



NGAL, CYSTATIN C AND INTERLEUKIN-18 ARE DETECTED EARLIER THAN CONVENTIONAL BIOMARKERS IN CONTRAST INDUCED NEPHROPATHY

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ABSTRACT

Stress is a leading cause of cardiovascular disorders, as more number of patients with cardiovascular diseases are undergoing interventional procedures such as cardiac catheterization; use of contrast media has become common in medical practice to diagnose the blockades of arteries. Use of these contrast media is more prone to Contrast Induced Nephropathy (CIN). Recently, NGAL (Neutrophil Gelatinase Associated Lipocaline), Cystatin C and Interleukin-18 (IL-18) are emerging as novel biomarkers for the early detection of Contrast Induced Nephropathy (CIN) and has been investigated intensively. The present study proved that serum NGAL, Cystatin C and Interleukin-18 are early elevated serum markers when compared to urea and creatinine hence could serve as better markers of Contrast Induced Nephropathy (CIN). Thirty male Wistar rats were randomly distributed into 10 different cages, having 3 animals each and cages were randomly divided into 5 different groups and were injected 0.6 mL of iohexol contrast (350 mg Iodine/kg) intraperitoneally. Blood samples were obtained before and after inducing contrast and centrifuged. Results have shown that the NGAL, Cystatin C and Interleukin-18 were early to rise in serum when compared to Urea and Creatinine. The raise in levels of Interleukin-18 and Cystatin C were observed to be elevated at 3 hours and NGAL at 6 hours where as there is no change in Creatinine levels although urea has shown little change to before and after values and statistically significant $P < 0.05$ with paired 't' test. The present study evaluated the early raise of serum NGAL, Cystatin C and Interleukin-18 in Contrast Induced Nephropathy (CIN) when compared to urea and creatinine are delayed markers.

KEY WORDS: Contrast Induced Nephropathy (CIN), Creatinine, Cystatin C, Glomerular Filtration Rate (GFR), Interleukin-18, Iohexol, NGAL (Neutrophil gelatinase Associated Lipocaline), Reactive Oxygen Species (ROS) and Urea.



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INTRODUCTION

Early detection of Contrast Induced Nephropathy (CIN) is gaining momentum in clinical settings as treatment is completely dependent on diagnostic approaches such as computed tomography and interventional angiographic procedures and techniques. Special contrast agents, ionic and non ionic are administered orally, rectally or intravenously. Contrast enables various tissues, blood vessels and organs to be identified more distinctly and specifically for any lesions. Use of these contrast reagents may damage renal tissue in various ways by bringing about vasoconstriction by releasing Reactive Oxygen Species (ROS).¹ Early detection of CIN is very important in clinical settings. Raise of traditional markers, urea and creatinine in serum contribute to delayed diagnosis of CIN. Recent novel bio markers have shown promise for earlier detection of CIN.² There is a need for a simple, accurate and rapid endogenous marker of GFR which can detect small changes in GFR. Detecting appropriate and precise marker for tubular toxicity has been a major limiting factor in clinical practice and research. Due to inaccuracies associated with methods used to estimate creatinine and urea in serum and its association with other diseases, the raise of these biochemical parameters are time dependent. The early detection of CIN may allow for timely preventive or therapeutic measures to be adopted reversing the conditions of CIN. Serum Creatinine and Urea are the most commonly used filtration markers in day to day clinical practice but its accuracy is significantly hampered by diet, age, gender and muscle mass³ and assay interference and the confounding factors. There are several well reported difficulties concerning its analysis.^{4,5} Cystatin C is freely filtered by glomerulus. NGAL and interleukin-18 are released when there is a tubular damage.⁶⁻⁹ The aim of the present study was designed by keeping in view of paucity of research on early detection of CIN. The present research demonstrates NGAL, Cystatin C and Interleukin as better and earlier markers to rise in serum, and hence can be used as reliable markers for CIN.

MATERIALS AND METHODS

Animals

The experimental protocol was executed with 30 male Wistar albino rats at Department of Research and Development, Saveetha University, Chennai after obtaining the institutional animal ethical clearance (SU/BRULAC/RD/ 001/2014). The rats were procured from Department of Research and Development, Saveetha University well maintained with free access to food and water ad libitum. Animals were kept in a natural dark light cycle.

METHODS

1. NGAL Rapid ELISA Kit- Bioporto® Diagnostics
2. Cystatin C Turbidimetric Immunoassay (Auto Pure, Sphera system Pack) procured from Accurex Biomedical Pvt Ltd, India.

3. Interleukin-18 sandwich ELISA method. (Elabscience Biotechnology Co.Ltd)
4. Creatinine modified Jaffe's method Assay procured from ChemCHEK™ AGAPPE.
5. Urea, Enzymatic method of urea by glutamate dehydrogenase (kinetic method) measured by urease and glutamate dehydrogenase (GLDH) enzymatic method. Chemistries were processed on RX imola (RANDOX) clinical chemistry analyser and ELISA reader was used for ELISA methods.

Experimental design

Thirty male Wistar rats were randomly distributed into 10 different cages having 3 animals in each cage 5 groups in order to evaluate 0,3,6,12,24 and 48 hours time point. Every group has one cage. The groups were divided according to the time duration of sampling after inducing contrast ranging from 3 to 48 hours in ascending order of time 1 to 5 groups respectively. All the groups of animals received 0.6 mL of iohexol contrast intraperitoneally. Animals were euthanized and blood samples were collected by bleeding retroorbital plexuses before and after inducing contrast at 3,6,12,24 and 48 hours for groups 1 to 5 respectively. Blood was centrifuged and serum was stored at -20°C for further analysis. Care was taken to achieve good results and avoid bias in research.

STATISTICAL ANALYSIS

Analysis of data was done by using Sigma Stat (13), USA and Graphpad Prism (6), USA. Values were given as Mean±SEM. Paired 't' test was performed to draw statistical significance for before and after values for individual parameters. Percentage change in individual parameter was compared with 0 hour pooled up values of before contrast for all parameters individually. Statistical significance was assumed at P<0.05.

RESULTS

The present study clearly indicates statistical significance between before and after values at different time intervals. NGAL at 3 hours after the contrast was significant with before contrast and at 6 hours there was a raise of NGAL after contrast when compared to before contrast. Cystatin C has showed raise in 3 hours and 12 hours after contrast when compared to before. There was an increase in Interleukin-18 values at 3 and 6 hours after contrast when compared to before. There was no change in levels of NGAL, Cystatin C and Interleukin-18 values in 24 hours and 48 hours after contrast. The conventional biomarkers urea and creatinine raise has occurred at 12 hours after the contrast, only 12 hours urea before and after contrast was statistically significant. It observed in 24 and 48 hours after contrast there was a decrease or small change in creatinine and urea which was statistically not significant. NGAL and interleukin-18 were increased at 3 hours and 6 hours after contrast, but the raise of Cystatin C was observed at 3 hours and 12 hours after contrast. (Table 1 and 2; Figure 1)

Table 1
Serum levels of NGAL, Cystatin C, Interleukin-18, Creatinine and Urea before and after contrast in male Wistar rats

Duration of sample collection	sample	NGAL	Cystati C	Interleukin-18	Creatinine	Urea
3 hours	before	0.0±0.0	0.212±0.212	8.3 ± 0.9	0.45±0.01	34±3.2
	after	6.7±2.1*	0.392±0.198	12.3 ± 4.5	0.44±0.04	46±7.1
6 hours	before	5.0±2.2	0.150±0.091	3.9 ±1.6	0.41±0.01	35±0.7
	after	13.3±7.6	0.145±0.137	7.8 ±2.1	0.44±0.05	38±4.4
12 hours	before	10±0.0	0.055±0.032	3.2 ± 1.4	0.46±0.02	34±2.7
	after	10±6.3	0.448±0.227	4.4 ± 0.9	0.61±0.07	53±5.3*
24 hours	before	10±2.6	0.068±0.035	1.1 ±0.3	0.47±0.03	34±4.5
	after	5.0±3.4	0.120±0.048	1.3 ± 0.2	0.35±0.03	28±1.0
48 hours	before	6.7±3.3	0.198±0.090	1.3 ± 0.2	0.47±0.03	42±3.6
	after	10±2.6	0.110±0.057	0.8 ± 0.4	0.40±0.08	36±6.0

Data presented as Mean±SEM. Paired t-test. p<0.05 considered as significant.
 * indicates statistical significance with before value. N=6

Table 2
Comparison of novel biomarkers and conventional biomarkers before and after contrast in male Wistar rats

s.no	Duration of sample collection	F value	P value
1	0 hours	-	-
2	3 hours	1.697	0.182
3	6 hours	0.638	0.640
4	12 hours	1.053	0.400
5	24 hours	0.168	0.953
6	48 hours	3.113	0.033

Statistical significance. Oneway ANOVA.
 Pearson correlation. p<0.05 considered as significant. N=6

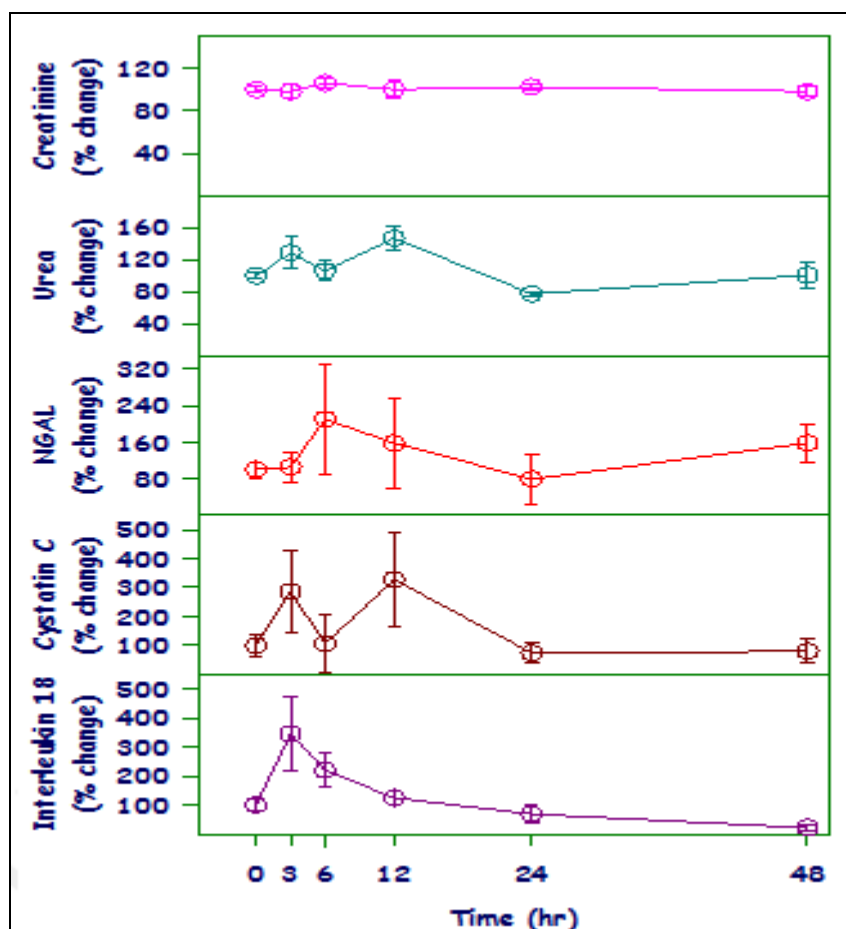


Figure 1

Comparison and correlation of percent change of novel (NGAL, cystatin C and Interleukin) and conventional (Urea and Creatinine) biomarkers in Contrast Induced Nephropathy (CIN) at different time intervals. Paired 't' test was used for statistical significance and p<0.05 was considered to be significant. Comparison and correlation was done with 0 hour values with 3, 6, 12, 24 and 48 hours after contrast. N=6.

DISCUSSION

Increase in Contrast Induced Nephropathy (CIN) cases in hospital setups is a growing concern to researchers and clinicians which has made medical fraternity to identify the early markers to detect CIN.¹⁰ The diagnosis of acute kidney injury (AKI), previously termed acute renal failure, is based typically on an elevation in the serum creatinine (sCr) concentration. A 50% reduction in creatinine clearance from baseline will lead eventually to a 100% increase in serum creatinine, irrespective of the presence or absence of Chronic Kidney Disease (CKD).¹¹ The steady state, however, may not be reached for several days after an episode of AKI.^{8,9} Findings from the present study clearly indicate that NGAL represents a novel risk biomarker of contrast induced AKI. Serum NGAL was found to be a most impressive predictive marker even after adjustment for eGFR. This suggests that NGAL would not be a simple surrogate index of baseline eGFR, but also a marker on its own predicting AKI progression. Different studies have underlined the crucial role played by the renal tubule in the genesis of progressive acute and chronic kidney disease and its evolution to terminal stage.¹¹ The search for early, specific substances in order to reveal the onset of acute kidney injury has uncovered NGAL as one of the most promising biomarkers in the future of clinical nephrology.¹¹ This small 25-kD protein, belonging to the "lipocalins" super family, is massively released into blood and urine from injured tubular cells after various conditions, and potentially detrimental to the kidney in experimental and human clinical models.¹¹ NGAL released from renal tubules occurs soon after tubular damage, notably preceding the rise in serum creatinine and thus allowing the initiation of preventive therapeutic measures in a timely manner.¹¹ On the basis of these unique properties, recent works have validated the reliability of NGAL as a specific, sensible, and early predictor of AKI after cardiac surgery, contrast administration, septic shock and even renal transplantation. Findings from the present study also indicate cystatin C is early marker to detect in contrast induced AKI with small changes in GFR. Previous investigations have suggested that cystatin C might be a superior indicator of GFR when compared with creatinine.¹¹⁻¹³ In the present study, it was observed that the levels of Cystatin C were increased after inducing contrast when compared to that of before values in 3 hours and 12 hours respectively. Kyhse-Anderson et al.² included twenty seven healthy controls and twenty four patients with reduced GFR and found a significant correlation of serum cystatin C to GFR than serum creatinine. This revealed that the diagnostic accuracy of serum cystatin C for reduced GFR was superior to that of serum creatinine. Randers et al.¹⁵ found that cystatin C was more sensitive than creatinine for mild decrease in GFR by 99 mTc-DTPA clearance as evidenced by ROC curve analysis. Newman et al.¹⁶; Hojs et al.¹⁷ concluded that in addition to being a better estimator of GFR than creatinine, cystatin C was more sensitive marker than creatinine for even analysing small changes in GFR. Kumerasen.¹⁸ concluded that the correlation between 99mTc-DTPA renal scan and serum cystatin C was better than that 99mTc-DTPA renal scan and serum creatinine. The results of our study indicated that there

was an increase of serum cystatin C levels after contrast and is in support of other studies. In the present study, 3 and 6 hours has shown an increase in serum levels of IL-18 after inducing contrast on prior to contrast. Generally an increase in serum IL-18 levels indicates ischemic renal tissue injury, injury to heart, brain, inflammation and T-cell mediated immunity.¹⁹ Potent vascular and tubular factors, as well as inflammatory processes, are involved in the pathogenesis of renal ischemia.^{19,20} Macrophages are mediators of AKI in mice and rats and interleukin -18 is a mediator of ischemic AKI in mice.²⁰ A recent study demonstrated that IL-18 derived primarily from cells of bone marrow origin contributes to the renal damage observed during ischemic AKI in mice.²¹ Zubin et al.²² tested that the macrophages are the source of IL-18 in ischemic AKI. IL-18 may be activated in the proximal tubules and directly contributed to tubular injury. The role of caspase-1 and IL-18 in hypoxia induced membrane injury of freshly isolated mouse proximal tubules was studied invitro²³ that supports our findings. In the present study, there was raise of serum creatinine at 12 hours after inducing contrast, an increase of 0.15 mg/dL when compared to before contrast. Generally, an increase in serum creatinine levels indicates ischemic renal tissue injury further, in our study creatinine kinetics have uncovered a non significance increase at 12 hours when compared to 3 hours, 6 hours, 24 hours and 48 hours after contrast. Previous studies by other researchers had shown an increase of serum creatinine at 72 hours after contrast insult.²⁴ But, in our study an increase of serum creatinine level was observed in 12 hours of duration after inducing contrast that may probably suggest the injury to kidney due to oxidative stress. Numerous epidemiologic studies and clinical trials have used different cut-off values for serum creatinine to quantitatively define AKI.²⁵ Serum creatinine and blood urea nitrogen (BUN) have typically been used to diagnose AKI. Serum creatinine is dependent on nonrenal factors such as age, sex, race, muscle mass, nutritional status, infection and volume of distribution independent of kidney function.²⁶ The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and prerenal condition where blood urea nitrogen (BUN)-creatinine ratio is increased.²⁷ The BUN level may reflect functioning of the liver and kidneys. Increased blood urea nitrogen is seen associated with kidney disease or failure, blockage of the urinary tract by a renal stone, congestive heart failure, dehydration, fever, shock and bleeding in the gut.²⁸ The high BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods.²⁷ If the BUN level is higher than 100 mg/dL it points to severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use.²⁸ In previous study, dehydrated patients presented with an elevated serum urea level showed an increase in renal reabsorption of urea mediated by antidiuretic hormone (ADH).²⁸ The present study revealed that there was an increase in serum urea levels at 12 hours after inducing contrast when compared to before contrast. The raise in urea levels at

12 hours in this study may be due to dehydration and hemoconcentration which is in support of previous study²⁹ or may probably be due to decrease in tubular excretion of urea and tubular toxicity due to contrast. The suitability of the biomarkers can be ascertained by ROC curve with a larger sample size.

CONCLUSION

The present study concludes that novel biomarkers (NGAL, Cystatin C and Interleukin-18) may probably

help in diagnosing Contrast Induced Nephropathy earlier than conventional biomarkers (Urea and Creatinine). The raise of novel biomarkers is at 3 and 6 hours although the conventional biomarkers are delayed. Interleukin-18> NGAL=Cystatin C> Creatinine >Urea.

CONFLICT OF INTEREST

Conflict of interest declared none.

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