Int J Biol Med Res. 2012; 3(3): 2241-2246

BioMedSciDirect Publications

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Evaluation of urinary tubular enzymes for the detection of early kidney injury due to cisplatin chemotherapy

Saleena U V* [a] , MS Athiyaman [b] , Donald F [b] , BM Vadhiraja [b] , R Prabhu [c], Nalini K [d]

[a]*Dept. of MLT, Manipal College of Allied Health Sciences,*
[b]*Dept. of Radiotherapy and Oncology,* [c]*Div. of Nephrology,* [d]*Dept. of Biochemistry, KMC Manipal, Manipal University, Manipal, INDIA – PIN: 576 104*

ARTICLE INFO

Keywords:
Kidney Injury
Tubular enzyme
α-Glutathione-s-transferase
Nephrotoxicity
Cisplatin

ABSTRACT

This study was designed to evaluate the efficacy of urinary renal tubular cell specific enzymes α-Glutathione-s-transferase(α-GST) and Gamma glutamyl transpeptidase (GGT) for predicting kidney injury in cisplatin treated cancer patients. The urinary levels of these enzymes, studied in a timely manner may help in identifying patients who may benefit from early interventions. Methods: After obtaining Institutional ethical clearance, venous blood samples were collected from all the patients, 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. α –GST and GGT was estimated in all the urine samples by colorimetric kinetic assay using NBD-Cl and Gamma-ghitamylp-nitroanilide respectively. 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(≥0.3mg/dL) within the first 48 hours. The mean urinary α-GST levels at different time intervals show a clear temporal rise, especially from 6hrs after cisplatin administration, till 12hrs and at a slower rate thereafter. The AUC of >0.8 for α-GST in all the timed urine samples after cisplatin administration indicated its good performance in predicting kidney injury. Urinary GGT levels showed a steep increase upto 6 hours before gradually declining over the next 48 hours after cisplatin administration among patients who eventually developed AKI. Conclusion: Urinary levels of proximal tubular enzymes, α-GST and GGT are useful in predicting early kidney injury induced by cisplatin. As the test method is simple and cheaper, the estimation of α-GST in random urine samples may be particularly useful for identifying

1. Introduction

Cisplatin (cis-dichlorodiamimine-platinum II) is an effective antineoplastic agent used to treat various solid tumors. Its chief dose limiting side effect is nephrotoxicity, which usually hinders the use of higher doses[1]. Acute kidney injury (AKI) is a common condition with a high risk of death. The most common etiologies of AKI include nephrotoxin administration and ischemia due to infections, cardiac disease, including myocardial infarction, cardiogenic shock, congestive heart failure, sepsis, unresolved pre-renal factors and liver diseases [2-6]. The frequency of AKI is increasing all over the world. Despite significant improvements in therapeutics, the mortality and morbidity associated with AKI remains high[7],[8] . In critically ill patients AKI is multi-factorial in origin and carries a high mortality.

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 early diagnosis of AKI are serum creatinine and creatinine clearance. It has been recognized these routinely used measures of renal function, significantly increase only after substantial kidney injury occurs and with a time delay [8 -11]. The lack of early biomarkers of AKI has impaired our ability to initiate potential therapeutic or preventive interventions in the nephrotoxicity in a timely manner. Early intervention can significantly improve the prognosis. A major reason for the mortality and morbidity is the lack of early markers for AKI, resulting in an unacceptable delay in initiating therapy [12-15].

* Corresponding Author : Saleena Ummer V
Assistant Professor – Senior scale
Dept. of MLT
Manipal College of Allied Health Sciences
MANIPAL UNIVERSITY
Manipal – 576 104
Karnataka, INDIA
E.mail: finduvsaleena91@gmail.com

Increased excretion of urinary enzymes is often used to detect nephrotoxicity and renal disease. Such measurements of enzyme activity in urine provide a sensitive assessment for renal tubular cell damage, thereby enabling detection of sub-clinical tubular injury also[16],[17]. Glutathione-S-Transferase(GST) enzymes are important in the conjugation of many electrophilic substances, including cytotoxic drugs, herbicides, and carcinogens, with reduced glutathione. In man alpha isoenzyme of GST (α GST) is present in the proximal convoluted tubules of kidney. The Alpha-GST is not normally present in urine, but appears in various forms of tubular injury, including ischaemia, cis-platinum toxicity, Gentamicin toxicity and after exposure to heavy Metal[16-19].

Gamma Glutamyl Transpeptidase(GGT) is an enzyme primarily located in the brush border of the proximal convoluted tubules of the kidney. Its unique localisation in the renal cells made easily damaged by ischaemia or nephrotoxin and its ease of assay provides the rationale for its use in the measurement of renal injury. Increased excretion of GGT in urine implies injury to the brush border membrane with loss of microvillous structure[16],[17],[20].

Cisplatin induced kidney injury is found to be a better model for studying the efficacy of different options for detecting kidney injury as the time of exposure of cisplatin is known, and the onset of injury and its progress also can be monitored . Hence we designed this study to evaluate the efficiency of renal tubular cell specific enzymes α-Glutathione-s-transferase(α-GST) and Gammaglutamyl transpeptidase(GGT) in detecting kidney injury in cancer patients treated with cisplatin, by studying the urinary excretion of α-GST and GGT at different time intervals and by comparing that with the changes in serum creatinine in cisplatin treated patients.

Cell specific biomarkers can predict toxic insults earlier and can provide information on sites of injury. Hence the urinary levels of α-GST and GGT, 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. Patients with any pre-existing renal insufficiency and on any other nephrotoxic drugs are excluded from the study. All the included patients were planned for treatment with concurrent chemo-radiotherapy. The main outcome measure was the identification of patients with clinically diagnosed AKI based on AKIN criteria [21].

2.1. Chemotherapy

All the selected patients were planned for concurrent chemo-radio therapy with single agent cisplatin, administered at a dose of 100 mg/m^2, 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 cisplatinum infusion. 40 mEq of potassium (KCl) and 5mg of magnesium ($MgSO_4$) salts also were added to each litre of the intravenous hydration fluid.

2.2 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 adminsitration 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.

The serum creatinine was estimated using buffered kinetic Jaffé reaction without deproteinization by fully automated Cobas 6000 (C501) auto analyzer. α GST activity was estimated in all the urine samples by colorimetric kinetic assay using 7- chloro-4-nitrobenzo-2-oxa-1,3-diazole(NBD-Cl)[18]. Urine samples were analysed for GGT activity by the method of colorimetric kinetic assay using gamma-glutamal-p-nitroanilide and glycylglycine [19,20]

2.3 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 ROC plot. An AUC of >0.7 was considered as the good performance of the markers in predicting AKI. 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 chemo-radiation 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. 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.

Fig. 1a – Temporal patterns of urinary α-GST in AKI and Non-AKI

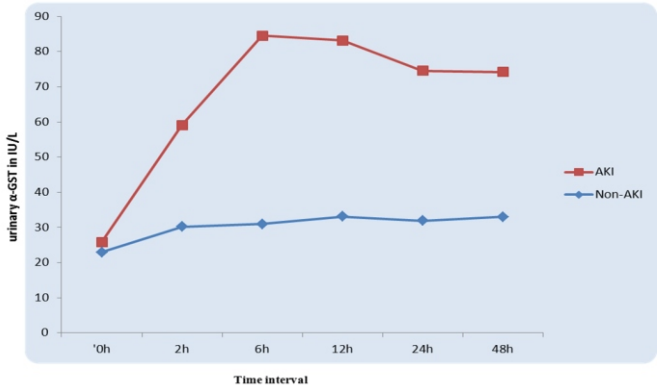


Fig. 1b – Temporal patterns of urinary GGT in AKI and Non-AKI

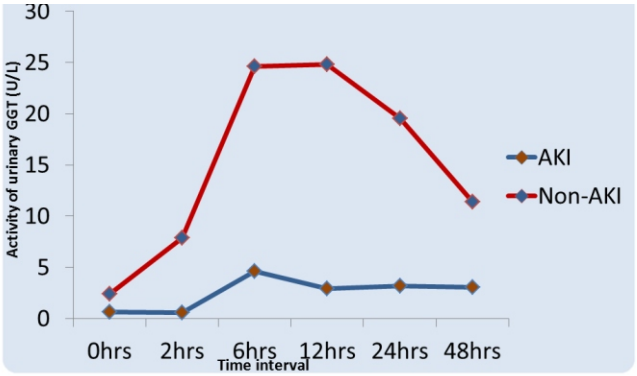
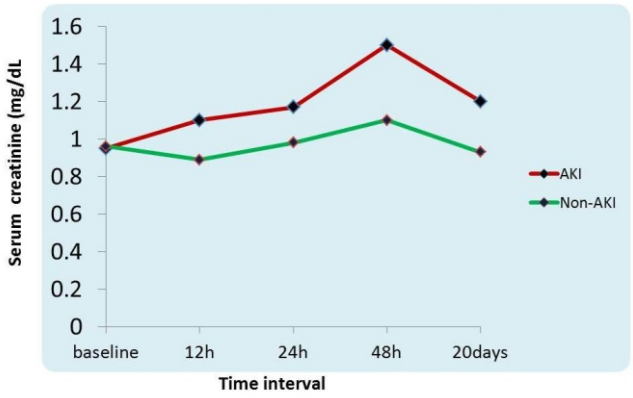


Fig. 1c – Temporal patterns of serum creatinine in AKI and Non-AKI



3.2 Evaluation of urinary levels of tubular enzymes:

All the 438 urine samples, 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 α-GST and GGT activities. Using elevation of serum creatinine at 48 hours as the standard, the sensitivity, specificity, positive predictive value and negative predictive value of both the urinary markers for all the six samples were studied The mean urinary enzymes' activities in AKI group at different time intervals show a clear temporal rise, ie, α-GST activity was increased after 2 hours after cisplatin administration and reached peak at 6h, and at a slower rate thereafter (Figure. 1a) and GGT levels showed a steep increase upto 6 hours before gradually declining over the next 48 hours after cisplatin administration among patients who eventually developed AKI(Figure. 1b). Whereas the significant increase in serum creatinine was observed only after 48hrs (Figure. 1c). In Non-AKI group there was no much difference in mean enzyme activities before and after cisplatin administration.

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

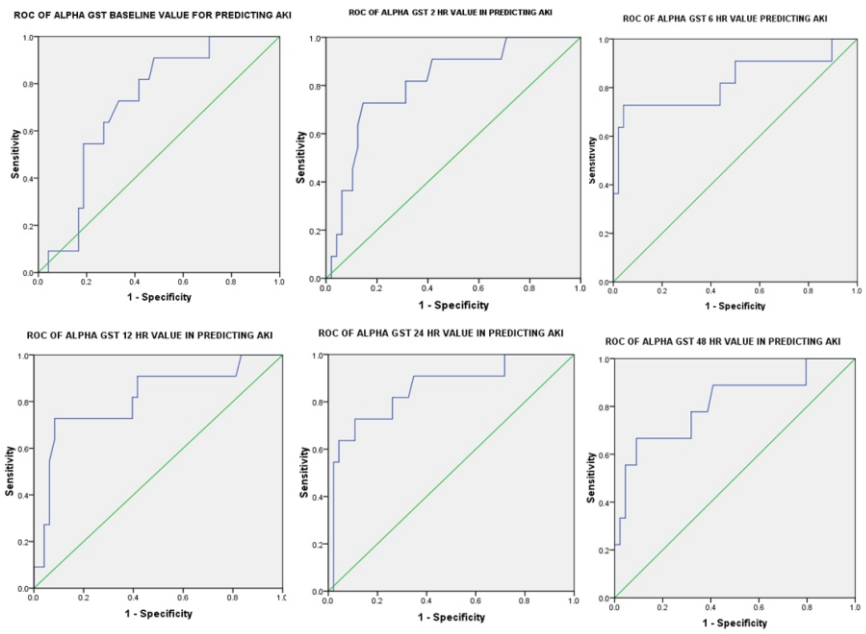


Table 1: The performance charcteristics of urinary αGST

Time Interval	Area Under ROC	Optimal Cut Off (JU/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	56.7	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. 3 – ROCs with AUC of urinary GGT at different time intervals

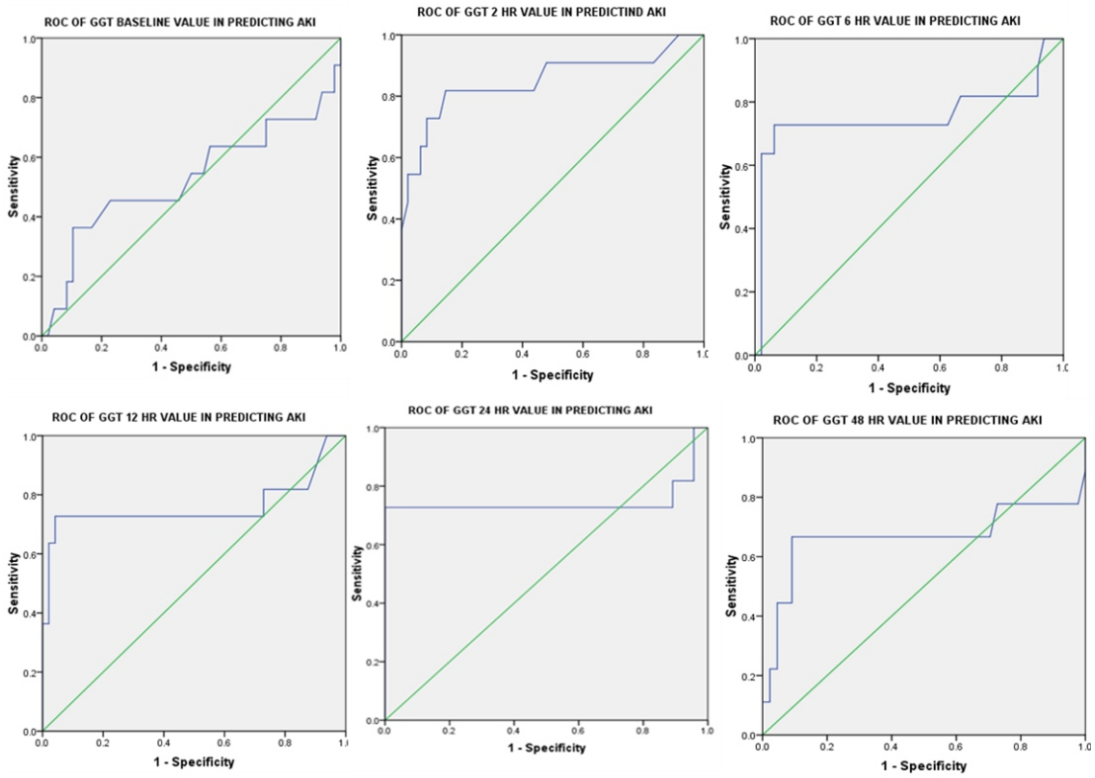


Table. 2 – Performance of urinary GGT as a marker for AKI

GGT	AUC	Optimal cut off (IU/L)	Sensitivity (%)	Specificity (%)	Positive predictive value %)	Negative predictive value %)
GGTb	0.527	≥5.6	54.5	45.8	18.8	81.5
GGT 2h	0.850	≥8.1	81.8	66.0	56.2	95.3
GGT 6h	0.755	≥14.7	72.7	66.7	61.5	93.5
GGT 12h	0.759	≥24.6	72.7	64.6	80.0	93.9
GGT 24h	0.745	≥34.2	72.7	63.0	91.2	93.9
GGT 48h	0.667	≥20.9	66.7	68.2	60.0	93.0

The performance of urinary enzyme activities was studied by estimating the area under ROC (AUC) at different time intervals. AUC > 0.7 indicate good performance of the marker. For urinary α-GST an AUC > 0.81 was obtained for all the timed samples after 2hrs. Therefore urinary α-GST from 2hrs till 48hrs after cisplatin administration performed as a good predictor of AKI(figure. 2).

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, whereas the specificity (from 57% to 61%) was comparatively less (Table.1).The sample collected at 24hours showed a better sensitivity and specificity in detecting acute kidney injury.

For urinary GGT, an AUC of more than 0.75 was obtained for all the timed samples after 2hrs till 24hrs. But the AUC of urinary GGT after 48hrs was less than 0.7(0.66). Therefore urinary GGT from 2hrs till 24hrs after cisplatin administration performs as a good predictor of AKI, whereas that may not be able to detect AKI after 48hrs (figure. 3).

The sensitivity of GGT ranged from 66% to 82% between the different temporal groups, whereas the specificity from 63% to 68.2% (Table.2).The sample collected at 6hours showed a better sensitivity and specificity in detecting acute kidney injury.

4.Discussion

Head and neck cancers are known to increase in incidence as age increases. The mean age of our patients in the study was 46.4 years. When sex wise distribution was assessed, we found that nearly 90% of the patients were males. It is known that the head and neck cancers are more common in males as supported by the fact that the use of tobacco and alcohol is more common in male population among Indian patients. Concurrent chemoradiation has become the mainstay of treatment for loco-regional advanced head and neck cancers. Cisplatin is the most commonly used chemotherapeutic agent with radiotherapy. It is a well-known nephrotoxic drug. In our study we have used cisplatin (100mg/m^2) as the chemosensitizer. This dose of cisplatin administered is known to affect the incidence of nephrotoxicity

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 [21]. In our study the selected patients were grouped into, those with AKI and without AKI, based on the AKIN criteria. We 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[6-8]. 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 been 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 [17], there is no reported data of urinary α-GST and GGT 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 and GGT levels and a direct comparison with subsequent changes in serum creatinine.

5.Conclusion

Our results clearly indicate that urine α-GST and GGT are useful early predictors of AKI that precedes the increase in serum creatinine by several hours. The magnitude of rise supports the notion that urinary enzymes α-GST and GGT are highly discriminatory biomarkers. And also urinary diagnostics have several advantages, including the non-invasive nature of sample collection and the reduced number of interfering proteins.

Even though, 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. It will be also important to confirm our findings in documented high risk settings. It is possible that a collection of other strategically selected candidates along with these enzymes will prove of value for early and rapid diagnosis of AKI.

Acknowledgements

We are thankful to **Dr. B Rajashekhar,** DEAN, MCOAHS-Manipal, MU and **Dr.(Sr.) Licy CD**, Prof. and Head Dept. of MLT, MCOAHS-Manipal, MU for their support.

6.References

[1] Ash DC. Cis-platinum(II) diamminedichloride: clinical review of a new cancer chemotherapeutic agent. J. Clin. Hematol. Oncol. 1980; 10: 55–62.

[2] Vishal S Vaidya, Sushrut S Waikar et al. Biomarkers for sensitive and specific detection of acute kidney injury in humans. Clin transl sci. 2008 Dec ;1(3):200-208

[3] Boventre J. Diagnosis of acute kidney injury : from classic parametres to new biomarkers; acute kidney injury. Contrib. Nephro.2007; vol 156:p 213-219.

[4] Jorge Cerda, Norbert lameire. Epidemiology of acute kidney injury. Clin J Am Soc Nephro. 2008;3: 881-886.

[5] Al-homrany M . Epidemiology of acute renal failure in hospitalized patients ; Experiences from sourthern Saudi Arabia, East Meditterr Health J.2003; 9: 1061-1067.

[6] Kohli HS, Bhat A, Jairam A, Aravindan AN, Sud K, Gupta , Sakhuja V. Predictors of mortality in acute renal failure in a developing country ; a prospective study, renal fail.2007; 29:4-469.

[7] Sushrut S, Waikar , Kathleen D Liu, Glen M Chertow. Diagnosis, epidemiology and outcomes of acute kidney injury. Clin J Am Soc Nephro. 2008; 3(13):844-861.

[8] Chertowgm, Burdick E, Honour M , Bonventre JV, Bates DW. Acute kidney injury ,mortality, length of stay, and costs in hospitalized patients . J Am Soc Nephro. 2005; 16:3365-3570.

[9] Wang Y, Cui Z, Fan M. Hospital acquired and community acquired acute renal failure in hospitalized chinese ; a ten year review, renal fail. 2007; 29:163-169.

[10] Jaya Mishra, Kiyoshi Mori, Qing Ma, Caitlin Kelly, Janathan Barasch, Devrajan. Neutrophil gelatinase associated lipocalin ; A novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephro. 2004; 24:307-315

[11] Bruce A, Molitoris , Melnikov et al .Technology insight; biomarkers development in acute kidney injury – what can we anticipate ? Nat Clin pract Nephro. 2008; 4(3) nature publishing group.

[12] Cerda J, Bagga A , Kehr V , Chakravathi RM. The contrasting characteristics of acute kidney injury in developed and developing countries . Nat Clin pract Nephro. 2008

[13] Nickolos , Thomas L , Barasch , Jonathan , Devarajan. Biomarkers in acute and chronic kidney disease. Current opinion in nephrology and hypertension. 2008 March ; vol 17 (2): p-127-132

[14] Devrajan . Proteomics for biomarkers discovery in acute kidney injury . Seminars in Nephro . vol 27(6): page 637-651.

[15] Mai T, Nguyen and Prasad Devrajan. Biomarkers for early detection of acute kidney injury. Pead Nephro. 2008 Dec; vol 23: no.12.

[16] Wilfred. K. Gatua, Joseph N, Makumi , Eliud M Njagi , Christine S Kigondu, Seth O Mcligeyo, Stanley K Waithaka. Evaluation of Urinary Tubular Enzymes as Screening Markers of Renal Dysfunction in Patients Suffering from Diabetes Mellitus. Asian Journal of Medical Sciences. 2011; 3(3): 84-90.

[17] Justin Westhuyzen1, Zoltan H Endre, Graham Reece , David M Reith , David Saltissi , Thomas J Morgan. Measurement of tubular enzymuria facilitates early detection of acute renal impairment in the intensive care unit. Nephrol Dial Transplant. 2003; 18: 543–551.

[18] Amanda JW, Branten Theo PJ, Mulder Wilbert HM, Peters Karel JM, Assmann Jack FM Wetzels. Urinary Excretion of Glutathione –S-Transferases Alpha and Pi in Patients with Proteinuria: Reflection of the Site of Tubular Injury. Nephron. 2000;85:120–126

[19] Banibrata Mukhopadhyay, Shashikant Chinchole, Valentine Lobo, Sishir Gang, Mohan Rajapurkar. Enzymuria pattern in early post renal transplant period: Diagnostic usefulness in graft dysfunction. Indian Journal of Clinical Biochemistry. 2004; 19 (2): 14-19

[20] Vivek Ambade, Parduman Singh, BL Somani, Dashrath Basannar. Urinary n-acetyl beta glucosaminidase and gamma glutamyl transferase as early markers of diabetic nephropathy. Indian Journal of Clinical Biochemistry. 2006; 21 (2): 142-148

[21] Mehta RL, Kellum JA, Shah S, Molitoris BA, Ronco C, et al. Acute Kidney Injury Network (AKIN): report of an initiative. In AKIN Summit. Amsterdam:AKIN Comm.2006.