive i.e., using easily accessible samples such as blood or urine. Determination of biomarkers from blood or urine samples is a promising approach for assessing drug induced renal toxicity [27].

Development of omics technology has led to the identification of several urinary protein biomarkers and transcriptional biomarkers which enable earlier detection of kidney injury. Novel kidney injury biomarkers in both serum and urine, have demonstrated superiority in detecting kidney injury, much ahead of changes in conventional parameters like serum creatinine or BUN.

1.2 Urinary Protein Biomarkers

Urinary protein biomarkers have great benefit of easy availability of urine and lack of sample preparation and showing good predictive power. Enhanced sample preparation effort and limitation in tissue availability restrains the routine use of transcriptional biomarkers compared to urinary protein biomarkers. Most promising urinary protein biomarkers of nephrotoxicity include kidney injury molecule (Kim-I), clusterin, neutrophil gelatinase associated lipocalin or lipocalin-2(NGAL/Lcn-2), interleukin-18 (IL-18), vanin, insulin-like growth factor binding protein 7 (IGFBP7), liver-type fatty acid binding protein (L-FABP) etc., have been identified in kidney tissues, serum and in urine using ELISA, immunohistochemistry and quantitative real time PCR techniques.

2. MOST PROMISING URINARY PROTEIN BIOMARKERS AND RECENT REPORTS

2.1 Kidney Injury Molecule-1 (KIM-1)

Kidney injury molecule-1(Kim-1) is a type I transmembrane glycoprotein with an immunoglobulin-like domain, consisting of six unusual cysteine and a long mucin-like domain
in extracellular region, localized to the apical membrane of exclusively surviving proximal tubular epithelial cells after injury [28]. It is expressed as low level in normal kidney tissue but dramatically up regulated and is easily detected in the urine upon nephrotoxicity.

Kim-1 has been considered as a non-invasive biomarker for human renal proximal tubular damage [29]. Dana Hoffsmn et al. [30] analyzed Kim-1, lipocalin-2, clusterin, Timp-1 in kidney and confirmed that Kim-1 and clusterin as early, sensitive and noninvasive markers of renal injury, as it reflected changes in gene /protein expression and histopathological alterations in absence of functional changes [30]. Previous studies in rats and in patients with AKI, demonstrated significantly higher sensitivity and specificity of urinary Kim-1 and clusterin as markers of renal injury than traditional clinical chemistry parameters [28,31-34]. Kim-1 is not detected in normal tissue or urine, but highly expressed in proximal tubular epithelial cells after toxic injury [28]. Urinary Kim-1 levels were significantly elevated within 12h after aristolochic acid administration, whereas significant increase in the BUN levels were observed only on day 5, with no changes in urinary N-acetyl-β-glucosaminidase (NAG) and total protein throughout the study [35]. However, urinary Kim-1 is reported to increase after the peaks of urinary NAG and NGAL in patients with AKI after cardiac surgery [32].

2.2 Neutrophil Gelatinase Associated Lipocalin or (NGAL/Lcn-2) or Lipocalin-2

NGAL is also known as human neutrophil lipocalin, lipocalin-2, siderocalin belongs to well defined super family of proteins called lipocalins. NGAL is a protein that binds to gelatinase in particular neutrophil granulocytes and it is upregulated during inflammation or tumorogenesis [36]. NGAL may be produced in many cells including kidney tubule and might be readily detected in urine. NGAL level predicts the future appearance of acute kidney injury after treatments which adversely affects kidney.

NGAL was easily detectable in urine of mouse with cisplatin-induced nephrotoxicity preceding the appearance of NAG and β2microglobulin, suggesting as an early, non-invasive biomarker for ischemia and nephrotoxic renal injury [37]. In a previous study in which rats were administered with gentamicin, and found that urinary Lcn2 performed better than Kim-1 [38] NGAL/lipocalin-2 was found to be rapidly increased and secreted into urine in a range of preclinical and clinical studies on acute kidney injury [39,40]. However, it has also been demonstrated that urinary concentrations of Lcn2 was observed to be increased in liver fibrosis induced by carbon tetrachloride [41] elevated in patients with pneumonia [42] and in inflammatory bowel disease [43] suggesting that it may not be specific for kidney injury but also occur in response to systemic inflammation or tissue damage at other site or organs.

2.3 Clusterin

Clusterin is sulphide-linked heterodimeric glycoprotein consisting of α and β subunits [44,45] present in the cytoplasm of proximal convoluted tubule or at the end of distal convoluted tubule (DCT) including connecting tubule in the kidney cortex. It can be used as a potential marker of nephrotoxicity since it is upregulated and is detected in the urine of patients with acute kidney injury.
Increased expression of clusterin was found in tubules showing signs of cell death and regeneration indicating its role in tissue remodeling after damage [46]. Clusterin has been implicated in apoptosis and growth control, cell adhesion and tissue remodeling and significantly upregulated in response to cellular stress [47]. Clusterin was significantly increased in patients with bladder cancer raising the concern that the presence of clusterin may not be linked only to kidney toxicity [48].

2.4 Interleukin-18 (IL-18)

IL-18 is a widely expressed pro-inflammatory cytokine, produced by renal tubular cells and by macrophages, formerly known as interferon-gamma-inducing factor, which induces interferon-gamma production in T cells and natural killer cells. IL-18 has an active role in apoptosis, ischemia, infection, malignancy and autoimmune condition [49].

John M et al. [50], measured the concentration of IL-18 in the urine samples of 95 patients with AKI stage after cardiac surgery and found IL-18 was the best predictor, and L-FABP, NGAL, Kim-1 were also good predictors. Liu Y et al. [51] concluded IL-18 as a useful biomarker of AKI with moderate predictive value across all clinical settings. In a cross sectional study, urine IL-18 levels were markedly elevated in patients with established AKI, but not in subjects with UTI, CKD and nephrotic syndrome [52]. Urinary IL-18 was found to be significantly upregulated in patients with acute respiratory distress syndrome who developed AKI [53]. Urinary IL-18 level was significantly elevated within 6 h in children undergoing cardiopulmonary bypass (CPB) who developed AKI [54]. Moreover, IL-18 is a predictive biomarker in kidney transplantation [55]. Overall, IL-18 appears to be more specific to AKI and has been anticipated as a possible early marker.

2.5 Vanin-1

Vanin-1 is an epithelial ectoenzyme with pantetheinase activity, which catalyzes the conversion of pantetheine into pantothenic acid and cysteamine. Lack of cysteamine is associated with enhanced γ-glutamyl cysteine synthetase activity leading to elevation of endogenous glutathione stores in tissues which protect tissue against the degenerating effects of oxidative damage by scavenging free radicals [56]. Higher serum and urinary concentration of vanin-1 were observed in ethylene glycol treated groups compared to control group, suggesting it as a useful and rapid biomarker for renal tubular injury [57].

A recent study has conducted to determine whether increase in urinary vanin is detected before the elevations of serum creatinine, urinary NAG, Kim-1, and NGAL in animal models induced nephrotoxicity by cisplatin and gentamicin. Urinary vanin-1 was detected earlier than other biomarkers after the administration of higher doses of both drugs. This result suggest that compared with urinary Kim-1 and NGAL, urinary vanin-1 is an earlier and equally sensitive biomarker for drug induced AKI [58]. Urinary vanin-1 was found to be increased in patients with diabetic nephropathy [59] and the protein levels of renal vanin-1 was increased in rats with streptozotocin-induced diabetic nephropathy. Therefore, it is anticipated that urinary vanin-1 is a potential biomarker of early detection of AKI.

2.6 Insulin-like Growth Factor Binding Protein-7 (IGFBP7)

IGFBP7 involved in G1 cell cycle arrest during early phase of cell injury and may prevent the division of cells with damaged DNA until DNA damage is repaired [60,61].
Previous study investigated concentration of urinary IGFBP7 and Timp-1 in 50 patients undergoing cardiac surgery. Results indicated that both served as sensitive or specific biomarker to predict AKI early after cardiac surgery and to predict renal recovery. AUC level was significantly higher for IGFBP7 and Timp-1, i.e., superior to other biomarkers like Kim-1, NGAL, cystatin C, IL-18 to predict AKI [37,62,63].

2.7 Tissue Inhibitor of Metalloproteinase -1 (Timp-1)

Timp-1 are endogenous, specific inhibitors of matrix metalloproteinase (MMPs) by forming high affinity complexes and thereby blocking binding of MMPs to the substrate. MMPs are family of proteolytic enzymes that degrade various types of extracellular matrix components (ECM). Timp-1 promotes renal fibrosis through inhibition of matrix metalloproteinase and accumulation of collagen. Down regulation of MMPs and up regulation of TIMPs lead to accumulation of ECM proteins and further to progression of chronic renal failure (CRF) which is characterized by tubulointerstitial fibrosis and glomerulosclerosis [64].

Enhanced urinary excretion of Timp-1 was seen in high dose animals but the overall performance of Timp-1 as an early indicator of proximal tubular injury was poor compared to Kim-1 and clusterin [30]. Elevated levels of Timp-1 have been observed in models of kidney injury and in urine of patients with renal diseases as compared to healthy controls [65].

2.8 Liver-type Fatty acid Binding Protein (L-FABP)

L-FABPs are small cytoplasmic proteins abundantly expressed mainly in proximal tubule of kidney and also in tissues with active fatty acid metabolism. Primary function is the facilitation of long chain fatty acid transport.

Increased urinary expressions have also been described in animal models of AKI [66-68]. In hospitalized patients with established AKI, the AUC of urinary L-FABP for prediction of AKI was 0.97, and higher in patients with poor outcome [69]. In patients with specific shock and AKI, urinary L-FABP levels were significantly higher [70], in addition to elevated levels in CKD [71]. Urinary L-type fatty acid binding protein was shown to increase within 2 h of cisplatin administration and correlated with histological injury score than BUN [68], indicating L-FABP as a promising and sensitive biomarker.

3. FUTURE PROSPECTS

Present use of troponins as biochemical markers of myocardial injury rather than the measurement of aspartate amino transferase leads to a progress in the diagnostic ability and a parallel improvement in treatment and survival after cardiac injury.

Most of the research studies have focused only on Kim-1 and it would be wrong, as it indicates the renal damage only on S3 segments [72]. Recent reports also indicate other biomarkers like IL-18, IGFBP and vanin as more sensitive biomarkers than Kim-1. In future, it may be necessary to validate the sensitivity and specificity of these markers in clinical samples from large cohorts and in multiple clinical situations. This may help to provide further information about the specificity of these biomarkers in acute kidney injury. To date only limited data are available regarding gender difference in drug induced renal injury.

4. CONCLUSION

Markers of organ damage are better than functional markers. For the last 10 decades, serum creatinine was used as a major determinant of kidney function. RIFLE scheme (risk, injury,
failure, loss and end stage renal diseases), AKIN scheme (acute kidney injury network) were options used for the classification of renal injury. These methods were based on the specific cut off values of serum creatinine, GFR or urinary output. Different definitions of AKI in the published literatures rely on changes in SCr. Therefore, there is an urgent need for a standard definition which is not based on serum Cr concentration.

Difficulties in the early or accurate detection of AKI have led to significant problems in patient care and drug development. The data generated during the preclinical toxicity testing has to be as detailed and informative as possible. It has been observed that the incidence of patients in intensive care units developing AKI is about 30-50%, but only 7% of new drug candidates fail in the preclinical trials because of toxicity [73]. This discrepancy may be due to the underestimation of nephrotoxicity in preclinical trials. Determination of novel biomarkers from blood or urine samples rather than conventional parameters may be a promising approach for assessing AKI in very early stage.

Better biomarkers will help drug developers to make appropriate decisions and may prevent the entry of nephrotoxic drug into the market or it facilitate early management of patients who suffer from kidney injury. Therefore, these promising experimental biomarkers of kidney damage require further confirmation of its use in routine clinical use.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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