Urinary Cystatin C as an Early Biomarker of Acute Kidney Injury in Critically ill Patient

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Abstract

Introduction: Acute kidney injury is associated with high mortality in critically ill patients. We usually measure serum creatinine to detect AKI, but it is a poor predictor of accuracy particularly in the early stages of AKI(1). We studied the utility of urinary cystatin C and its significance in AKI.

Materials and Methods: This prospective observational study was done in 83 critically ill patients admitted in ICU. Serum creatinine was done on day 1 and day 3 which was compared with Urinary Cystatin C done on day 1.

Results: Out of the 83 patients, 36 (43.3%;) developed AKI. Serum creatinine on day 1 and day 3 were compared with urinary cystatin C done on day 1. We observed that in the AKI group there was significant elevation of urinary cystatin C (15% (p=0.001)). On contrary in the non AKI group there was a marginal level of increase in urinary cystatin C. As the urinary cystatin C is the early excretory product it can be used as an early biomarker of AKI.

Conclusions: We found that the urinary cystatin C levels were significantly elevated in AKI groups when compared with the creatinine levels. Hence we suggest that urinary cystatin C is a significant early biomarker for AKI. Moreover, further studies are warranted with large number of sample size with multicentric population to confirm these results

Keywords: urinary cystatin C, acute kidney injury, critically ill patients.

Introduction

Acute kidney injury (AKI) is a sudden incident of kidney failure or kidney damage that happens within a few hours or a few days. Acute kidney injury is common in patients who are in the hospital, in intensive care units, and in older adults. AKI is significantly associated with increased morbidity; mortality rate depends on the length of hospital stay and cost effective of treatment(2). Current diagnosis method to detect the AKI in humans is rise in serum creatinine (Scr) concentration. However, the serum creatinine is an unreliable indicator since the several factors are regulating the levels of serum creatinine and urine(3).
The incidence of AKI varies across the world depending on the definitions they used and the populations studied. Very few studies have systematically explored the epidemiology of AKI in the critically ill population or have used validated criteria, such as RIFLE or AKIN and found that the incidence of AKI of about 10–40% in India.

There are multiple risk factors like patient specific, treatment specific, and other risk factors in ICU which act together to cause AKI in ICU. Prerenal azotaemia is defined as a functional decline in glomerular filtration associated with renal under perfusion and is a leading cause of AKI in the general and geriatric populations (4).

Although classically associated with hypovolemia and resulting from failure of normal adaptive responses to maintain GFR, prerenal AKI also commonly develops in the setting of effective intravascular volume depletion associated with congestive heart failure (cardiorenal syndrome) and liver disease.

A detailed history and physical examination is critical in differentiating the aetiologies of AKI. Initial diagnostic studies should include a urinalysis including urine sediment examination, urine chemistries (urine sodium and creatinine) and renal ultrasound. If the aetiology of AKI remains unclear following a careful history, physical examination, and laboratory work-up, or if the work-up suggests the presence of acute glomerular disease, consideration of a kidney biopsy is warranted.

Serum creatinine (SCr) is an apoor predictive accuracy for renal injury, particularly in the early stages of AKI (1). There are several biomarkers which are useful in diagnosis of AKI. Newer biomarkers for detecting AKI in various clinical situations are urinary cystatin C, IL 18, KIM1 and NGAL.

NGAL (Nuetrophil Gelatinase Associated Lipocalin) was considered a useful predictor in the early phase of AKI which has prognostic value in clinical outcomes: such as the need for dialysis and mortality. Unfortunately, the large extrarenal production in response to systemic stress can increase its urinary excretion in the absence of AKI, and may increase in CKD -and not only in the acute stage, which can confuse its interpretation(5).

KIM-1 Human - Kidney Injury Molecule-1
It is expressed at low levels in normal kidneys. However in acute renal injury it is dramatically upregulated in regenerating proximal tubules. It increases after 24-48h in the proximal tubule of the post-ischemia. KIM 1 facilitates the early diagnosis of AKI but it is also produced in other conditions like chronic proteinuria, inflammatory and fibrotic disease states in humans.

Inter Luekin (IL) 8
IL 8 seems to be a candidate biomarker in defining AKI, but its pro-inflammatory properties and its high levels in inflammatory diseases may limit its use vis-à-vis its sensitivity and specificity (5,6).

Cystatin C
Cystatin C is a cysteine protease inhibitor, synthesized by all nucleated cells in the body. It is freely filtered by the glomerulus, fully reabsorbed and not secreted. The urinary excretion of low molecular weight cystatin C protein, which is an endogenous marker of renal dysfunction correlates with the severity of acute tubular injury. As blood levels of cystatin C are not significantly affected by age, gender, race, or muscle mass in general, it is a marker for estimating the glomerular function in cachectic patients or early AKI, in which serum creatinine could underestimate the true renal function. The costs for analysis are still considered high, which limits its use in clinical practice, and factors such as thyroid dysfunction, obesity, use of corticosteroids and inflammation can interfere in its serum levels(5,7).

Aim
The aim of this prospective cohort study was to evaluate the urinary cystatin C (UCysC) as early biomarkers of AKI in an unselected, heterogeneous group of patients admitted critically ill to the emergency department in a large tertiary care
hospital. The objectives of current study was to assess the urinary cystatin C and its significance in Acute Kidney Injury. Also Serum creatinine levels were measured at different times and its significant correlation in Acute Kidney Injury was analysed.

Finally urinary cystatin C was compared with serum creatinine to find out which is the early biomarker for AKI.

**Materials and Methods**

This study is a prospective observational study in which eighty three critically ill people admitted in the ICU during January 2017 to October 2017. The important laboratory parameters such as serum creatinine (day 1), serum creatinine (day 2), Urinary Cystatin C (day 1), serum electrolytes and haemoglobin levels were measured. Furthermore, the age, gender and glomerular filtration rate were calculated using the standard MDRD formula. All the critically ill patients were classified into two groups: with development of AKI and without development of AKI based on serum creatinine and urinary cystatin C levels. Finally statistical analysis were performed between the groups to find the potential diagnostic marker for AKI.

All patients admitted in medicine ICU during January 2017 to October 2017 were included in this study. patients who were less than 18 years or more than 80 years, without any indwelling catheter; had obvious haematuria, rhabdomylosis and polycythemia, receiving cytotoxic chemotherapy or renal replacement therapy or assessed to need RRT in 48 hours, expected to leave ICU within 72 hours or not expected to survive 72 hours and patient who presented with established AKI were excluded in the study. The study was approved by the institutional review board (IEC No: CSP-MED/16/JAN/27/27).

The blood samples were processed immediately to measure the all clinical parameters and the urine samples were stored in for while and once all the target urinary samples collected, these were processed by ELISA method to find the cystatin C levels. The quantitative determination of creatinine in serum, plasma and urine was measured using the CREA method which employs a modification of Jaffe kinetic reaction. The advantage of this method is to eliminate interference from non creatinine Jaffe positive compounds. Human cystatin C is measured by ELISA method. Results are reported as total concentration of Cystatin C (ng/ml) in urine samples.

The Human urinary cystatin C values that provided 95% sensitivity, 95% specificity, and optimal sensitivity and specificity using the ROC curve at the best time-point. All statistical tests were two-tailed and P<0.05 was considered significant. The analyses were performed using the SPSS software, version 16.0.

**Results**

A total of 83 patients, 57 (68.6%) males and 26 (31.3%) females, were included in the study. 36 (43.3%;) patients developed AKI and 47 (56.7 %) did not develop AKI. The demographic, clinical, and laboratory data between the AKI and Non-AKI groups are shown in Table 1. Among the studied characteristics the creatinine level day 1 and creatinine level day 3 were correlated with urinary cystatin C. The comparison is given in the table2.

In patients who developed AKI on day 3 detected by raise in serum creatinine was found to be significant as reflected by p value .Urinary cystatin C level was measured in AKI group increased by 15% from the reference level (p=0.001). Other studied characteristics such as the total count (TC) (p=0.198), sodium (p=0.30), potassium (p=0.267), chloride (p=0.237), bicarbonate (p=0.083) and haemoglobin (HB) (p=0.519) were also measured.

Among the patients (n = 36) enrolled in our study, most common cause of AKI was sepsis (30.5%) followed by ALD (11.1%) followed by acute pancreatitis (8.3%). Among the sepsis urinary
tract infections was the most common source followed by respiratory infection. The observations regarding the etiology is summarised in figure 1.

Among the non AKI group we compared creatinine level day 1 with urinary cystatin C done on day 1 and found that there is a marginal level of increase in urinary cystatin C. Similarly we compared creatinine level on day 3 with urinary cystatin C done on day 1 in the AKI group and found that there was significant elevation of urinary cystatin C. These results suggest that the urinary cystatin C estimated on day 1 was the early excretory product, hence we can use urinary cystatin C level in critically ill patients as a early biomarker of AKI. (figure2)

**Table 1:** Demographic characteristics, clinical, and laboratory data of the critically-ill patients between the AKI and Non-AKI groups

| CHARACTERISTICS          | NON AKI (N=47) Mean ±SD | AKI (N=36) Mean ±SD | P value |
|--------------------------|--------------------------|---------------------|---------|
| Haemoglobin (g/dl)       | 11±2.5                   | 10.7±1.2            | 0.519   |
| Total Count              | 13572.1±5195.7           | 14980±4672.8        | 0.198   |
| Sodium (mmol/l)          | 135.5±4.13               | 134.3±5.9           | 0.3     |
| Potassium (mmol/l)       | 4.1±0.89                 | 3.9±0.58            | 0.267   |
| Chloride (mg/dl)         | 103.2±4.1                | 105.1±8.69          | 0.237   |
| Bicarbonate (mmol/l)     | 23.6±3.7                 | 32±2.3              | 0.083   |
| Creatinine day 1 (mg/dl) | 0.8±0.2                  | 1±0.17              | 0.001   |
| Creatinine day 3 (mg/dl) | 0.7±0.31                 | 1.73±0.36           | 0.001   |
| Urinary cystatin C       | 61.0±20.6                | 150.0±42.0          | 0.001   |

**Table 2:** Comparison between groups with creatinine levels and urinary cystatin C

| Group     | Creatinine level (Day 1) | Creatinine level (Day 3) | Urinary Cystatin C (day 1) | Percentage of urinary cystatin C compare with reference level |
|-----------|--------------------------|--------------------------|-----------------------------|---------------------------------------------------------------|
| Non-AKI   | 0.8 ± 0.2                | 0.7 ± 0.31               | 61.0 ± 20.6                 | 6% increase of Cystatin C                                      |
| AKI       | 1.0 ± 0.17               | 1.73 ± 0.36              | 150.4 ± 42.0                | 15% increase of Cystatin C                                    |

**Figure 1:** Etiology of AKI
Figure 2: Comparison between groups with creatinine levels and urinary cystatin C

Discussion
Monitoring the renal function is very important for patients admitted in Intensive care unit. However the accurate diagnosis of AKI is especially problematic in critically-ill patients in whom renal function is in an unsteady state. Potentially effective therapeutic interventions for AKI may currently fail because they are applied late in the course of injury. The ideal endogenous marker has not yet been identified. In our study, we observed that the significant elevated levels of urinary cystatin C when compared to creatinine levels day 1 and creatinine levels day 3. This indicates urinary cystatin C may be early predictive marker for AKI of critically ill patients. Furthermore, our study revealed that the sepsis was the primary cause of AKI.

A cohort study by Ron Wald et al., in 2010 included 150 critically ill patients in different health care centres, found that the postoperative AKI occurred in 47 (31.3%) patients and was diagnosed a median of 1 day from surgery. AKI was diagnosed in 29 patients on the first postoperative day, 11 patients on the second postoperative day and in 7 patients on the third postoperative day. Three patients’ required renal replacement therapy and 6 died in the hospital. Furthermore, they revealed that the serial measures of plasma CysC are highly correlated with the development of AKI. Similarly, the other authors also found that serum Cys-C is a better marker of GFR than creatinine, even in cases of sub-clinical renal dysfunction. An Indian study conducted by Murty et al., in 2013...
that included 200 healthy subjects and 130 patients of AKI. This study found that the serum cystatin C is a better marker of renal function in early stages of AKI and is less affected by age, gender, muscle mass, and ethnicity. Furthermore, the use of serum cystatin C-based GFR may be more accurate and useful for early therapeutic intervention and possibly for a favourable outcome (14).

Unfortunately, in our study, we have not measured the serum cystatin C level to compare with the respective day of creatinine. However present study found that there is elevation in the levels of urinary Cystatin C among AKI patients. This observation indicates that the human urinary cystatin C was the early biomarker of AKI in critically ill patients. This is well supported by the studies of Park et al and Nejatet al (15,16).

Limitations
This study has important limitations. First, is the small size of the study group. Second, this was a short term study of AKI in critically-ill patients. We did not follow-up patients to determine the decline or increase or normalization of cystatin C in comparison with creatinine regarding improvement of AKI or deterioration. Furthermore, we have not analysed the serum cystatin C to compare with the urinary cystatin C. This will be an important future study.

Summary and Conclusions
The present study has investigated the association of urinary cystatin C concentration with Non-AKI and AKI groups. We found that the urinary cystatin C levels were significantly elevated in AKI groups when compared with the creatinine levels (day 1 and day3). Furthermore, present study found that the sepsis was the most common cause of AKI in our populations. This study yielded that the urinary cystatin C as significant early biomarker for AKI. Moreover, further studies are warranted with large number of sample size with multicentric population to confirm these results.

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