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Urinary enzymes as early diagnostic marker for cisplatin induced renal damage : a comparison with Cystatin C

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Abstract

Background. Serum cystatin C have been proposed to be early diagnostic marker for cisplatin induced renal injury, but it is not widely available. Urinary enzymes which are markers of tubular damage viz. urinary GGT/ALP/LDH and their creatinine standardized ratio can serve as early diagnostic markers for renal tubular damage as seen in cisplatin induced renal injury.

Methods. Serum and urine samples were taken from 30 cancer patients on Day 1 Day 3 Day 7 after cisplatin first dose. Determination of serum creatinine, cystatin c and urinary ALP, LDH and GGT creatinie ratio was done .

Results. Standardised GGT,ALP,LDH and serum creatinine was significantly higher at day 3 and day 7 compared to day 1 ($p < 0.0001$) also on day 7 was significantly higher compared to day 3 ($p < 0.0001$). Cystatin C was significantly higher at day 3 and day 7 compared to day 1 ($p < 0.05$) also on day 7 was significantly higher compared to day 3 ($p < 0.05$).

Conclusions. Urinary GGT, ALP and LDH creatinine standardised ratio and serum cystatin C are early diagnostic maker for cisplatin induced renal injury but GGT and ALP have better diagnostic importance on day 3 and day 7 respectively.

Keywords. Cisplatin renal injury, cystatin C, creatinine, urinary enzymes cratinine ratio.

Introduction

Cis -diammine- dichloro-platinum (cisplatin) has had a major impact in cancer medicine, changing the course of several solid tumors, such as those of ovary, testes, and the head and neck.(1) Cisplatin chemotherapy is limited by tumor cells resistance and severe side effects such as nephrotoxicity, neurotoxicity, ototoxicity, and emetogenicity.(2) Among these factors, nephrotoxicity has been reported as the major limiter in cisplatin therapy.(3) In fact, it has been reported that the concentration of cisplatin in epithelial tubular cells is fivefold higher than in blood.(4) Impairment of the renal function is found in approximately 25–35% of patients treated with a single dose of cisplatin.(5) Compounding the clinical problem is the fact that the primary indicator of nephrotoxicity is an increase in serum creatinine, which is insensitive and occurs relatively late for intervention to occur.(6)

Serum Cystatin C represents a more sensitive clinical marker than serum creatinine for the early assessment of GFR damage caused by cisplatin.(7) Serum Cystatin-C does not depend on muscle mass, diet, or gender. (8) The urinary GGT activity after cisplatin administration was significantly increased.(9) The urinary activity of GGT, ALP and LDH raises after cisplatin adjuvant chemotherapy.(10) Alkaline phosphatase a phosphohydrolase enzyme attached to the cell wall by glycosyl phosphatidyl inositol anchors, and lactate dehydrogenase, a key enzyme in energy metabolism located in the cell cytoplasm, are more prominent even before the appearance of microalbuminuria.(11) Gamma glutamyl transferase in urine originates from the surface of brushy border of epithelial cell membrane in the proximal tubules lumen. Gamma GT is a specific and sensitive indicator of these tubular cell damages.(12) Serum Cystatin C have been proposed to be early diagnostic marker for cisplatin induced renal injury, but it is not widely available. Urinary enzymes which are markers of tubular damage viz. urinary GGT/ALP/LDH creatinine standardized ratio can serve as early diagnostic markers for ischemic tubular damage as seen in Cisplatin induced renal injury.

Materials and methods

The study was conducted in the Department of Biochemistry, Pt. J.N.M. Medical College and DR. B.R.A.M. Hospital, Raipur, C.G. from June 2019 to August 2019 among patients who admitted in Radiotherapy ward, Cancer Hospital, DR. B.R.A.M. Hospital, Raipur (C.G.) 30 subjects undergoing cisplatin based chemotherapy**.** Study was conducted after the approval of institutional scientific and ethical committee.

The following patients were included in study.

- Patients older than 18 years having indication for cisplatin based chemotherapy.
- Histologically diagnosed non-small cell lung cancer, bladder cancer or head and neck cancer, gynaecological cancer.

The following patients were excluded from this study

- Those who have already received cytotoxic therapy for other conditions.
- Those have an estimated GFR <45 ml/min (according to CKDEPI equation).
- Known case of nephropathy of other cause eg. Diabetic nephropathy, hypertensive nephropathy etc.

Specimen collection and preparation

Three ml of venous blood samples after overnight fasting in plain vacutainer and early morning second urine sample – spot will be collected in a sterile container on Day 1, Day 3 and Day 7. Serum will be separated after allowing the samples to clot for 30 minutes at room temperature and then centrifuging the samples at 1500 rpm for 10 minutes. The serum thus obtained will be utilized for determination of serum urea, serum creatinine. Serum sample will be stored in labled aliquoted tubes and stored in a refrigerator at -20 degree celcius for estimation of serum cystatin C as per the manufacturer protocols.

Assay/ Reagents

- **Serum Cystatin C** was measured by the sandwich enzyme immunoassay for the quantitative measurement of human Cystatin C.
- **Urine Gamma Glutamyl transferase** was measured by Szazs/Persijn method using fully automated biochemistry I-Lab 650 autoanalyzer.

- **Urine Alkaline Phosphate** was measured by kinetic method recommended by IFCC, using fully automated biochemistry I-Lab 650 autoanalyzer.
- **Urine Lactate Dehydrogenase** was measured by optimized method according to "Deutsche Gesellschaft für klinische Chemie" (DGKC). using fully automated biochemistry I-Lab 650 autoanalyzer
- **Serum Urea** was measured by Urease / GLDH methodology, using fully automated biochemistry I-Lab 650 autoanalyzer.
- **Serum and urinary Creatinine** was measured by - Modified Jaffe's method, using fully automated biochemistry I-Lab 650 autoanalyzer.

Results

The present study was carried out on 30 patients with first cycle of cisplatin chemotherapy. The blood and urine samples collected on Day 1, Day 2 and Day 7 after first dose of 50mg cisplatin.

Out of 30 cancer patients 26 were of carcinoma cervix, 3 were oral cancer and 1 of tongue cancer.

Serum creatinine was done at day0, day1, day3, day7. On day 0 minimum 0.5mg/dL and maximum 1.1mg/dL with mean 0.76 and standard deviation of 0.14. On day1 min. 0.5 mg/dL and max. 1.1 mg/dL with mean 0.75 mg/dL and std. deviation of 0.13. On day 3 min. 0.6mg /dL and max. 1.2 mg/dL with mean 0.84 mg/dL and std. deviation of 0.12. On day 7 min. 0.8 mg/dL and max. 1.3 mg/dL with mean 0.97 mg/dL and std. deviation 0.13. Comparison of serum creatinine at different interval of time was performed using ANOVA for Paired measures. Serum creatinine was significantly higher on day 3 compared to day zero($p < 0.0001$) and day one($p < 0.0001$). Also serum creatinine was significantly higher on day 7 compared to day 0 ($p < 0.0001$), day 1($p < 0.0001$) and day 3 ($p < 0.0001$).

Standardised GGT (GGT/Creatinine) at Day 1, Day 3 and Day 7 . On day 1 minimum zeroIU/g and maximum 76.27IU/g with mean 11.22IU/g and standard deviation 13.46. On day 3 minimum zeroIU/g and maximum 237.5IU/g with mean 64.73IU/g and standard deviation 55.58. On day 7 minimum 17.24IU/g and maximum 332.02IU/g with mean 120.90IU/g and standard deviation 72.30. Comparison of standardised GGT (GGT/Creatinine) at different time interval from cisplatin therapy was performed using Friedman test. Significant difference was observed thus post hoc anlaysis further revealed that standardised GGT was significantly higher at day 3 and day 7 compared to day 1 ($p < 0.0001$) . Also STD GGT on day 7 was significantly higher compared to day 3 ($p < 0.0001$).

Standardised ALP (ALP/Creatinine ratio) at Day 1, Day 3 and Day 7 from cisplatin therapy. On day 1 minimum zeroIU/g and maximum 15.25IU/g with mean 3.73IU/g and standard deviation 3.67. On day3 minimum zeroIU/g and maximum 48.89IU/g with mean 18.60IU/g and standard deviation 12.16. On day 7 minimum zeroIU/g and maximum 197.92IU/g with mean 61.13IU/g and standard deviation 47.91. Comparison of standardised ALP (ALP/Creatinine ratio) at different time interval from cisplatin therapy was performed using ANOVA for repeated measures. Significant difference was observed thus post hoc analysis further revealed that standardised ALP was significantly higher at day 3 and day 7 compared to day 1($p < 0.0001$) also STD ALP on day 7 was significantly higher compared to day 3($p <$

0.0001).

Standardised LDH (LDH/Creatinine ratio) at Day 1, Day 3 and Day 7 from cisplatin therapy. On day 1 minimum 1.68IU/g and maximum 520.27IU/g with mean 44.78IU/g and standard deviation 96.40. On day 3 minimum 7.77IU/g and maximum 2023.47IU/g with mean 196.75IU/g and standard deviation 366.08. On day 7 minimum 26.64 IU/g and maximum 1945.10IU/g with mean 329.46IU/g and standard deviation 423.44. Comparison of standardised LDH (LDH/Creatinine) at different time interval from cisplatin therapy was performed using Friedman test. Significant difference was observed and post hoc anlaysis further revealed that standardised LDH was significantly higher at day 3 and day 7 compared to day 1($p < 0.0001$) also STD LDH on day 7 was significantly higher compared to day 3($p < 0.0001$).

Cystatin C at Day 1, Day 3 and Day 7 from cisplatin therapy. On day 1 minimum 495ng/mL and maximum 1874ng/mL with mean 814.97ng/mL and standard deviation 262.80. On day 3 minimum 622ng/mL and maximum 5971ng/mL with mean 1204.50ng/mL and standard deviation 937.72. On day 7 minimum 701ng/mL and maximum 9953ng/mL with mean 1558.90ng/mL and standard deviation 1622.66. Comparison of serum cystatin C at different time interval from cisplatin therapy was performed using Friedman test. Significant difference was observed and post hoc anlaysis further revealed that serum cystatin C was significantly higher at day 3 and day 7 compared to day 1($p<0.05$) also serum cystatin C on day 7 was significantly higher compared to day 3($p<0.05$).

Table 1: ROC curve analysis for diagnostic significance of various paramters at day 1 post cisplatin therapy.

Test Result Variable(s)	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval		Cutoff	Sensitivity	Specificity
				Lower Bound	Upper Bound			
SCr D1(mg/dl)	.315	.151	.300	.019	.610	1.0	100	7.4
STD GGT D1(IU/g)	.790	.116	.104	.563	1.017	8.545	100	55.6
STD ALP D1(IU/g)	.494	.118	.972	.263	.725	5.385	100	29.6
STD LDH D1(IU/g)	.506	.150	.972	.213	.799	35.865	100	29.6
CYS D1 (ng/ml)	.667	.135	.351	.402	.931	837	100	40.7

At day 1 AUC of cystatin c (0.667), standardised GGT (0.79), standardised ALP (0.494), standardised LDH (0.506) and creatinine (0.315).

Table 2: ROC curve analysis for diagnostic significance of various paramters at day 3 post cisplatin therapy.

Test Result Variable(s)	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval		Cut-off	Sensitivity	Specificity
				Lower Bound	Upper Bound			
SCr D3(mg/dl)	.589	.117	.678	.360	.818	0.85	39.3	100
STD GGT D3(IU/g)	.732	.100	.280	.536	.929	41.6	64.3	100
STD ALP D3(IU/g)	.625	.201	.561	.230	1.020	4.39	89.3	50
STD LDH D3(IU/g)	.643	.235	.506	.183	1.103	14.75	96.4	50
CYS D3 (ng/ml)	.536	.096	.868	.348	.723	1041.5	50	100

At day 3 AUC of cystatin c (0.536), standardised GGT (0.732), standardised ALP (0.625), standardised LDH (0.643) and creatinine (0.589).

Table 3: ROC curve analysis for diagnostic significance of various parameters at day 7 post cisplatin therapy.

Test Result Variable(s)	Area	Std. Error	Asymptomatic Sig.	Asymptomatic 95% Confidence Interval		Cut-off	Sensitivity	Specificity
				Lower Bound	Upper Bound			
SCr D7(mg/dl)	.337	.110	.223	.121	.553	1.25	100	0
STD GGT 7(IU/g)	.771	.118	.043	.539	1.003	88.6	83.3	70.8
STD ALP 7(IU/g)	.854	.082	.008	.694	1.014	45.83	100	58.3
STD LDH 7(IU/g)	.722	.130	.097	.467	.978	155.8	83.3	62.5
CYS D7 (ng/ml)	.444	.129	.678	.192	.697	1509.5	83.3	25.0

At day 7 AUC of cystatin c (0.444), standardised GGT (0.771), standardised ALP (0.854), standardised LDH (0.722) and creatinine (0.337).

Discussion

The present study was done with an aim to evaluate urinary enzymes as early diagnostic marker for cisplatin induced renal damage. Serum creatinine was significantly higher on day 3 compared to day zero($p < 0.0001$) and day one($p < 0.0001$). Also serum creatinine was significantly higher on day 7 compared to day 0 ($p < 0.0001$), day 1($p < 0.0001$) and day 3 ($p < 0.0001$).

Comparison of standardised GGT (GGT/Creatinine) at different time interval from cisplatin therapy was performed using Friedman test. Significant difference was observed thus post hoc anlaysis further revealed that standardised GGT was significantly higher at day 3 and day 7 compared to day 1 ($p < 0.0001$) . Also STD GGT on day 7 was significantly higher compared to day 3 ($p < 0.0001$).

Comparison of standardised ALP (ALP/Creatinine ratio) at different time interval from cisplatin therapy was performed using ANOVA for repeated measures. Significant difference was observed thus post hoc analysis further revealed that standardised ALP was significantly higher at day 3 and day 7 compared to day 1($p < 0.0001$) also STD ALP on day 7 was significantly higher compared to day 3($p < 0.0001$).

Comparison of standardised LDH (LDH/Creatinine) at different time interval from cisplatin therapy was performed using Friedman test. Significant difference was observed and post hoc anlaysis further revealed that standardised LDH was significantly higher at day 3 and day 7 compared to day 1($p < 0.0001$) also STD LDH on day 7 was significantly higher compared to day 3($p < 0.0001$).

Comparison of serum cystatin C at different time interval from cisplatin therapy was performed

using Friedman test. Significant difference was observed and post hoc anlaysis further revealed that serum cystatin C was significantly higher at day 3 and day 7 compared to day 1($p<0.05$) also serum cystatin C on day 7 was significantly higher compared to day 3($p<0.05$).

According to Table 1: ROC curve analysis for diagnostic significance of various paramters at day 1 post cisplatin therapy. Maximum diagnostic significance at day 1 was shown by standardised GGT (AUC=79.0%) , AUC of cystatin c was less (66.7%), AUC of standardised LDH (AUC=50.6%), AUC of standardised ALP (AUC=49.4%) and AUC of serum creatinine is 31.5%. Diagnostic cut off of GGT was found to be 8.54 IU/g of creatinine and sensitivity at this cut off was 100% and specificty was 55.6%. Diagnostic cut off of Cystatin C was found to be 837 ng/mL and sensitivity at this cut off was 100% and specificty was 40.7%. Diagnostic cut off of LDH was found to be 35.86 IU/g of creatinine and sensitivity at this cut off was 100% and specificty was 29.6%. Diagnostic cut off of ALP was found to be 5.38 IU/g of creatinine and sensitivity at this cut off was 100% and specificty was 29.6%. Diagnostic cut off of serum creatinine was found to be 1.0mg/dL and sensitivity at this cut off was 100% and specificty was 7.4%.

In present study according to Table 2: ROC curve analysis for diagnostic significance of various paramters at day 3 post cisplatin therapy. On day third maximum diagnostic significance was noted with Standardised GGT (AUC=73.2%). It was even higher than Cystatin C (AUC =53.6), AUC of Standardised LDH (64.3%), AUC of Standardised ALP(62.5%) and AUC of serum creatinine(58.9%). The diagnostic cutoff of GGT was found to be at 41.6IU/g of creatinine and sensitivity was 64.3 % while specificity was 100% at this cut off. The diagnostic cutoff of LDH was found to be at 14.75U/g of creatinine and sensitivity was 96.4 % while specificity was 50% at this cutoff. The diagnostic cutoff of ALP was found to be at 4.39IU/g of creatinine and sensitivity was 89.3 % while specificity was 50% at this cutoff. The diagnostic cutoff of serum creatinine was found to be at 0.85mg/dL and sensitivity was 39.3 % while specificity was 100% at this cutoff. The diagnostic cutoff of CystatinC was found to be at 1041.5ng/mL of creatinine and sensitivity was 50% while specificity was 100% at this cut off.

In present study according to Table 3: ROC curve analysis for diagnostic significance of various paramters at day 7 post cisplatin therapy. Maximum diagnostic significance at day 7 was shown by standardised ALP (AUC 85.4%) while standardised GGT showed AUC=77.1% , AUC of cystatin c was even less (44.4%), AUC of standardised LDH(72.2%) and AUC of serum creatinine(33.7%). Diagnostic cut off of standardised ALP was found to be 45.83 IU/g of creatinine and sensitivity at this cut off was 100% and specificity was 58.3%. Diagnostic cut off of standardised GGT was found to be 88.6 IU/g of creatinine and sensitivity at this cutoff was 83.3% and specificity was 70.8%. Diagnostic cut off of standardised LDH was found to be 155.8 IU/g of creatinine and sensitivity at this cutoff was 83.3% and specificty was 62.5%. The diagnostic cutoff of CystatinC was found to be at 1509.5ng/mL of creatinine and sensitivity was 83.3% while specificity was 25% at this cutoff. The diagnostic cutoff of serum creatinine was found to be at 1.25mg/dL and sensitivity was 100 % while specificity was 0% at this cutoff.

Conclusions.

Serum creatinine is levels are significantly raised compared to baseline on day 3 and day 7.

There is significant increase in standardised urinary GGT, ALP, LDH on Day 3rd and Day 7th

compared with their day 1. There is also significant increase in serum cystatin C on day 3rd and Day 7th compared with its day 1.

Maximum diagnostic significance at day 1 is shown by standardised GGT (AUC=79.0%) , AUC of cystatin c is less (66.7%). On day third maximum diagnostic significance is noted with Standardised GGT (AUC=73.2%). It is even higher than Cystatin C (AUC =53.6). Maximum diagnostic significance at day 7 is shown by standardised ALP (AUC 85.4%). It is even higher than Cystatin C (AUC =44.4%).

Conflict of interest-

Acknowledgements-

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