INTRODUCTION

The nephrotoxicity of radiographic contrast agents remains a serious clinical problem. Radiographic procedures utilizing contrast media are increasing for both diagnostic and interventional procedures, and may cause contrast-induced nephropathy (CIN) which is the third most common cause of hospital-acquired acute kidney injury (AKI). It is defined as an absolute increase in serum creatinine concentration of greater or equal to 0.5 mg/dL or by a relative increase of 25% or more from the baseline value within 48 hours. The current definition of CIN is based on the changes in serum creatinine levels after administration of intravenous iodinated contrast media. However, serum creatinine is more a marker of glomerular function rather than kidney injury. Factors such as changes in glomerular filtration rate (GFR), rate of tubular secretion, rate of generation and volume of distribution affect the rise in serum creatinine levels after AKI. Hence, large changes in GFR may be associated with relatively small changes in serum creatinine in the first 24-48 hours following AKI. This leads to not only in delay...
in diagnosis and intervention but also in estimating the degree of injury. Certain novel biomarkers have been identified for early detection of renal injury like neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), and cystatin C which have been proved to be specific and sensitive. However, these markers are costly and cannot be applied in routine clinical practice. Contrast media have been shown to be toxic to the renal tubular cells. Tubular injury can lead to excretion of lysosomal and brush border enzymes into the ultra filtrate and thus cause enzyme activities to increase in urine. Studies have demonstrated the clinical utility of urinary N-acetyl-β-D-glucosaminidase (NAG) in predicting AKI. However, the diagnostic utility of increased NAG after contrast administration in patients undergoing coronary interventions has not been evaluated. Hence the present study was taken to assess the utility of urinary enzymes i.e., NAG, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) as markers of tubular injury along with urinary microalbumin (MA) levels as markers of glomerular injury after contrast administration in patients undergoing coronary angiography (CAG) and/or angioplasty.

**MATERIAL AND METHODS**

Consecutive patients scheduled to undergo CAG and/or angioplasty in the Department of Cardiology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati during the period from January to December 2012 were considered for recruitment into the study. Patients scheduled for elective CAG, with or without angioplasty who had a baseline serum creatinine of ≤ 1.2 mg/dL (males) and ≤ 1.1 mg/dL (females) were included in the study. Patients with pre-existing renal disease, hypotension, hyperthyroidism, hypothyroidism, those on glucocorticoid therapy, cardiogenic shock, or allergy to contrast media were excluded. The study was approved by the Institutional Ethical Committee. Of the 2565 patients screened, 560 met the inclusion criteria. Of these, 315 were excluded as they were not willing for multiple sample collection and 100 patients were not willing to give the sample due to be collected at 48 hours. Of the 145 recruited patients, successive timed samples could not be collected in 25 patients due to non-compliance. The study could be completed in the remaining 120 patients. Written informed consent was obtained from all study participants. As per the institutional protocol, all the patients included in the study were recommended liberal oral intake of fluids as prophylaxis.

Baseline data was obtained from all subjects including height, weight, history of drug intake, history of co-morbid conditions like diabetes mellitus, hypertension, history of tobacco smoking and alcohol consumption were noted. Body mass index (BMI) (kg/m²) was calculated. A low-osmolal contrast agent, iohexol (Omnipaque® 320mg iodine/mL; Wipro GE Healthcare Private Limited, New Delhi) was administered and the amount of contrast medium used for each patient was recorded after the procedure.

Five mL of peripheral venous blood samples and urine sample were collected from all patients, just before the procedure (0 hours) and subsequently at 4, 24 and 48 hours after contrast administration. Serum was separated and stored at −80 °C until analysis. The urine was centrifuged at 3000 rotations per minute (rpm) for 10 min to remove any particulate material and stored at −80 °C until analysis. The u-NAG was measured by spectrophotometric stop rate reaction using a substrate 4-nitrophenyl N-acetyl-β-D-glucosaminide [PNP-NAG (N9376); Sigma-Aldrich, Co; St. Louis, MO, USA]. Absorbance at 420 nm was measured using a Lambda 25 UV-visual double beam spectrophotometer (Perkin ELMer, Singapore).
The u-ALP, u-LDH, u-MA and serum and urinary creatinine were measured using Beckman system packs on Synchron CX9 fully automated analyser (Beckman-coulter, CA, USA). Creatinine was measured by Jaffe’s rate method with calibration traceable to isotope dilution mass spectrometry (IDMS) reference method using the National Institutes of Standards and Technology (NIST) Standard Reference Material 967.9

Estimated glomerular filtration rate (eGFR) was calculated for all patients according to the Cockcroft-Gault formula (CG) [eGFR (mL/min/1.73m²) = (140–age) × weight (Kg) 72 × serum creatinine (mg/dL) × (0.85 if female)].9 CIN was defined as a 25% increase in serum creatinine concentration from the baseline value, or an absolute increase of at least 0.5 mg/dL within 48 hours after the administration of contrast media.2

Statistical analysis

Statistical analysis was performed using Microsoft Excel, MedCalc (version 13.2.2, Belgium) and Statistical Package for Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc, Chicago, IL, USA). Continuous variables which were normally distributed were expressed as mean ± SD and median (inter-quartile range) for those not having normal distribution. Categorical variables were expressed as frequency. Urinary analytes were corrected for creatinine to nullify the effect of urine volume changes over time which can influence their interpretation. The data were transformed to percentages taking the 0 hour value as 100% in order to remove the bias of confounding variables. The time course changes of each marker were compared using repeated measures analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test or non-parametric Friedman’s test and Wilcoxon’s signed-rank test for paired comparisons, as appropriate. Mann-Whitney U test was used to test the difference in medians between groups for timed samples. A p-value less than 0.05 was considered as statistically significant.

RESULTS

Baseline and clinical characteristics of the study population are presented in Tables 1 and 2. The CIN and non-CIN groups were comparable except that use of statins was more frequent in patients with CIN.

Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>CIN group (n=27)</th>
<th>Non-CIN group (n=93)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>52.8 ± 8.8</td>
<td>50.3 ± 8.9</td>
<td>0.194</td>
</tr>
<tr>
<td>Male, gender†</td>
<td>26 (96.2)</td>
<td>91 (97.8)</td>
<td>0.652</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>23.8 ± 3.6</td>
<td>23.8 ± 2.8</td>
<td>0.970</td>
</tr>
<tr>
<td>Presence of diabetes mellitus†</td>
<td>15 (55.5)</td>
<td>45 (48.4)</td>
<td>0.846</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td>118.1 ± 12.7</td>
<td>118.5 ± 12.8</td>
<td>0.911</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)*</td>
<td>76.3 ± 8.4</td>
<td>76.8 ± 7.9</td>
<td>0.768</td>
</tr>
<tr>
<td>Presence of hypertension†</td>
<td>1 (3.7)</td>
<td>7 (7.5)</td>
<td>0.473</td>
</tr>
<tr>
<td>Tobacco smoking†</td>
<td>8 (29.6)</td>
<td>48 (51.6)</td>
<td>0.052</td>
</tr>
<tr>
<td>eGFR by CG equation (mL/min)*</td>
<td>97.6 ± 25.8</td>
<td>89.1 ± 26.7</td>
<td>0.153</td>
</tr>
<tr>
<td>Presence of congestive heart failure†</td>
<td>4 (14.8)</td>
<td>16 (17.2)</td>
<td>0.771</td>
</tr>
</tbody>
</table>

* data are expressed as mean ± standard deviation
† data are expressed as No. (%)
CIN = contrast-induced nephropathy; eGFR = estimated glomerular filtration rate; CG equation = Cockcroft-Gault equation
The mean age of the study subjects was 51.3 ± 8.9 years. Most of the study subjects [n=117 (97.5%)] were males. All had normal baseline renal function with mean eGFR of 89.4 ± 26.0 mL/min. Among the 120 patients, 49 (40.8%) underwent coronary angiography, while 71 (59.1%) underwent angioplasty. The mean dose of contrast medium administered to the patients was 61.2 ± 25.9 mL (range 35-110 mL).

The time course changes of each marker in patients with and without CIN are summarized in the Table 3. A significant increase in serum creatinine was seen as early as 4 hours after contrast administration (p=0.003) which further increased at 24 hours (p<0.001) and 48 hours (p<0.001) in the CIN group. However, in the non-CIN group no significant increase was seen after contrast administration when compared to baseline levels (Table 3). A significant increase in u-NAG corrected for creatinine (u-NAG/Cre) was observed in CIN group after contrast administration when compared to baseline [Figure 1(a)]; however, no significant increase was observed in the non-CIN group. Between the two groups, u-NAG/Cre levels were significantly higher in the CIN group at 4 hours (p=0.041) compared to the non-CIN group. A statistically significant increase in u-ALP/Cre [Figure1B ] u-LDH/Cre [Figure 1C ] and u-MA/Cre [Figure 1 D] were observed in both CIN and non-CIN groups when compared to baseline level. No significant difference was observed in the markers between the two groups.

**DISCUSSION**

European Society of Urogenital Radiology (ESUR) guidelines\(^2\) define CIN as an absolute increase in serum creatinine of more than or equal to 0.5 mg/dL or relative increase of serum creatinine more than or equal to 25% from the baseline value within 48 hours. CIN has been recently termed as contrast induced AKI (CI-AKI) by the Kidney Disease Initiative Global
Table 3: Time-course changes in biochemical markers studied in patients with and without CIN

<table>
<thead>
<tr>
<th>Time point</th>
<th>CIN group (n=27)</th>
<th>Non-CIN group (n=93)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 4 hours</td>
<td>Baseline 4 hours</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)*</td>
<td>0.80 ± 0.14</td>
<td>1.03 ± 0.20</td>
</tr>
<tr>
<td>Urinary NAG (U/L)§</td>
<td>12.1 (4.6-17.8)</td>
<td>11.8 (6.9-19.3)</td>
</tr>
<tr>
<td>Urinary NAG (U/g cre) §</td>
<td>7.5 (5.5-14.4)</td>
<td>9.8 (6.16-16.5)</td>
</tr>
<tr>
<td>Urinary ALP (U/L)§</td>
<td>2.0 (3.0-5.0)</td>
<td>2.0 (3.0-4.0)</td>
</tr>
<tr>
<td>Urinary ALP (U/g cre) §</td>
<td>2.9 (1.6-4.9)</td>
<td>2.3 (1.6-3.8)</td>
</tr>
<tr>
<td>Urinary LDH (U/L)§</td>
<td>10.0 (7.0-14.0)</td>
<td>11.0 (8.3-13.0)</td>
</tr>
<tr>
<td>Urinary LDH (U/g cre) §</td>
<td>12.3 (5.3-15.4)</td>
<td>13.5 (5.3-14.2)</td>
</tr>
<tr>
<td>Urinary micro albumin (mg/dL)§</td>
<td>1.2 (0.5-3.1)</td>
<td>1.2 (0.4-3.3)</td>
</tr>
<tr>
<td>Urinary micro albumin (mg/g cre)§</td>
<td>9.9 (3.7-25.5)</td>
<td>9.8 (4.8-28.1)</td>
</tr>
<tr>
<td>Urinary creatinine (g/dL)</td>
<td>0.12 ± 0.09</td>
<td>0.13 ± 0.08</td>
</tr>
</tbody>
</table>

Urinary analytes were corrected for urinary creatinine and percentage transformed taking the baseline value as 100%

* data are expressed as mean ± standard deviation
† p<0.05 when compared to baseline levels (0 hours Vs 4 hours)
‡ p<0.01 when compared to baseline levels (0 hours Vs 24 hours)
§ data are expressed as median (IQR)
NAG = N-Acetyl β-D-glucosaminidase, ALP = alkaline phosphatase, LDH = Lactate dehydrogenase, CIN = contrast-induced nephropathy, U/g cre = units per gram creatinine, U/L = Units per liter; IQR = inter-quartile range
Figure 1: Box-whisker plots showing timecourse changes in NAG (A); ALP (B); LDH (C); and MA (D) in patients with and without CIN.

Boxes indicate 25th percentile (bottom line), median (middle line) and 75th percentile (top line)

CIN = contrast induced nephropathy; NAG = N-acetyl-β-D-glucosaminidase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; MA = microalbumin; NS = not significant
Outcome (KDIGO) guidelines,\textsuperscript{11} and is defined as an increase in serum creatinine by 0.3 mg/dL or an increase in serum creatinine by 1.5 times the baseline value within 48 hours.\textsuperscript{11} We have defined CIN based on ESUR guidelines\textsuperscript{2} in the present study and observed that 22.5\% patients developed CIN.

Reported incidence of CIN in patients with normal baseline renal function varies from 2\%-50\%. It is dependent on the presence of other risk factors like presence of diabetes mellitus, the type and volume of contrast medium used as well as the pre-procedure prophylaxis given to these patients. High and low-osmolal, non-ionic contrast media have been shown to be more nephrotoxic compared to iso-osmolal contrast medium.\textsuperscript{12} In a study\textsuperscript{12} comparing these two types of contrast media, the incidence of CIN was found to be 26\% in patients who received low-osmolal, non-ionic contrast media compared to only 3\% in those who received iso-osmolal contrast medium in patients with stable baseline renal function (serum creatinine 1.5 to 3.5 mg/dL). The incidence of CIN was found to be 51.4\% in a recent study\textsuperscript{13} with use of high osmolal contrast medium. The type of prophylaxis given prior to contrast administration also influences the outcome. A study\textsuperscript{14} comparing the effects of two prophylactic measures on the development of CIN showed a greater incidence of CIN (34.6\%) in patients who received unrestricted oral fluids as against 3.4\% in patients receiving IV normal saline.

We found a significant increase in u-MA/Cre 4 hours and 24 hours after contrast administration in both CIN and non-CIN groups when compared with baseline levels. Transient proteinuria has been reported after ionic contrast media injection.\textsuperscript{15} It has been proposed that ionic contrast medium causes alterations in glomerular basement membrane charge which leads to increased glomerular permeability to albumin and defective proximal tubular reabsorption and degradation of albumin. Experimental studies show microalbuminuria to be a response to injury to the kidney. In an experimental AKI model in mice, the albumin gene, which is normally silent in the kidney, has been observed to be rapidly induced by AKI, as indicated by increases in renal cortical albumin mRNA expression.\textsuperscript{16}

Negligible effect of non-ionic contrast media on microalbuminuria in patients undergoing coronary angiography has been reported in another study\textsuperscript{17} which is contradictory to our finding. The difference could probably be due to the differences in time points studied. The time point studied in our patients was at baseline (0 hour) and 4 hours after contrast administration, whereas another study\textsuperscript{17} had evaluated the patients immediately after the procedure.

Experimental studies have shown u-MA/Cre to be a useful marker in diagnosing AKI.\textsuperscript{16} However, no significant difference was observed in u-MA/Cre levels between the CIN and non-CIN groups (p=0.409). Thus, urinary microalbumin excretion (u-MA/Cre) is not able to differentiate patients with and without CIN.

A significant increase in u-NAG/Cre was observed at 4 hours and 24 hours in the CIN group compared to baseline. NAG is one of the important markers of tubular damage as it is found predominantly in the proximal tubular epithelial cells. Its large molecular weight (> 130 Kilo Daltons (kD)) prevents its filtration by the glomerulus.\textsuperscript{18} Thus the increased excretion of the tubular enzyme u-NAG/cre reflect active tubular damage due to toxic insult caused by the administration of contrast media. Our findings are in accordance with previous reports\textsuperscript{19,20} which have shown increased enzymuria following contrast administration.

Four hours after contrast administration we found a significantly increased u-NAG/Cre
excretion in CIN group at compared to non-CIN group. It has been recommended that urinary enzyme excretion should be adjusted to reference parameter, in order to reduce the variation of the analyte concentration due to the inconstant dilution/concentration of urine samples. The ideal reference parameter for adjustment is creatinine. Also, patients with diabetes are known to have increased urinary NAG levels representing tubular dysfunction in these patients. Both these factors were taken care of in the present study. The first issue was taken care of by correcting the urinary enzyme excretion for urinary creatinine levels. The second issue of the confounding effect of the presence of diabetes was taken care by converting the baseline values to 100% and changes at 4 hours and 24 hours were calculated based on percentage change with respect to baseline so as to represent only changes occurring due to contrast administration. Thus, the increase in NAG excretion can be attributed to the toxic effects of contrast media on proximal tubular cells which was significant in the CIN group. Urinary NAG has been found to be useful in differentiating CIN and non-CIN groups.

The other urinary enzymes studied i.e., u-ALP/Cre and u-LDH/Cre were found to be increased at 4 hours and 24 hours in CIN and non-CIN groups when compared to baseline levels. This is in agreement with previous reports. ALP is found in the proximal part of nephron which constitutes an integral part of brush border membrane while, LDH is a cytosolic enzyme which is uniformly distributed along the nephron and urinary tract. The enzymes leak from the cells as a result of increased tissue damage, hence increased excretion of urinary enzymes represent renal tubular cell damage. Contrast media produce toxic damage to renal tubular cells causing release of brush border enzyme ALP and the cytosolic enzyme LDH from the damaged cells. Several experimental studies have also shown increased enzymuria following contrast administration. This enzymuria has been shown to be dose dependent in experimental models. Renal cortical LDH content has been shown to be an accurate indicator of the extent of renal damage in experimental AKI models. LDH has been shown to be released from cortical cells following toxic or ischaemic renal injury with more than 65% LDH release following severe AKI. The cortical LDH loss was found to correlate with the degree of simultaneous elevation in urinary and serum LDH levels.

No significant difference in u-ALP/Cre and u-LDH/Cre excretion was found between the CIN and non-CIN groups. Hence, they represent the toxic insult of the contrast agents on renal tubular cells but cannot differentiate between CIN and non-CIN. To the best of our knowledge, there are no clinical studies which have assessed the diagnostic utility of these markers for CIN.

Effect of contrast media on renal function has also been shown to be related to the baseline renal function and the presence of risk factors. Contrast administration has been shown to produce negligible effect in low-risk individuals and in those with normal baseline renal function. The effect is found to be higher in patients with chronic kidney disease (CKD). Greater incidence of nephropathy in CKD patients has been attributed to an exaggerated release of endothelin following contrast administration. However, in the present study significant enzymuria and microalbuminuria was seen despite the patients having normal baseline renal function.

This is the first study to show marked albuminuria following administration of nonionic low-osmolal contrast medium in patients undergoing coronary interventions with normal baseline renal function. Also the changes in marker concentration was studied
Urinary enzymes and microalbuminuria after contrast administration

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after correcting for the confounding factors i.e., underlying disease, baseline renal function and changes in urine volume.

The findings of the present study show that low osmolal, non-ionic contrast medium produces toxic insult to the glomeruli as well as renal tubules as evidenced by marked increase in microalbuminuria and enzymuria respectively even in patients with normal baseline renal function. However, only u-NAG was able to differentiate patients with and without CIN. Urinary NAG is, thus, a useful marker for identification of CIN.

REFERENCES