Is Kidney Injury Molecule 1 a Valuable Tool for the Early Diagnosis of Contrast-Induced Nephropathy?

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Aim/Scope: Contrast-induced nephropathy (CIN) is a common complication of diagnostic/therapeutic procedures. Serum creatinine levels are sensitive but often lead to diagnostic delays in acute kidney injury and potential misclassification of actual injury status. Kidney injury molecule (KIM-1) is a novel early marker of acute kidney injury. The aim of our study was to evaluate the KIM-1 levels in patients with CIN. We performed a single-center, nested case-control study.

Materials and Methods: Three thousand two hundred patients who had undergone coronary angiography were included in the study. Thirty-two patients were diagnosed with CIN. Twenty patients who had undergone coronary angiography but did not have CIN were evaluated as a control group (n = 20). The diagnosis of CIN was performed according to the KDIGO 2012 Acute Kidney Injury Guideline criteria. Urinary KIM-1 levels were measured by enzyme-linked immunosorbent assay before as well as on the 6th and 48th hours of contrast exposure. Serum creatinine levels were measured before as well as on the 24th and 48th hours after angiographic procedure.

Results: We demonstrated that KIM-1 levels increased in the patients with CIN significantly on the sixth hour when compared with the baseline ($P < 0.01$; median levels, 0.27 and 0.70 mg/dL) but not in the controls ($P = 0.107$). The precontrast and 48th-hour KIM-1 levels were median ones and were also significantly different ($P = 0.001$, the median levels were 0.27 and 0.60 mg/dL, respectively).

Conclusions: Because creatinine is a sensitive but a late marker of CIN, KIM-1 may be used for early diagnosis and early initiation of treatment and may reduce risk for morbidity.

Key Words: acute kidney Injury, contrast-induced nephropathy, kidney injury molecule 1

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Contrast-induced nephropathy (CIN) is usually recognized by an acute deterioration in renal function 2 to 7 days after contrast exposure in the absence of an alternative cause of acute renal damage. It is the third most common cause of acute renal failure (ARF) in hospital patients.[1] The incidence of CIN in the general population is 1.7% to 2.3%, but when focusing on specific high-risk patients, especially diabetes mellitus (DM), the incidence can increase to more than 50%.[2] Contrast-induced nephropathy has been shown to be associated with an increased risk of a prolonged hospital stay, an increased need for dialysis, a potential risk of nosocomial complications, expensive health care costs, and mortality.[3,4]

The most frequently accepted clinical standard for the definition and diagnosis of CIN is usually defined by an increase of serum creatinine or a reduction of urine output, which was a definition proposed by the Kidney Disease Global Improving Outcome (KDIGO).[5] Although very small increments in serum creatinine have a significant impact on morbidity, the diagnosis can be made by a 0.3-mg/dL increase in serum creatinine levels after 48 to 72 hours of exposure.[6] This condition causes a delay for the required treatment modalities such as volume expansion. In addition, because of its poor sensitivity and specificity, serum creatinine is incapable of comprehensively reflecting the time and type of renal injury. Moreover, serum creatinine is also affected by some other factors, such as age, as well as acute and chronic renal failure.[5] These data suggest that more reliable and efficient measurement for CIN diagnosis is urgently required.[7]

Kidney injury molecule 1 (KIM-1), a type 1 transmembrane protein, was originally found as a putative epithelial cell on the proximal tubules of the kidney,[8] which was absent in normal function, but markedly up-regulated in the S3 segment after ischemic injury.[9] It was proposed as a novel indicator of acute kidney injury over the conventional biomarker such as blood urea nitrogen, serum creatinine (Cr), urinary albumin, low-molecular-weight excreted proteins (α1- and β2-microglobulins [MG]) and tubular enzymes *N*-acetyl-β-D-glucosaminidase (NAG).[10,11] This molecule has a markedly increased urinary secretion in cisplatin, cyclosporin, chrome, cadminium toxicity, and acute allograft rejection.[12] However, there is very limited report of KIM-1 study in the human population being exposed to contrast media. Thus, we aimed to show the relationship between CIN and urinary KIM-1 levels.

MATERIALS AND METHODS

Study Population

This prospective study was conducted between January 2012 and July 2013 at the Division of Coronary Angiography, Department of Cardiology, Turgut Ozal University Hospital, Ankara, Turkey. Subjects who had undergone coronary arterial intervention, which needed contrast use, were enrolled into the study. Iodixanol was used as a contrast material in all patients. Contrast-induced nephropathy is defined according to the KDIGO 2012 criteria as an increase in serum creatinine by 0.3 mg/dL from a baseline level. These criteria are graded to require a larger increase in serum creatinine according to the baseline value. Thirty-two patients were diagnosed as having CIN. A total of 120 control blood and urine samples were collected, but we failed to obtain informed consent from 94 patients to publish the medical records. Because of that, they were excluded from the study. Six blood samples that belonged to different patients were hemolysed, and we could not study their serum creatinine levels. After these exclusions, we have compared the patient group with 20 controls. Written informed consent was obtained from all the participants, and the institutional review board for each participating center approved the study protocol.

Study Design

The exclusion criteria included age of less than 18 years, chronic dialysis therapy, having unclear history of any renal

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TABLE 1. Demographic and Clinical Characteristics of Patients According to Study Groups

Variables	CIN Group (n = 32)	Control Group (n = 20)	*P*
Age, mean (SD), y	67.5 (10.1)	61.6 (9.4)	0.041
Sex, n (%)			0.070
Men	11 (34.4)	12 (60.0)	
Women	21 (65.6)	8 (40.0)	
Coexisting DM, n (%)	26 (81.3)	17 (85.0)	1.000
Coexisting HT, n (%)	—	1 (5.0)	0.385
Coexisting HL, n (%)	17 (53.1)	11 (55.0)	0.895
Coexisting PVD, n (%)	2 (6.3)	1 (5.0)	1.000

HL indicates hyperlipidemia; HT, hypertension; PVD, peripheral vascular diseases.

disease and primary percutaneous intervention because of myocardial infarction. The patients who were receiving comfort measures only and those for whom therapeutic interventions were considered futile by the caregivers were also excluded.

The KDIGO 2012 Acute Kidney Injury criteria were used to diagnose CIN. The urinary KIM-1 and the serum creatinine were measured before as well as on the 24th and 48th hours of contrast media exposure.

Data Collection

The medical records of the study participants were reviewed prospectively to retrieve hospitalization data, including baseline demographic characteristics; coexisting diseases, especially DM; and renal variables, including serum creatinine values and urinary sediment findings. At the time of enrollment, the contributing causes of ARF were determined according to the notes of the internal medicine consulting service.

Processing and Storage of Urinary Samples

Fresh urinary samples were obtained at the time of enrollment as well as at the 6th and 48th hours of contrast exposure, and these were centrifuged immediately to remove any insoluble elements after the routine test-strip urinalysis. The supernatant was stored at −80°C until assayed.

Measurement of Urinary KIM-1 Level

The urinary KIM-1 measurements were performed using microsphere-based Luminex xMAP technology with polyclonal antibodies raised against the human KIM-1 ectodomain. This technique is an adaptation of the sandwich ELISA assays (Human KIM-1 ELISA kit; SunRed Biotechnology Company, Shangai, China). For measurements, 40 μL of the urine samples were analyzed in duplicate.

The lowest limit for the detection of this assay was 0.125 ng/mL. The interassay and intra-assay variability was less than 10%. The urinary KIM-1 level was expressed in absolute terms and also normalized to the urinary creatinine concentration.

Statistical Analyses

The SPSS for Windows 11.5 packet program was used to analyze the data. The Kolmogorov-Smirnov test was used to investigate whether the continuous and discrete numeric variables were close to normal.

The difference between the groups regarding the mean value was assessed with a Student *t* test, whereas the significance of the difference in the median values was assessed by the Mann-Whitney *U* test. The categorical variables were analyzed using the Pearson and Fisher χ^2 test. To see whether there was statistically significant correlation between the continuous and discrete numeric variables, the Spearman correlation test was used. The Wilcoxon sign test was used to analyze the significant difference among the baseline, 6th-hour, and 48th-hour laboratory tests of the CIN group.

The results were expressed as mean (SD) or percentages. The figures were graphically displayed as box and whisker plots. The differences were considered statistically significant at $P < 0.05$.

RESULTS

Overall Characteristics of the Cohort

A total of 3200 patients were enrolled between January 2012 and July 2013 in the coronary angiography unit. The subjects from whom baseline, 6th-hour, and 48th-hour blood and urine samples were obtained were regularly assessed for urinary KIM-1 levels (n = 120, 3.75% of total). Thirty-two patients were evaluated as having CIN according to the KDIGO 2012 guidelines (1% of the overall patients, and 26.6% of the study group). The controls were subjects who had been exposed to contrast but not the altered renal function tests (n = 20). The mean (SD) age was 64.5 (9.7) years; 44.2% were male (n = 23), and 55.8% were female (n = 29). No significant difference was observed according to sex ($P = 0.70$), but the CIN group had a significantly higher mean age when compared with the controls ($P = 0.041$). Baseline hemoglobin level was 13.1 (2.2) mg/dL for CIN group 13.8 (1.8) mg/dL for the control group ($P = 0.200$). In addition, the groups were similar in terms of coexisting diseases such as hypertension, hyperlipidemia, and DM. The demographic and clinical features of the patients are compared in Table 1.

Characteristics of the Study Group According to KIM-1 Levels

Figures 1 and 2 display the characteristics of the study group according to the urinary KIM-1 levels. At the baseline, the patients

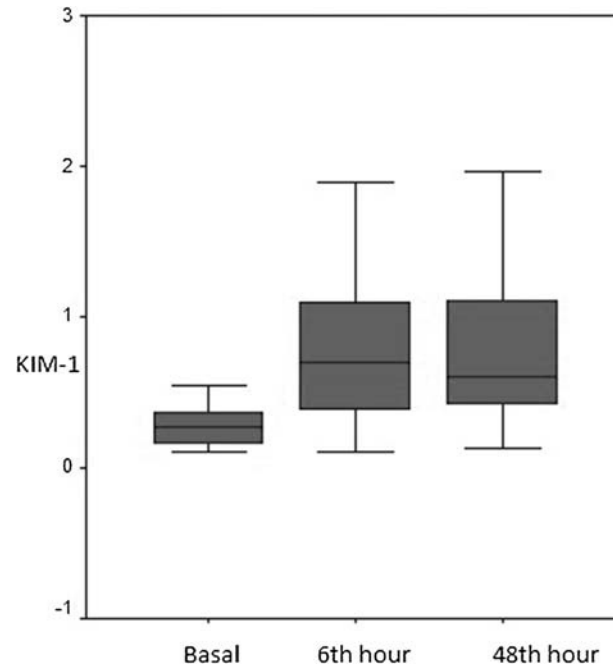

FIGURE 1. Distribution of baseline, 6th-hour, and 48th-hour KIM-1 levels in the CIN group.

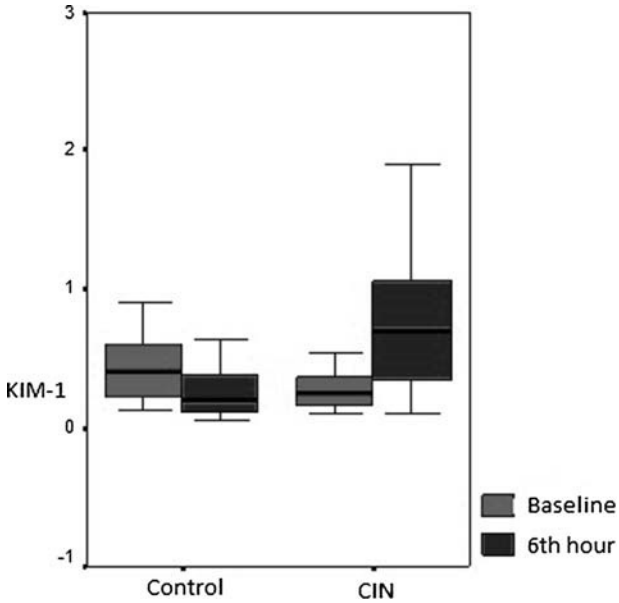

FIGURE 2. Distribution of baseline and sixth-hour KIM-1 levels between patients and controls.

and controls had similar urinary KIM-1 levels, but the CIN group had significantly elevated urinary KIM-1 levels after the 6th and 48th hours of contrast exposure (Table 2, $P < 0.001$). The serum creatinine levels were elevated in the 48th-hour specimens, but not those in the 6th-hour specimens. The 6th- and 48th-hour urinary KIM-1 levels were similar in the CIN group ($P = 0.249$). In addition, the diagnostic performance values of the sixth-hour KIM-1 levels were also significant (Table 3).

We analyzed the area under the curve with receiver operating characteristic analysis to determine whether urinary KIM-1 levels were statistically decisive for differentiating groups. The best cut-off point for the sixth-hour urinary KIM-1 levels was determined by using Youden index. Negative and positive predictive values, sensitivity, and specificity were significant in this value (Fig. 3).

According to these data, there may be a relationship between the CIN and KIM-1 levels. However, there were also some coexisting conditions such as DM, hypertension, and angiotensin-converting enzymes. These factors may also have affected the renal function tests and urinary KIM-1 levels besides the contrast exposure. When we excluded the contrast exposure, there was no relationship between these coexisting factors and the KIM-1 levels

TABLE 2. Follow-up Measurements of Serum Creatinine, Urea, and Urinary KIM-1 Levels in the CIN Group

Follow-up	KIM-1	Creatinine	Urea
Baseline	0.27 (0.10–0.94)	0.9 (0.5–1.9)	37 (20–197)
6th hour	0.70 (0.11–5.40)	1.0 (0.9–2.6)	43 (25–167)
48th hour	0.60 (0.13–1.97)	1.2 (0.6–2.9)	40.5 (24–290)
Multiple comparisons*			
Baseline to 6th hour	$P < 0.001$	$P = 0.1$	$P = 0.004$
Baseline to 48th hour	$P < 0.001$	$P < 0.001$	$P = 0.002$
6th hour to 48th hour	$P = 0.608$	$P < 0.001$	$P = 0.249$

*$P < 0.017$ was accepted as statistically significant according to Bonferroni.

TABLE 3. Diagnostic Performance Value of Sixth-Hour KIM-1 Levels to Distinguish CIN and Control Groups

Indicators	Definitions	6th-Hour KIM-1
Area under the curve		0.797
95% confidence interval		0.677–0.917
P		<0.001
Best breakpoint		>0.366
No. cases	N	52
Sensitivity	TP / (TP + FN)	24/32 (75.0%)
Specificity	TN / (TN + FP)	15/20 (75.0%)
PPV	TP / (TP + FP)	24/29 (82.8%)
NPV	TN / (FN + TN)	15/23 (65.2%)
Accuracy	(TP + TN) / (N)	39/52 (75.0%)
P		<0.001

FN indicates false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

(Table 4). However, the correlation between the age and the KIM-1 levels continued to exist after excluding the other factors (Table 5).

DISCUSSION

The present study described the urinary KIM-1 level as an early marker for the diagnosis of CIN. This report examined the utility of the kidney injury marker for the diagnosis at a clinically relevant point in the course of the CIN and the time of the nephrology consultation. The results indicate that in patients with established CIN, the urinary KIM-1 level is a useful surrogate for the diagnosis of CIN and has a facilitative utility that is similar to or better than conventionally used markers such as the urine output and serum creatinine level.

These findings are of particular relevance because in clinical practice, nephrologists often encounter patients with established CIN, namely, after a rise in serum creatinine. Moreover, the diagnosis of CIN may be missed, especially in outpatients who use

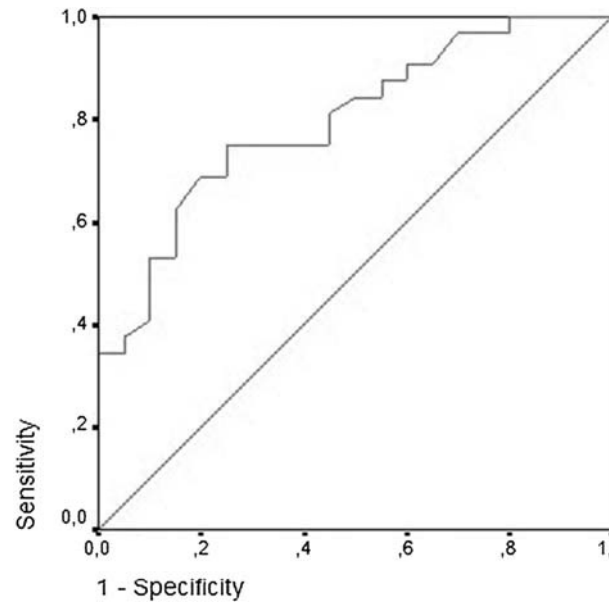

FIGURE 3. Receiver operating characteristic curve of sixth-hour KIM-1 levels to differentiate the CIN group.

contrast-exposed diagnostic/therapeutic procedures such as coronary angiography. Early precise estimation of the diagnosis of CIN can potentially facilitate the targeted introduction of conventional and novel biologic therapies to patients who are most likely to benefit.

Urinary KIM-1, however, is a more recently described type 1 cell membrane glycoprotein that is expressed in humans and rodents when the injured renal proximal tubule assumes a dedifferentiated phenotype.[13] As previously reported, KIM-1 is strongly up-regulated in the proximal tubular epithelial cells during various states that are characterized by epithelial cell dedifferentiation, including ischemia, toxic renal injury, polycystic kidney disease, and renal cell carcinoma.[14,15] The preliminary data from humans have revealed that KIM-1 is a sensitive and specific marker for the early detection of acute kidney injury after cardiopulmonary bypass surgery and, in that context, is superior to urinary NAG activity.[16] Although this molecule is effective in determining pure ischemic damage to the kidney and CIN has a complex pathophysiology,[17] the dominant mechanism is medullary ischemia in the renal damage of CIN.

There are some studies that test the hypothesis of whether urinary KIM-1 levels are associated with the early diagnosis of CIN. In the rat model, the urinary KIM-1 levels were elevated in the second hour of contrast exposure.[18] Although KIM-1 levels were elevated at the 24th hour of cardiac catheterization in one study,[19] we found that the urinary KIM-1 levels were significantly elevated at the 6th hour of contrast exposure ($P < 0.01$). Because the timing of enrollment depended on the nephrology consultation, there was likely to be considerable heterogeneity in the time lapsed from the occurrence of acute kidney injury, to the development of ARF, and to the urine sampling.

Advanced age is an important risk factor for CIN.[20,21] In our study, the CIN group had a significantly higher mean age when compared with the controls ($P = 0.041$; 67.4 vs 61.6 years). Undoubtedly, the incidence of other risk factors for CIN such as coronary artery disease, hypertension, and DM increases with age.

TABLE 4. Baseline and Sixth-Hour KIM-1 Levels According to Coexisting Diseases and Angiotensin-Converting Enzyme Inhibitors Use

Variables	Baseline	6th Hour
Sex		
Male	0.31 (0.10–0.90)	0.29 (0.10–2.16)
Female	0.30 (0.12–0.94)	0.64 (0.05–5.40)
*P**	0.641	0.043
Coexisting DM		
Excluded	0.25 (0.12–0.48)	0.68 (0.10–1.11)
Included	0.32 (0.10–0.94)	0.38 (0.05–5.40)
*P**	0.404	0.377
Coexisting HL		
Excluded	0.31 (0.10–0.94)	0.40 (0.10–5.40)
Included	0.30 (0.12–0.90)	0.45 (0.05–2.23)
*P**	0.835	0.474
ACEI use		
Excluded	0.24 (0.10–0.74)	0.28 (0.05–1.13)
Included	0.32 (0.13–0.94)	0.46 (0.06–5.40)
*P**	0.187	0.108

*$P < 0.017$ was accepted as statistically significant according to Bonferroni.

HL indicates hyperlipidemia.

TABLE 5. Evaluation of Sixth-Hour KIM-1 Levels on CIN Corrected According to Other Risk Factors by Multivariate Linear Regression Analysis

Variables	Odds Ratio	95% Confidence Interval		*P*
		Lower Limit	Upper Limit	
Age	1.099	1.010	1.195	0.028
Female factor	2.383	0.474	11.969	0.292
HCT	1.053	0.898	1.235	0.523
NLR	1.602	0.645	3.980	0.310
LDL	1.018	0.993	1.044	0.158
6th-hour KIM-1 > 0.366	9.630	1.811	51.210	0.008

Hct indicates hematocrit; LDL, low-density lipoproteins; NLR, neutrophil-lymphocyte ratio.

When we corrected the relationship between the risk of CIN and advanced age according to other risk factors with the multivariate analysis of linear regression, the significant relationship was proven ($P = 0.011$). Consequently, we showed that advanced age is important for the contrast-related renal damage.

Diabetes mellitus has been reported as a risk factor for CIN in many studies.[4,22,23] However, whether diabetic subjects who have a glomerular filtration rate of greater than 60 mL/min have an increased risk is controversial.[24] Nikolsky et al.[25] proved that diabetic patients who had a normal renal function test result had a 15% risk of CIN, and the insulin requirement is a risk factor for CIN. However, the general view is that DM is an accelerating but not an independent risk factor for CIN.[26] In our study, all the subjects had at least 1 risk factor, 83% having DM (81.3% in patients vs 85.3% in controls), and all of them had a glomerular filtration rate of greater than 60 mL/min. No significant difference was observed between the groups concerning DM. Although predominantly, they had DM and this may affect the comparisons, our results supported this contention.

CONCLUSIONS

Urinary KIM-1 seems to be a promising early prognostic marker in patients with CIN; especially, outpatients who take contrast need to be diagnosed for CIN on the same day of exposure. However, further investigation is required to establish the temporal factors that govern urinary KIM-1 excretion.

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