

Urinary α -Glutathione-s-transferase Variations in Cisplatin Treated Cancer Patients with and without Kidney Injury

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Abstract. Introduction: One of the most common causes of Acute Kidney Injury is nephrotoxin administration. Platinum compounds like cisplatin are very effective as chemotherapeutic agents but the risk of nephrotoxicity frequently hinders the use of its higher dose to maximise its antineoplastic effects. The lack of early biomarkers of AKI has impaired our ability to initiate the preventive interventions in the nephrotoxicity caused by these agents in a timely manner. This study was designed to evaluate the variation of urinary renal tubular cell specific enzyme α -Glutathione-s-transferase(α -GST) in cancer patients treated with cisplatin chemotherapy. The urinary levels of α -GST, studied in a timely manner may help in identifying patients who may benefit from early interventions. **Methodology:** This is a prospective observational cohort study in patients with head and neck malignancies qualified for three weekly chemosensitiser with cisplatin(dose 100mg/m²). Venous blood samples were collected from all the patients, (after obtaining Institutional ethical clearance) before the administration of cisplatin (baseline), and at 12 hours, 24 hours, 48 hours and 20days after cisplatin infusion and a random urine sample was collected before and at 2 hrs , 6 hrs, 12 hrs, 24hrs and 48hrs after cisplatin administration. Serum creatinine was estimated by Jaffe's method using commercial reagent kit. and α -GST was estimated in all the urine samples by colorimetric kinetic assay using NBD-Cl. **Results:** There was a 20.5% incidence of acute kidney injury after cisplatin administration as suggested by a significant rise in the serum creatinine levels within the first 48 hours. The mean urinary α -Glutathione S Transferase levels at different time intervals show a clear temporal rise, especially after 2 hours after cisplatin administration, and at a slower rate thereafter. **Discussion:** We observed that, in AKI group, there was a substantial rise in the enzyme values following cisplatin administration. The peak rise was seen around 6 hrs after cisplatin infusion, which was followed by a steady fall. Whereas the significant increase in serum creatinine was observed only after 48hrs. Hence an earlier detection of rise in enzyme levels could help in prompt intervention and prevention of further renal damage.

Keywords: Acute Kidney Injury, α -Glutathione-s-transferase, Nephrotoxicity, Acute Kidney Injury Network, Cisplatin Applications

1. Introduction

Acute kidney injury (AKI) is an increasingly common and potentially catastrophic complication in hospitalised patients. The frequency of AKI is increasing all over the world. Despite significant improvements in therapeutics, the mortality and morbidity associated with AKI remains high (1,2). The most common etiologies include nephrotoxin administration, cardiac disease, including myocardial infarction , cardiogenic shock, congestive heart failure, sepsis, unresolved pre-renal factors and liver diseases (3-7) . AKI in critically ill patients is multi-factorial in origin and carries a high mortality, which increases with increasing number of failed organs (8).

One of the most common causes of AKI is nephrotoxin administration. Platinum compounds like cisplatin are very effective as chemotherapeutic agents but the risk of nephrotoxicity frequently hinders the use of its higher dose to maximise its antineoplastic effects. The lack of early biomarkers of AKI has impaired our ability to initiate the preventive interventions for the nephrotoxicity caused by these agents in a timely manner. (9). To improve the identification of patients at risk of AKI and their care, novel approaches for early diagnosis and risk stratification are needed. The most widely used biomarkers for the diagnosis of

AKI are serum creatinine and creatinine clearance which significantly increase only after substantial kidney injury occurs and with a time delay (10-12).

Early intervention can significantly improve the prognosis. However, the paucity of early, predictive, non-invasive biomarkers has impaired our ability to institute potentially effective therapies for these common clinical conditions in a timely manner (13). A major reason for the mortality and morbidity is the lack of early markers for AKI, resulting in an unacceptable delay in initiating therapy (14-16).

This study was designed to evaluate the variation of renal tubular cell specific enzyme α -Glutathione-s-transferase(α -GST) in cancer patients treated with cisplatin chemotherapy. Cell specific biomarkers can predict toxic insults earlier and can provide information on sites of injury and optimal dosing. Hence the urinary levels of α -GST, studied in a timely manner may help in identifying patients who may benefit from early interventions thus reducing the rate of morbidity and mortality associated with kidney injury.

2. Methodology

This project was designed as a prospective observational cohort study in patients with head and neck malignancies qualified for three weekly chemosensitizer with cisplatin (dose 100mg/m²). Patients with any pre-existing renal insufficiency, diabetes mellitus, and peripheral vascular diseases and on any other nephrotoxic drugs are excluded from the study. Patients were planned for treatment with concurrent chemoradiotherapy. The main outcome measure was the identification of patients with clinically diagnosed AKI based on AKIN (modified RIFLE) criteria (17).

2.1. Chemotherapy

All the selected patients were planned for concurrent chemotherapy with single agent cisplatin, administered at a dose of 100 mg/m², given every 3 weeks, on day 0, day 22 and day 43 respectively. Cisplatin dose was calculated as per the patient's body surface area and the total dose was mixed into 250cc normal saline and the infusion was delivered over one hour. The patients were adequately pre-hydrated with at least 2 litres of intravenous normal saline 12 hours prior to the start of cisplatin infusion. Adequate post-chemotherapy hydration with at least 2 litres of intravenous normal saline was started immediately after completion of cisplatin infusion. 40 mEq of potassium (KCl) and 5mg of magnesium (MgSo₄) salts also were added to each litre of the intravenous hydration fluid.

Evaluation of the temporal pattern of serum creatinine and urinary α -GST.

Venous blood samples were collected from all the patients (after obtaining institutional ethical clearance- UEC/30/2009) in a 2 ml syringe before the administration of cisplatin (baseline), and at 12 hours, 24 hours, 48 hours and 20days after the cisplatin infusion. Similarly, a random urine sample was collected in a 50 ml sterile plastic container before the cisplatin administration and at 2 hrs, 6 hrs, 12 hrs, 24hrs and 48hrs after cisplatin administration.

Serum creatinine was estimated by Jaffe's method using commercial reagent kit and **α -GST** was estimated in all the urine samples by colorimetric kinetic assay using NBD-Cl (18).

2.2. Statistical analysis

The performance characteristics of α -GST as a marker for kidney injury was studied by constructing receiver operating characteristics curve (ROC). The area under curve (AUC) is calculated from a standard receiver- operating characteristic (ROC) plot. A probability value of 0.05 or less was considered as statistically significant. Analyses were performed with SPSS, ver.16.

3. Results

A total of 73 patients with head and neck cancer who were planned for treatment with concurrent chemoradiation with 3-weekly cisplatin sensitizer (>100mg/m²) and matching the eligibility criteria were recruited into the study. All the patients were above 20 years and below 70 years. Majority of the patients were in the 41-50 year age group, constituting 27% of all the patients. The great majority of the patients recruited were males, constituting more than 88% of the study population. Nearly 50% of the patients had cancer of the oral

cavity. Hypopharyngeal and oropharyngeal cancers were the 2nd and the 3rd most frequent sites of cancer. In that Stage III and stage IV patients constituted the majority.

3.1. Incidence of acute kidney injury:

Based on the serum creatinine levels, kidney injury was defined as an elevation of serum creatinine ≥ 0.3 mg/dL within 48 hours from the baseline (AKIN criteria). Accordingly there were 15 patients out of the total 73 showed kidney injury. This indicates a 20.5% incidence of AKI after cisplatin administration as suggested by a significant rise in the serum creatinine levels within the first 48 hours.

Among the total male population (64), AKI was observed in 11 patients. And in the female group (9), 4 of them showed AKI. That indicates a high incidence of kidney injury in females (44.4) compared to males (17.2%). The age of the patients did not seem to have any impact on the incidence of AKI. Nearly 20% of the patients in each age group had developed AKI.

3.2. Evaluation of urinary levels of α -Glutathione-S-transferase

All the 438 urine samples, which are collected before cisplatin administration (base line) and 2 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs after cisplatin administration from the 73 patients were analysed for urinary α -Glutathione-S-transferase. The mean urinary α -Glutathione S Transferase levels at different time intervals show a clear temporal rise, from 2 hours after cisplatin administration (Figure. 1). The ROC curves of α -GST at different time points have showed in Figure. 2. The performance characteristics of urinary α -GST have shown in Table. 1. The area under curve (AUC) of more than 0.7 was obtained for all of the timed sample groups. The optimal cut off values has been calculated as per the Youden index. Accordingly, the sensitivity of α GST ranged from 73% to 91% between the different temporal groups. The sample collected at 12 hours shows the highest sensitivity and specificity in detecting acute kidney injury.

4. Discussion

According to Acute Kidney Injury Network (AKIN) criteria, kidney damage has been categorised into three stages, the last two of which are identical to the RIFLE criteria. 'Risk', the Stage.1 of AKIN criteria defines AKI by an absolute increase of serum creatinine by 0.3mg/dl or a 50% increase(17). In our study the selected patients were grouped into, those with AKI and without AKI, based on the AKIN criteria. We could found the incidence of kidney injury in these patients after cisplatin administration is around 20%. In more recent experience, incidences of 20-30% renal insufficiency have been reported using saline hydration and diuresis(9). Ronald et al studied the early clinical use of cisplatin and saw dose-related cisplatin-induced acute renal failure in 14 to 100% of patients, with the incidence, varying with the cumulative dose. Another study also has seen reported severe renal dysfunction in 20% of patients receiving high-dose cisplatin. This indicates that our findings are in agreement with other reports on cisplatin induced nephrotoxicity.

Though the studies on enzymuria in renal insufficiency have been started many years back(19), there is no reported data of urinary α -GST in humans, administered with cisplatin. In this study we observed that, in AKI group, there was a substantial rise in the enzyme values following cisplatin administration. The peak rise was seen after 6hrs after cisplatin infusion, which was followed by a steady fall. Whereas the significant increase in serum creatinine was observed only after 48hrs. Hence an earlier detection of rise in enzyme levels could help in prompt intervention and prevention of further renal damage.

We believe few factors which give strength for our results. Like prospective recruitment of a relatively homogeneous cohort of adult subjects in whom the only obvious etiology for AKI would be the result of cisplatin. All subjects started with normal kidney function, and the study design allowed for the precise temporal definition of altered urinary α -GST levels and a direct comparison with subsequent changes in serum creatinine. Our results clearly indicate that urine α -GST is an early predictor of AKI that precedes the increase in serum creatinine by several hours. The magnitude of rise supports the notion that urine α -GST is a highly discriminatory biomarker with a wide dynamic range and cut off values that allow for easy risk stratification. And also urinary diagnostics have several advantages, including the non-invasive nature of sample collection and the reduced number of interfering proteins.

Eventhough, these findings certainly need to be validated in a larger randomized prospective trial, including adults with the usual confounding variables and comorbid conditions that normally accumulate with increasing age. And also it will be important to confirm our findings in documented high risk settings. It is possible that a collection of other strategically selected candidates along with this enzyme will prove of value for early and rapid diagnosis of AKI.

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Figure. 1- The temporal pattern of urinary α -GST in AKI and Non- AKI groups

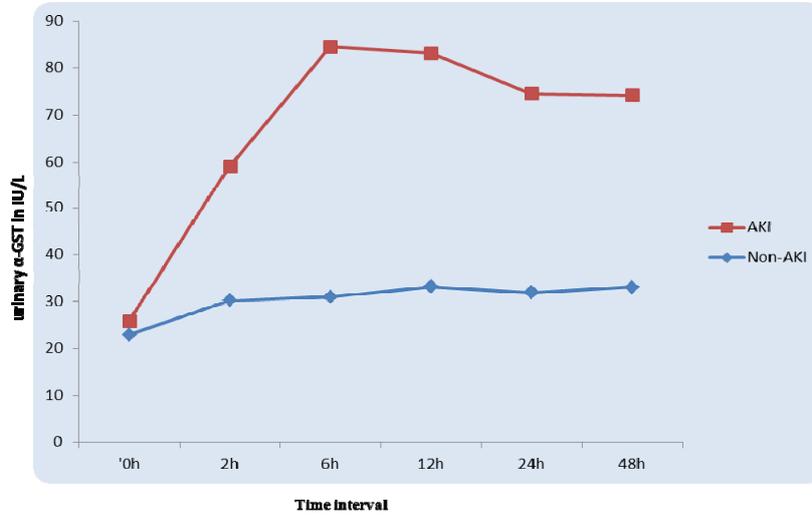


Table. 1 - The performance characteristics of urinary α -GST

Time interval	Area Under ROC	Optimal cut off (IU/L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
b	0.717	≥ 22.2	72.7	58.3	28.6	90.3
2h	0.811	≥ 27.10	81.8	60.4	100	84.2
6h	0.824	≥ 29.75	81.8	56.8	30	92
12h	0.812	≥ 32.2	81.8	60.4	32.1	93.5
24h	0.855	≥ 29.7	90.9	60.9	80	93.6
48h	0.809	≥ 30.3	88.9	56.8	57.1	89.1

Figure. 2 - ROC curves of urinary α -GST at different time points with AUC

