Urine Ratio of Neutrophil Gelatinase-associated Lipocalin to Creatinine as a Marker for Early Detection of Cisplatin-associated Nephrotoxicity

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Introduction. Cisplatin is a widely used chemotherapeutic agent with a major side effect of nephrotoxicity. Delayed increase in serum creatinine after cisplatin injection makes serum creatinine not to be an ideal marker for early detection of cisplatin nephrotoxicity. Recently several new biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) have been proposed for early detection of acute kidney injury (AKI). This study assessed kinetic of urine NGAL-creatinine ratio in patients who received cisplatin-containing chemotherapy.

Materials and Methods. Patients with a glomerular filtration rates greater than 45 mL/min who received cisplatin-containing chemotherapy were included. Urine creatinine and NGAL concentrations were measured before cisplatin infusion and 6, 24, 48, and 72 hours after cisplatin administration. To minimize hydration effects, urine NGAL levels were adjusted according to urine creatinine.

Results. Twenty-four patients were assessed. According to the Acute Kidney Injury Network criteria, 2 patients (8%) experienced cisplatin-associated AKI. The median increases in urine NGAL-creatinine ratio were 335% (interquartile range, 320% to 350%) in the patients with AKI and 100% (interquartile range, 73% to 190%) in those without AKI (P = .02) during the first 24 hours after cisplatin administration. A urine NGAL-creatinine ratio greater than 26.9 ng/mg 24 hours after cisplatin infusion had a sensitivity of 86% and a specificity of 50% to detect cisplatin-associated nephrotoxicity.

Conclusions. Urine NGAL-creatinine ratio significantly increased in patients with cisplatin-associated AKI. Urine NGAL-creatinine ratio within the first 24 hours after cisplatin infusion may better predict cisplatin-associated nephrotoxicity than serum creatinine level.

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INTRODUCTION

Cisplatin is a commonly used potent chemotherapeutic agent that has been proved to manage several solid tumors including bladder, cervical, endometrial, esophageal, lung, head, neck, ovarian, and testicular carcinoma.[1] Cisplatin-induced adverse effects such as gastrointestinal side effects, nephrotoxicity, ototoxicity, neurotoxicity, and myelosuppression may complicate chemotherapy courses.[1,2] Kidney is

the major organ for cisplatin elimination. Cisplatin accumulates in the proximal and distal tubule cells and causes a range of mild reversible to irreversible renal impairment. Therefore, cisplatin nephrotoxicity is a treatment-limiting toxicity that may manifest as increased serum creatinine and blood urea nitrogen concentrations, urinary electrolyte wasting, and decreased renal blood flow and glomerular filtration rate (GFR).[2,3] Patients' hydration and management of serum electrolyte disturbances resulted in decreased incidence of cisplatin nephrotoxicity from 70% in earlier reports to 20% to 30% in more recent studies.[4]

Increased serum creatinine concentration and decreased GFR usually happens 48 to 72 hours after cisplatin administration[5]; therefore, serum creatinine is not an ideal marker for early detection of cisplatin nephrotoxicity. Recently several new biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), ratio of serum to urine cystatin C, urinary kidney injury molecule-1, and interleukin-18 have been proposed for early detection of acute kidney injury (AKI).[6] Neutrophil gelatinase-associated lipocalin is produced by injured proximal renal tubular epithelial cells.[7] Since cisplatin affects the proximal and distal epithelial cells, urine NGAL concentration may increase following cisplatin administration.[1] This study was designed to evaluate kinetics of urine NGAL-creatinine ratio in patients who received cisplatin-containing chemotherapy.

MATERIAL AND METHODS

Patients

This cross-sectional study was conducted at Imam Khomeini Hospital Complex affiliated to Tehran University of Medical Sciences, Tehran, Iran. Patients with Karnofsky performance status greater than 70% and a GFR greater than 45 mL/min who received cisplatin-containing chemotherapy were included. Patients who were administered nephrotoxic agents including aminoglycosides, amphotericin B, acyclovir, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, nonsteroidal anti-inflammatory drugs, contrast media, or bisphosphonates were excluded from the study. All enrolled patients received 2 L of intravenous normal saline at the day before cisplatin infusion and 1 L of normal saline with 20 mEq of potassium as potassium chloride and 8 mEq of magnesium as magnesium sulfate on the day of cisplatin infusion as the department protocol. The study protocol was approved by the local Ethics Committee of Tehran University of Medical Sciences. All patients provided written informed consent forms.

Nephrotoxicity Assessment

Urine samples were collected from each patient just before cisplatin infusion, in order to assess urine creatinine and NGAL concentrations, and 6, 24, 48, and 72 hours after cisplatin administration. To minimize hydration effects, urinary NGAL levels were adjusted according to urine creatinine concentrations. Serum creatinine concentrations were measured before cisplatin infusion and 24, 48, and 72 hours after cisplatin administration. Patients were followed for cisplatin-induced AKI during hospitalization that was up to 72 hours after cisplatin administration. Patients were assessed 21 days after cisplatin administration upon their new admission for the next chemotherapy course regarding reversal of cisplatin-induced AKI of previous chemotherapy course. After collection, urine samples were immediately centrifuged at 10 000 rpm for 10 minutes and were frozen at -70°C for future analysis.

In the present study, the Acute Kidney Injury Network (AKIN) criteria were used for detection of cisplatin-associated AKI. Based on the AKIN criteria, stage 1 AKI includes patients who experience 0.3 mg/dL or greater increase in serum creatinine or at least 50% increase in serum creatinine level compared with baseline value or urine output of less than 0.5 mL/kg/h for 6 hours within 48 hours. Patients with more than 100% increase in serum creatinine concentration or urine output of less than 0.5mL/kg/h for at least 12 hours are categorized into stage 2 AKI. Patients with 3-time increase in serum creatinine level or serum creatinine level of at least 4 mg/dL or who need renal replacement therapy or suffering anuria for at least 12 hours are considered to be in stage 3 of AKI.[8] Based on the recommendation of the European Society of Clinical Pharmacy Special Interest Group on Cancer Care, we measured GFR using the Modified Diet in Renal Disease formula.[5]

Statistical Analysis

The Shapiro-Wilk test was used to assess normality of distribution of data. Baseline

characteristics and biochemical data of the patients with and without cisplatin-associated AKI were compared by the independent *t* test. Correlations between quantitative variables were assessed by the Pearson or the Spearman correlation test, where appropriate. The Fisher exact test was used for comparing nominal variables. Data from multiple urine NGAL-creatinine ratio samples were compared by the repeated-measure analysis of variance. These analyses were done using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, Ill, USA). The receiver operating characteristic curves were constructed with the Sigma Plot trial version 12.3. Data are expressed as mean ± standard deviation or median (interquartile range). *P* values less than .05 were considered significant.

RESULTS

A total of 24 patients with newly diagnosed cancer who received cisplatin-containing chemotherapy courses were included in the study. All of the patients received 50 mg/m^2 to 80 mg/m^2 of cisplatin in 21-day interval courses. Demographic and baseline biochemical characteristics of the participants are shown in Table 1. According to the AKIN criteria, 2 patients (8%) experienced cisplatin-associated AKI, 1 showed stage 1 and the other stage 2 of AKI. All baseline characteristics except for serum magnesium concentrations were similar between the patients with and without cisplatin-induced AKI (Table 1).

Compared to baseline values, GFR significantly decreased in the patients with AKI from 7.0 ± 13.13 mL/min before cisplatin administration to 47.5 ± 1.60 mL/min 48 hours after drug administration (*P* = .03) and to 1.33 ± 11.48 mL/min, 72 hours after cisplatin infusion (*P* = .03). Serum creatinine level was 0.78 ± 0.15 mg/dL in AKI patients versus 0.85 ± 0.21 mg/dL in non-AKI patients before cisplatin administration (*P* = .58) and 0.95 ± 0.49 mg/dL versus 1.05 ± 0.18 mg/dL 24 hours after cisplatin infusion (*P* = .85). The mean serum creatinine concentration was significantly higher in patients with cisplatin-associated AKI compared to patients without AKI 48 hours after cisplatin infusion (1.40 ± 0.05 mg/dL versus 1.02 ± 0.20 mg/dL; *P* =.02) and 72 hours after cisplatin infusion (1.30 ± 0.14 mg/dL versus 0.92 ± 0.19 mg/dL; *P* = .01). However, 21 days after cisplatin infusion, the mean serum creatinine concentrations were not different between the

Table 1. Baseline Characteristics and Biochemical Data of Studied Participants With and Without Cisplatin-associated Acute Kidney Injury (AKI)

Parameter	All patients	Patients With AKI (n = 2)	Patients Without AKI (n = 22)	*P*
Age, y	53.54 ± 8.33	49.8 ± 1.18	54.52 ± 7.7	.25
Female (%)	15 (63)	1 (50)	14 (64)	.99
Weight, kg	58.9 ± 7.8	6.2 ± 6.1	58.57 ± 8.3	.13
Malignancy				
Upper gastrointestinal	18	1	17	
Ovary	5	0	5	
Mesothelioma	1	1	0	.90
Serum creatinine, mg/dL	0.92 ± 0.18	0.78 ± 0.15	0.85 ± 0.21	.58
Glomerular filtration rate, mL/min	67.75 ± 15.76	7.00 ± 13.13	67.21 ± 16.37	.87
Serum sodium, mEq/L	139.82 ± 3.77	139.33 ± 3.28	143.50 ± 1.00	.06
Serum potassium, mEq/L	4.20 ± 0.38	4.05 ± 0.22	4.23 ± 0.40	.48
Serum magnesium, mg/dL	2.10 ± 0.18	2.08 ± 0.16	2.40 ± 0.10	.02
Serum calcium, mg/dL	8.60 ± 0.62	8.80 ± 0.77	8.64 ± 0.62	.84
Serum albumin, g/dL	4.07 ± 0.50	3.50 ± 0.28	4.10 ± 0.40	.48
Cisplatin dose, mg	82.91 ± 23.49	10.00 ± 12.20	78.42 ± 23.86	.07
Leukocyte count, ´ 10^9/L	7.44 ± 4.47	12.40 ± 6.74	6.10 ± 2.61	.01
Hemoglobin, g/dL	11.37 ± 1.11	11.66 ± 0.96	11.30 ± 1.16	.87
Platelet count, ´10^9/L	265.88 ± 88.29	294.80 ± 133.00	258.20 ± 75.74	.23
Serum aspartate aminotransferase, U/L	24.41 ± 8.11	26.20 ± 7.16	23.94 ± 9.13	.07
Serum alanine aminotransferase, U/L	23.12 ± 8.42	22.60 ± 8.80	23.26 ± 9.13	.53
Serum alkaline phosphatase, U/L	242.33 ± 82.71	283.8 ± 122.54	231.42 ± 69.28	.22
Total bilirubin, mg/dL	0.84 ± 0.25	1.08 ± 0.40	0.78 ± 0.15	.02

patients who experienced and those who did not experience cisplatin-associated AKI (1.00 ± 0.53 mg/dL versus 1.03 ± 0.18 mg/dL, respectively; *P* = .84). The mean urine NGAL-creatinine ratio at the initiation of the study was 11.33 ± 3.85 ng/mg in all the assessed patients. At the beginning of the study, urine NGAL-creatinine ratios were similar in patients with and without cisplatin-associated AKI (Table 2). Urine NGAL-creatinine ratio significantly increased in the patients with cisplatin-associated AKI, but not in patients without AKI within 24 hours after cisplatin infusion (Table 2 and Figure 1). The median increases in urine NGAL-creatinine ratio were 335% (320% to 350%) in the patients with AKI and 100% (72.8% to 190%) in the patients without AKI during the first 24 hours after cisplatin administration (*P* = .02).

The area under the receiver operating characteristic curves are shown in Figure 2 for urine NGAL-creatinine ratio at 24 hours after cisplatin administration (area, 0.80; 95% confidence interval, 0.6 to 0.95; *P* =.048). A urine NGAL-creatinine ratio of 26.9 ng/mg 24 hours after cisplatin infusion had a sensitivity of 86% and a specificity of 50% to detect cisplatin-associated nephrotoxicity.

Although not statistically significant, the mean administered dose of cisplatin was higher in patients who experienced cisplatin-associated AKI compared with cisplatin dose of patients without AKI (10.0 ± 12.2 mg versus 78.42 ± 23.86 mg; *P* =.07).

Except for serum albumin concentration, occurrence of cisplatin-associated AKI showed no correlation with the patients' demographic and biochemical data such as age, weight, body surface area, baseline GFR, or serum electrolytes levels (Table 3).

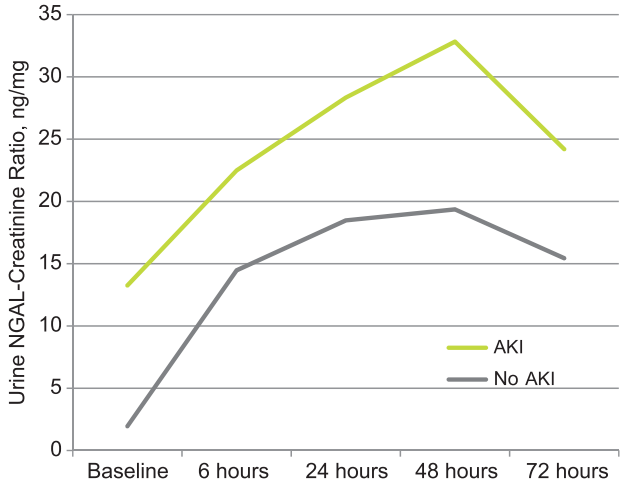

Figure 1. Urine neutrophil gelatinase-associated lipocalin (NGAL)-creatinine ratio since cisplatin administration in participants with and without cisplatin-associated acute kidney injury (AKI)

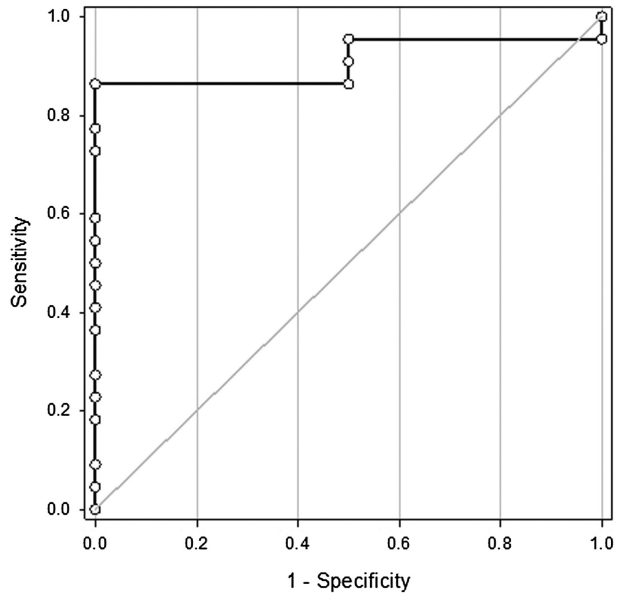

Figure 2. The receiver operating characteristic curve for neutrophil gelatinase-associated lipocalin (NGAL)-creatinine ratio 24 hours after cisplatin infusion (area under curve, 0.80; 95% confidence interval, 0.60 to 0.95; *P* = .048).

DISCUSSION

Cisplatin-associated nephrotoxicity occurs in 2 distinct phases; the first phase happens within the first 24 to 48 hours after cisplatin administration

Table 2. Urine Neutrophil Gelatinase-associated Lipocalin (NGAL)-Creatinine Ratio Since Cisplatin Administration in Participants With and Without Cisplatin-associated Acute Kidney Injury (AKI)

NGAL-Creatinine, ng/mg	Patients With AKI (n = 2)	Patients Without AKI (n = 22)	*P*
Baseline	13.25 ± 3.88	1.95 ± 3.73	.13
6 hours	22.49 ± 3.67	14.47 ± 5.31	.06
24 hours	28.34 ± 2.08	18.48 ± 5.75	.01
48 hours	32.83 ± 1.02	19.36 ± 6.67	.01
72 hours	24.20 ± 2.82	15.44 ± 5.19	.03

Table 3. Correlation Between Acute Kidney Injury and Patients' Demographic and Laboratory Data

Parameter	Correlation Coefficient	*P*
Age	-0.165	.44
Sex	-0.078	.72
Weight	-0.082	.70
Body surface area	0.008	.97
Serum albumin	-0.400	.04
Serum potassium	0.180	.48
Serum sodium	-0.410	.10
Serum magnesium	-0.360	.07
Serum calcium	0.050	.84

as polyuria and decreased urine osmolality, while GFR remains unchanged. During the second phase that happens 72 to 96 hours or later after drug infusion, serum creatinine gradually increases and GFR gradually decreases.[5] Therefore, detecting cisplatin-induced AKI using serum creatinine-derived GFR is a delayed finding. Some researchers have tried to find other markers for detection and severity stratification of cisplatin nephrotoxicity.[6,9]

Similar to Moon and coworkers' study,[10] only 8% of our study population experienced cisplatin-induced AKI. In contrast, Gaspari and colleagues[11] and Lin and colleagues[12] reported occurrence of cisplatin nephrotoxicity in 25% and 30% of their patients, respectively. This difference may be due to different definition of AKI in this study compared with others.[11,12] The two previous studies defined AKI as 25% increase in serum creatinine concentration from baseline values after cisplatin infusion.[11,12] This cutoff is not routinely used in clinic setting. During past several years more commonly used criteria for detection of AKI were the AKIN and the RIFLE.[8] We used AKIN criteria in this study that categorizes patients as suffering AKI with at least 0.3 mg/dL or 50% increased from baseline values in serum creatinine concentration. This higher cutoff change in serum creatinine for AKI definition resulted in lower number of patients with cisplatin- associated AKI in our study. If we used the previous definition,[11,12] 5 patients (20%) would be classified as showing cisplatin-associated AKI. Another reason for low incidence of cisplatin nephrotoxicity in this study may be the good performance status of our patients. Recently, low performance status of the patient has been proposed as a predisposing risk factor for cisplatin-associated nephrotoxicity.[13] In the present study, patients who experienced cisplatin-induced AKI showed increased urine NGAL-creatinine ratio within the first 24 hours after cisplatin infusion, while patients with stable kidney function did not show any significant changes in urine NGAL-creatinine ratio.

These results were consistent with the findings of a preliminary animal study by Mishra and colleagues.[14] They showed that urine NGAL concentration increased 3 hours after cisplatin injection.[14] We adjusted urine NGAL concentration by urine creatinine level to eliminate effects of patients' hydration status.[7] Although human data are limited, similar results were seen in 2 other small studies.[11,12] In the first human study, Gaspari and associates found that urine NGAL concentration increased over 1000% within the first day after cisplatin infusion in patients who suffered cisplatin-induced AKI, while no significant changes were seen in urine NGAL of patients with stable kidney function.[11] In the present study, urine NGAL concentration increased by about 3 times after cisplatin infusion. Less increase in urine NGAL concentrations in our patients may be due to including patients with good performance status and baseline normal kidney function or only mild kidney dysfunction.[13]

Lin and colleagues found that urine NGAL-creatinine ratio increased 12 hours after cisplatin infusion. The magnitude of increase in urine NGAL-creatinine ratio in their study was 64.6 ± 54.6 ng/mg 12 hours after cisplatin infusion.[12] Our results were more consistent with Lin and colleagues'[12] compared to Gaspari and colleagues' findings.[11] The present study showed that urine NGAL-creatinine ratio at a cutoff point of 26.9 ng/mg 24 hours after cisplatin injection has suitable sensitivity and specificity for early detection of cisplatin-associated nephrotoxicity. Our study, however, suffers several limitations including small sample size and short time of patients' follow-up of 72 hours that limited us to detect patients who showed AKI later.

CONCLUSIONS

Results of the present study showed that urine NGAL-creatinine ratio significantly increased in patients with cisplatin-associated AKI and this ratio may help us for early detection of cisplatin-associated nephrotoxicity. Urine NGAL-creatinine ratios greater than 26.9 ng/mg 24

hours after cisplatin infusion had a high and a moderate specificity to detect cisplatin-associated nephrotoxicity. However, further studies with larger sample sizes are needed to confirm our findings.

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CONFLICT OF INTEREST

None declared.

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