Original Article

Urinary Neutrophil Gelatinase Associated Lipocalin as an Early Biomarker of Contrast Induced Nephropathy

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ABSTRACT

Objective: The present study was aimed to evaluate urinary NGAL as an early biomarker of contrast induced nephropathy (CIN).

Methodology: This was a cross-sectional analytical study conducted at the Department of Biochemistry and Chemical Pathology Sheikh Zayed Medical Complex, Lahore. Fifty patients (33 males and 17 females) underwent coronary angiography with normal serum creatinine at baseline were included in this study.

Results: Ten patients out of fifty (20 %) developed CIN as defined by the gold standard criteria of 0.5 mg/dl or more rise in serum creatinine 24 to 48 hours after contrast media. Patients who developed CIN have a significant rise in their urinary NGAL levels from baseline 4 hours after contrast media. Results indicate that urinary NGAL at 4 hours after contrast correlates closely with serum creatinine levels at 24 to 48 hours after contrast.

Conclusion: Urinary NGAL is an early, sensitive and noninvasive biomarker of contrast induced nephropathy.

Keywords: *Contrast induced nephropathy. Serum creatinine. Urinary neutrophil gelatinase associated lipocalin (NGAL).*

INTRODUCTION

Contrast induced nephropathy (CIN) is defined as an absolute increase in serum creatinine of ≥ 0.5 mg/dl (44 µmol/l), or a relative 25% increase from the baseline value, assessed 24 to 48 hours following intravascular administration of contrast medium.[1] It is an acute injury to the kidney as a result of exposure to iodinated contrast medium.

Contrast induced nephropathy is the third most common cause of acute kidney injury in hospitalized patients after decreased renal perfusion and postoperative renal insufficiency.[2] It is associated with both short and long term adverse outcomes e.g. chronic renal dysfunction, increase health care burden, need for renal replacement therapy and mortality.[3] Two primary pathways are considered to be responsible for contrast induced nephropathy i.e. renal ischemia and direct toxicity to renal tubular epithelial cells. Contrast induced nephropathy is potentially a harmful condition, it is difficult to treat once develops. However, it can be prevented by several ways e.g. intravenous volume expansion, sodium bicarbonate is also renoprotective by alkalinizing renal tubular fluid, renal vascular vasodilators e.g. theophylline, use of antioxidants e.g. N-acetylcysteine and ascorbic acid, haemodialysis and haemofiltration.[4,5] Efforts to prevent acute kidney injury (AKI) after contrast administration are the focus of ongoing investigations, such as determination of early biomarkers of nephropathy e.g. Neutrophil gelatinase associated lipocalin (NGAL) and interleukin-18 (IL-18).[6]

Urinary NGAL has emerged as a sensitive, specific and early biomarker of AKI. It is a troponin like biomarker for AKI detection.[7] It is readily excreted and detected in urine due to its small molecular size and resistance to degradation.[8]

Acute kidney injury after contrast administration is currently diagnosed by measuring serum creatinine which is a late and unreliable indicator because it is affected by several non-renal factors like age, muscle mass, nutritional status, hydration status, comorbid conditions and drugs. Secondly, serum creatinine accumulates slowly making it of little use in nonsteady states e.g. AKI, a marked reduction in GFR can be present before it is reflected by a rise in serum creatinine. Up to 50% of the kidney function has already been lost before creatinine values might change.[9] This prevents timely diagnosis and estimation of the severity of the renal injury that causes a delay in appropriate treatment and leads to poor prognosis. Serum creatinine and creatinine clearance are late biomarkers of nephropathy.[9]

Acute kidney injury after contrast administration can be detected in an intermediate stage of kidney damage during which structural damage occurs without a functional injury. A number of interventions applied at this stage have been successful in preventing and treating acute renal failure. This stage can be detected by emerging structural biomarkers of injury like urinary NGAL.[10]

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Neutrophil gelatinase associated lipocalin (NGAL) derived from systemic sources does not affect urinary NGAL measurements since any filtered NGAL is rapidly and efficiently reabsorbed by the proximal tubule.[11] Therefore, enhanced urinary NGAL excretion most likely results from a combination of an early intrinsic tubular cell response to injury and a later component reflecting the inability of the damaged tubules to completely reabsorb filtered NGAL.[12]

Despite major advancements, the ability to effectively treat and improve the prognosis of renal injury has been disappointing. One of the major reasons is the reliance on current markers of renal dysfunction i.e. serum creatinine and creatinine clearance which are insensitive to minor changes in renal function. Neutrophil gelatinase associated lipocalin with its early and marked response to renal injury may prove to be an even more sensitive biomarker of renal injury.

METHODOLOGY

This cross-sectional analytical study was conducted in the Department of Biochemistry and Chemical Pathology Sheikh Zayed Medical Complex and patients were selected from Cardiology Department of Sheikh Zayed Medical Complex, Lahore. It was non-probability convenient sampling. Duration of study was 12 months. The study included 50 patients of both genders. The study was approved by the hospital ethical committee. Patients underwent contrast induced procedures were selected as per following inclusion criteria.

Inclusion Criteria

- Patients requiring intra-arterial angiography/ angioplasty.
- Patients with normal baseline serum creatinine levels.

Exclusion Criteria

- Acute renal failure.
- Evidence of rhabdomyolysis.
- Patients who have the end stage renal disease and on haemodialysis.
- Pregnancy.
- Contrast medium application within 7 days prior to intervention.

Those patients who fulfill the selection criteria were identified from cardiology department of Sheikh Zayed Hospital, Lahore. Written consents were taken from those who agreed as participants to enroll them in this study. Their socio-demographic information, detailed medical history including drug intake, findings in the available medical record, biochemical results and general physical examination were also recorded on already designed proforma. A 3 ml of blood sample was taken in disposable syringes from peripheral vein before contrast and 4 hrs, 24 hrs and 48 hrs after contrast and placed in plastic tubes allowed to clot for 1 ½ hour, centrifuged at 3000 rpm for 3 minutes to take serum that to be placed in tubes and kept frozen at -20°C for further analysis of serum creatinine.

Random spot urine samples were collected in aseptic containers before and 4 hrs after contrast. Urine was centrifuged to obtain a clear supernatant that was stored at -20^{0}C and repeated freeze-thaw cycles were avoided. Urinary NGAL, serum creatinine and estimated creatinine clearance were calculated before contrast administration and 4 hrs after contrast administration. Serum creatinine and estimated creatinine clearance were calculated 24 and 48 hrs after contrast administration.

STATISTICAL ANALYSIS

Data was entered and analyzed by using SPSS version 20.0. The data for age, creatinine, NGAL and eGFR was described by using mean $\pm$ S.D. Comparison of NGAL and creatinine was performed by using a t-test. The incidence of CIN at 24 hr and 48 hrs was reported by using frequency and percentage. The predictive power of urinary NGAL for severity of AKI after contrast administration was checked by logistic regression. A p-value $\leq$0.05 was considered significant.

RESULTS

The study included 50 patients (33 males and 17 females) who underwent coronary angiography. All cases were investigated for their serum creatinine level which was normal at baseline.

Serum creatinine levels were observed before contrast and 4 hours after contrast. The mean levels of creatinine were 1.07±0.21mg/dl and at 4 hours after contrast reached to 1.12±0.18 mg/dl. The difference was not clinically significant, though, statistically significant with p-value <0.001. The level raised to 1.43±0.38 mg/dl at 24 hours ranging between 0.9 and 3.3 mg/dl. This increase was significant with p-value <0.001. The average creatinine level after 48 hours reduced significantly to 1.29±0.50 mg/dl but the range was between 0.8 to 3.9 mg/dl.

Urinary NGAL was measured for these patients before contrast and 4 hours after contrast. It was observed the levels were 1.84±0.43ng/ml before contrast ranging between 1.0 and 3.0 ng/ml and reached to 6.79±6.41ng/ml after 4 hours and the range at this time was 1.75 to 29.0 ng/ml. This difference was highly significant with p-value <0.001.

As the creatinine levels changed between times, the creatinine clearance also a showed similar pattern. The clearance before contrast was 83.9±18.6 ml/min, it was recorded 78.1±14.6, 63.2±14.8 and 73.0±20.8 ml/min at 4, 24 and 48 hours after contrast respectively. The

difference between every two reading times were significant with p-values <0.001.

Patients were studied for CIN as per criteria of 0.5 mg/dl or more rise in serum creatinine at 4, 24 and 48 hours after contrast. There was no case of CIN at 4 hours, 10 (20.0%) at 24 hours and out of these 6 carried on nephropathy after 48 hours and remaining 4 recovered.

Taking CIN as creatinine raise by 0.5 mg/dl or more at 24 hours as gold standard urinary NGAL at 4 hours was evaluated for its diagnostic value for injury by using ROC curve analysis. The area under the curve was 0.971, and the ordinate observed and considered optimal was 9.40 ng/ml. At this cut-off the sensitivity of NGAL at 4 hours to predict injury at 24 hours was 90.0% (87.51 – 92.49), specificity recorded was 97.5% (96.82 – 98.18) so the accuracy rate of this cut off point was 96.0% (94.94 – 97.06).

DISCUSSION

In experimental and clinical studies, NGAL appears to be one of the most frequently investigated and most promising biomarker of AKI.[13,14] Acute deterioration in renal functions after contrast administration called CIN is generally mild and transient but may lead to irreversible renal damage and need renal replacement therapy which increases morbidity and mortality.[3] In this study, we tested the hypothesis whether urinary NGAL could be an early biomarker of CIN.

Neutrophil gelatinase associated lipocalin (NGAL), a member of the lipocalin family is a 25 kDa protein covalently bound to gelatinase in neutrophils and its mRNA is markedly induced in injured epithelia especially kidneys. NGAL releases from damaged renal tubules soon after injury, preceding the rise in serum creatinine.[13,15,16] Serum creatinine is most widely used biomarker for the diagnosis of CIN, but it is not sensitive because it takes 24 to 48 hours to be raised after renal damage which is too late for early intervention. So, new markers of kidney injury become a need of the hour which can detect CIN as early as possible.

The current work consisted of evaluating urinary NGAL as an early biomarker of CIN in patients undergoing coronary angiography. The study was conducted on 50 patients (33 males and 17 females) who underwent coronary angiography using low osmolar contrast media with normal baseline serum

Table:1 Mean levels of study variables of study subjects

Study variables	Mean levels
Creatinine	
Before contrast	1.07±0.21mg/dl
4 hours after contrast	1.12±0.18 mg/dl
24 hours after contrast	1.43±0.38 mg/dl
48 hours after contrast	1.29±0.5 mg/dl
Creatinine clearance	
Before contrast	83.9±18.6 ml/min
4 hours after contrast	78.1±14.6 ml/min
24 hours after contrast	63.2±14.8 ml/min
48 hours after contrast	73.0±20.8 ml/min
NGAL	
Before contrast	1.84±0.43 ng/ml
4 hours after contrast	6.79±6.41ng/ml

Table 2: Measures for predictive values with 95% confidence interval for urinary NGAL >9.40 ng/ml at 4 hours after contrast for CIN

Measure	Value	95% Confidence Interval
Sensitivity	90.0	87.51 – 92.49
Specificity	97.5	96.82 – 98.18
Positive Predictive Value	90.0	87.51 – 92.49
Negative Predictive Value	97.5	96.82 – 98.18
Accuracy	96.0	94.94 – 97.06

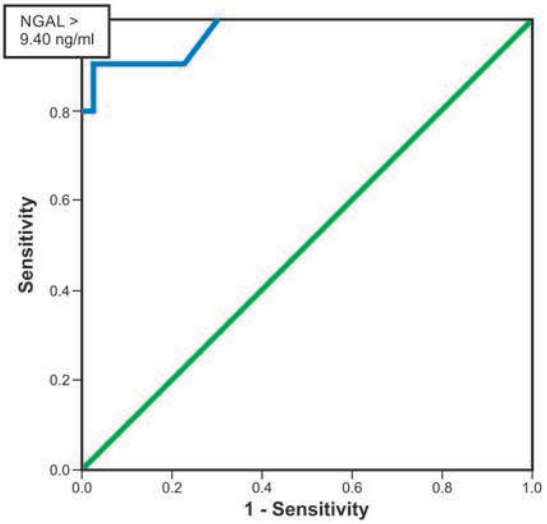

Figure 1: ROC curve presenting area under curve for urinary NGAL after 4 hours for CIN confirmed by creatinine raised by 0.5 mg/dl or more after 24 hours

creatinine.

The serum creatinine level was 1.07±0.21 mg/dl and 1.12±0.18 mg/dl before contrast and 4 hours after contrast respectively and it is significantly raised to 1.43±0.38 at 24 hours after contrast ranging between 0.9 and 3.3 (p-value <0.001). The average creatinine level after 48 hours was 1.29±0.50 ranging between 0.8 to 3.9 mg/dl.

As creatinine levels changed between times, the creatinine clearance also showed a similar pattern. The clearance before contrast was found 83.9±18.6 ml/min, this value was recorded 78.1±14.6, 63.2±14.8 and 73.0±20.8 ml/min at 4, 24 and 48 hours after contrast respectively. The difference between every two reading times was significant (p-values <0.001).

When we studied patients for CIN as per criteria of 0.5 mg/dl rise in serum creatinine there was no case among 50 patients who have rise in their serum creatinine >0.5 mg/dl from baseline at 4 hours after contrast while10 patients (20%) developed CIN as demonstrated by >0.5 mg/dl rise in serum creatinine from baseline at 24 hours after contrast and 6 patients out of these 10 carried on CIN as observed by rising in serum creatinine 48 hours after contrast.

This study had clearly shown that those patients who have increased serum creatinine level >0.5 mg/dl from baseline at 24 hrs after contrast administration have increased urinary NGAL level at 4 hours after contrast. This is in agreement with studies done by Shaker et al., Gajewska et al. and Ling et al.[6,17,18]

A characteristic trend of increase in urinary NGAL was seen in accordance with the severity of the renal impairment. Hence it is suggested that both serum creatinine and creatinine clearance which are gold standard markers for acute kidney injury are closely related to urinary NGAL. This study has proved that early diagnosis of CIN by urinary NGAL can detect CIN in its early stage when structural damage occurs without functional damage, timely diagnosis and intervention can prevent further progression of kidney damage to an irreversible stage. Urinary NGAL as seen might become a useful and noninvasive tool for evaluation of renal impairment after contrast media administration in diagnostic and therapeutic

procedures.

However, this was just a pilot study to evaluate urinary NGAL as an early biomarker of CIN. There are certain limitations in this study. Due to financial constraints, it was a single center study with a relatively smaller sample size which might not correctly estimate the relationships between urinary NGAL and any other parameter. A follow-up prospective study of longer duration and larger sample size may show the exact changes of the disease and may help to evaluate urinary NGAL more accurately as a diagnostic marker of contrast induced nephropathy.

CONCLUSION

The study showed patients who developed CIN 24 to 48 hours after contrast administration had significantly raised levels of urinary NGAL at 4 hours after contrast. The results demonstrated that urinary NGAL had a positive correlation with creatinine and negative correlation with creatinine clearance as calculated by cockcroft formula (eGFR) 24 to 48 hours after contrast administration. These findings suggest urinary NGAL measurement at 4 hours after contrast might become a useful, sensitive and noninvasive biomarker of kidney damage due to contrast media.

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