Neutrophil Gelatinase-Associated Lipocaline (NGAL) as an early predictive biomarker than Urea in contrast induced Nephropathy

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Abstract: Coronary angiography and percutaneous coronary interventions depend on iodinated and non-iodinated contrast media and consequently pose the risk of contrast induced acute kidney injury (AKI). This is an important complication that accounts for significant number of cases of hospital acquired renal failure, with adverse effects on prognosis and health care. Aim of this study is to evaluate Neutrophil Gelatinase-Associated Lipocalin (NGAL) as an early biomarker in AKI than that of urea. 30 animals were randomly divided into 10 different cages having 3 animals in each cage. Further cages were randomly divided into 5 groups (6 animals in a group). Dose of 0.5ml of iodoxol was intraperitoneally injected into animals. Blood samples were collected by bleeding retroorbital plexus before and after inducing contrast and centrifuged serum was stored at -20°C for further analysis. Increased levels of NGAL was indicated in group 2 (6 hours) and remained elevated in group 3 (12 hours) when compared to that of baseline levels before contrast. The levels of urea were increased in group 1 (3 hours) and group 3 (12 hours) after contrast when compared to level of baseline before contrast. Elevated levels of NGAL among the groups was statistically significant p<0.05. The increased level of urea at the end of 3 hours is probably due to dehydration after contrast induction. Estimation of serum NGAL after contrast induced radiological procedures especially after coronary angiogram may help to detect early kidney injury, so that preventive measures can be adopted to decrease the damage caused by the contrast induction.

Key words: Acute Kidney Injury (AKI), Blood Urea Nitrogen (BUN), Contrast Induced Nephropathy (CIN), Chronic Kidney Disease (CKD) Neutrophil Gelatinase Associated Lipocaline (NGAL), Proximal Convoluted Tubules (PCT), iodoxol, Urea.

Introduction
Contrast Induced Nephropathy has become an epidemic in patients with pre-existing impaired renal function and in patients getting exposure to new radiological diagnostic procedures. Various etiological factors are subjected to contrast studies, which provide specificity of diagnostic relevance in modern medicine. The use of radiological procedures using iodinated contrast media have increased accordingly leading to morbidity and mortality which have led to CIN in individuals if not diagnosed in time, due to dependence on conventional markers like creatinine and urea.

Increase of serum creatinine and urea were observed after 24 hours of contrast insult which resulted in AKI. As these parameters show an increase 24-48 hours after initial insult proceeding to kidney damage. Whatever might be the primary disease process, the rate of decline of the kidney function is recognized as strictly influenced by secondary components although hypertension, proteinuria, hyperlipidaemia and inflammation represents some important modifiable risk factors (1,2).

Recent studies have shown the role of renal tubules in the genesis and progression of kidney disease. The pathogenic mechanism which causes, tubular damage is said to be tubular hypoxia and peritubular capillary injury (3). With this, it is widely accepted that in some AKI- associated diseases like diabetic nephropathy and contrast induced nephropathy (CIN) the rate of deterioration in renal functions are associated with the degree of renal tubule-interstitial impairment.

In a recent study, it has been suggested that subjects with membranous nephropathy and impaired renal function showed exaggeratedly increased base line levels of NGAL (4). Keeping these assumptions, the aim of the present study was to detect the early detectable marker for AKI, by comparing serum levels of NGAL and Urea. According to previous literature, NGAL has emerged as a new biomarker to predict AKI in diagnosis of AKI. The study is done to trace the initial insult of the contrast within 24 hours.

Materials and Methods
30 Male Wistar albino rats (procured from Department of Research and Development, Saveetha university, Chennai) weighing about 160-210 grams were used in the study. The rats were maintained at Department of Research and Development, Saveetha University and animals were given pelleted diet and RO water. All animal experiments were conducted in compliance with the guidelines stated by Institutional Animal Ethical Committee (IAEC).

Experimental Groups:
30 male Wistar albino rats were randomly divided into 10 different cages having 3 animals in each cage. Cages were randomly divided into 5 groups...
on duration of sampling 3 hours, 6 hours, 12 hours, 24 hours and 48 hours and were grouped into group 1 to group 5. Animals were kept for 12 hours day and night cycle. The contrast iohexol (350mg iodine/ml), was calculated according to body weight of each animal and administered Intraperitoneally. The blood samples were collected by bleeding retroorbital plexus, under the influence of Isoflurane anesthesia, from each group according to time duration both before and after inducing the contrast. Serum was separated and stored at -20°C until further analysis. Kidneys were harvested for histopathological observation. Part of the kidney was fixed in paraformaldehyde and processed for hemotoxylin and eosin staining. Concentration of NGAL was determined by using Rapid ELISA Kit, Bioporto in accordance with manufacturer instruction provided with kit. Concentration of Urea was determined by enzymatic method of Urea by Urease and glutamate dehydrogenase (GLDH enzymatic method) using Randox kit (on Randox chemistry Analyser, Dayatona).

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism Version 6 package and data were presented as Mean±SEM. p<0.05 is considered as significant.

**Results**

It was observed that the level of NGAL after inducing contrast showed an increase in group 2 (6 hours) and continued to be elevated in group 3 (12 hours) when compared to the baseline level. Comparisons among the groups were conducted with one-way ANOVA, F value was significant and p< 0.05 Bartlets test corrected. Table 1, Figure 1. Serum Urea showed an increase at 3 hours (Group1) which declined at 6 hours (Group 2) but elevated at 12 hours (Group 3) and was statistically significant among the groups by using ANOVA with p value 0.01 (p<0.05). Table 1, Figure 1

**Table 1:** Levels of NGAL before and after inducing contrast

<table>
<thead>
<tr>
<th>Groups</th>
<th>NGAL</th>
<th>Mean ± SE</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3Hours before</td>
<td>0.02±0.0</td>
<td>1.040</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>6Hours after</td>
<td>6.7±2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12Hours after</td>
<td>13.7±6.7</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>24Hours after</td>
<td>102.0±2.3</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>48Hours after</td>
<td>50.3±3.4</td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ANOVA ordinary - **** statistically significant with Bartlet’s test (corrected), p<0.05

**Discussion**

Despite advanced treatment strategies for the patients the rate of mortality is as high as 60% (5). The poor clinical outcome of AKI patients partly reflects the pathophysiology of AKI. Study of NGAL, marks an important step towards the identification of biomarkers in AKI (6). Human NGAL was originally identified in neutrophils as a 25KDa protein in septic AKI (7). Increased levels of NGAL have been reported in systemic diseases, inflammation, hypertension and renal tubular injury (8). Findings, from the current study clearly highlights that NGAL is a better and earlier marker to establish contrast induced AKI, when compared with serum urea, which is also supported by previous studies, which suggests...
NGAL as a better marker of AKI (4). Table 1 Figure 1. Approximately 40-50% of filtered urea undergoes passive reabsorption in PCT. In states of intravascular volume depletion proximal sodium and water reabsorption increases coupled with the parallel increase in the reabsorption of urea, this results in a disproportionate raise in BUN levels. This elevation in the BUN to creatinine ratio is one of the laboratory indicators of decreased renal perfusion indicative of pre-renal injury (9). The raise in urea level at 3 hours in the current study may probably due to dehydration and hemocr-concentration, due to induction of contrast, which is supported by a previous study (9). Therefore, NGAL may be considered as a better marker over urea since
1. 3 hours raise in serum urea may be explained by dehydration.
2. Raise at 12 hours may be due to irreversible renal damage caused due to contrast induction. Table 2 Figure 2

**Conclusion**
The current study clearly demonstrated the raise of NGAL in 3 hours may probably be used as a novel biomarker in diagnosing contrast induced nephropathy (CIN), so that early preventive measures can be taken to avoid the progression of AKI to CKD.

**References**

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