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Serum Interleukin-18 and Serum NGAL Raise may Detect Contrast Induced Nephropathy Early in Randomized Animal Study?

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ABSTRACT

Introduction: Contrast-induced nephropathy (CIN) is a complication which is underestimated in clinical practice after contrast induction in diagnostic and therapeutic procedures. Uses of these radio opaque iodinated contrasts are increased in day to day clinical practice but its contraindications on kidney are neglected and less studied. Serum creatinine is a traditional biomarker and its early raise in serum is debatable. In recent years novel biomarkers like KIM 1, NGAL, Cystain C, IL-6 and IL-18 has marked their presences in identifying AKI. Recently, Interleukin-18 (IL-18) and NGAL (Neutrophil Gelatinase Associated Lipocaline) emerged as novel biomarkers in identifying CIN. But early detection of CIN has become a challenging task. The current study investigates whether serum IL-18 may be an early diagnostic biomarker than serum NGAL in CIN.

Methods: We performed randomized animal studies on adult male wistar rats weighing 160 g with a dose of 0.6 mL of Iohexol (350 mg I /mL) iodinated contrast given intraperitoneally to 30 animals. Each group has 6 animals, which were divided into 5 different groups in ascending order, as per the duration of sample collection 3, 6, 12, 24 and 48 h. Blood samples were drawn before and after the contrast induction by bleeding retroorbital plexus, serum analysis was done for IL-18 and NGAL. Biochemical analysis was performed with available standard ELISA kits of serum IL-18 and serum NGAL.

Results: Collected data presented as Mean ± SEM and was statistically significant with serum IL-18 when compare to the serum NGAL. An early raise in serum IL-18 was observed at 3 h found to be 48% increase and at 6 h 100% increase, but the raise in NGAL was 100% at 3 h and 50% at 6 h. Elevated levels of NGAL was observed at 6 h after the contrast induction but raise in serum IL-18 was found to be early at 3 h which was statistically significant with t' test (2.18) and P value< 0.05. Graphpad Prism (Version 7) was used for statistical analysis.

Conclusion: Current study demonstrates an early raise in serum interleukin-18 levels, suggests early ischemia of the renal tubular tissue and gives a good reflection towards the severity of damage caused due to contrast induction, may probably due to oxidative stress. Generally, an increase in serum IL-18 levels indicates ischemic renal tissue injury, injury to heart, brain, inflammation and T cell medicated immunity. These findings suggest that the IL-18 may be clinically useful, as an early diagnostic biomarker in CIN over NGAL.

Keywords: Acute Kidney Injury (AKI), Contrast Induced Nephropathy (CIN), Interleukin-18, Iohexol, Neutrophil Gelatinase Associated Lipocaline (NGAL)

INTRODUCTION

The use of contrast media in diagnostic medicine has taken slide increase to identify and treat the different types of diseases in clinical care settings. Repeated use of these contrast media may cause Acute Kidney Injury (AKI) to Chronic kidney disease (CKD) and now recognized as one of the leading cause of mortality globally [1] and it refers to an irreversible deterioration in renal function which classically develops over a period of years [2,3]. It is a severe condition that reduces life expectancy and typically progresses to end-stage renal disease (ESRD) and a need for renal replacement therapy [4]. Initially, it is manifested only as a biochemical abnormality, eventually, loss of the excretory, metabolic and endocrine functions of the kidney leads to the development of the clinical symptoms and signs of renal failure, which are referred to as uremia. The social and economic consequences of CKD are considerable [5]. Biochemical markers play an important role in accurate

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diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. Over decades research and utilization of biomarkers has evolved substantially [1]. As markers of renal function creatinine, urea, uric acid and electrolytes are for routine analysis whereas several studies have confirmed and consolidated the usefulness of novel biomarkers such as Cystatin C, β-Trace Protein, KIM-1, IL-18, IL-6 and NGAL6. But previous studies fail to identify the early raise among these novel biomarkers. Current research explores the possible early raise of the two novel biomarkers IL-18 and NGAL, and among these which one is early to rise and more reliable marker for identifying CIN early in animal model.

MATERIALS & METHODS

Study was conducted in the year of 2015 to 2017 at Department of Research and Development, Saveetha Medical College, Saveetha University, Chennai after obtaining animal ethical clearance from IRB. Randomized animal studies on 30 adults male wistar rats weighing about 160 g, injected with a dose of 0.6 mL of Iohexol (350 mg I/mL) Iodinated contrast intraperitoneally. Sample size was calculated by resource equation (E) where confidence interval (CI) was 95% with 80% power of the study. There was no attrition or death of animal in this study, therefore the attrition sample size calculation was neglected. Each group has 6 animals, which were divided into 5 different groups in ascending order, Group 1 (3 h), Group 2 (6 h), Group 3 (12 h), Group 4 (24 h) and Group 5 (48 h) as per the duration of blood sample collected. Animals were maintained at standard laboratory conditions were temperature, dark and light cycle was maintained, standard operating procedure was followed to avoid the experimental bias in current research. Blood samples were collected before and after the contrast induction by bleeding retroorbital plexus, serum analysis was done for IL-18 and NGAL. Pre and post contrast blood samples were collected at 3 h, 6 h, 12 h, 24 h and 48 h post contrast, centrifuged at 3000 rpm (37°C) for 15 min (Rotofix 32A, Hettich) to obtain clear supernatant serum and was stored at -20°C until further analysis. IL-18 before and after contrast was assessed by using sandwich Enzyme-Linked Immunosorbent Assay (ELISA, RayBio, Rat IL-18 ELISA, Catalog #: ELR-IL18) and NGAL was assessed by Bioporto. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The concentration of IL-18 and NGAL in the sample was proportional to the OD values and was calculated by comparing the OD of the samples to the standard curve.

Statistical analysis

GraphPad Prism version 7.0 was used to calculate p-value and $p<0.05$ was considered to be statistically significant. Paired t-test was used to compare the data, before and after administration of contrast in rats. Data was presented as Mean ± SEM and percent change.

RESULTS

In the current study, the serum IL-18 levels increased by 48% at 3 h, 100% at 6 h, 37% at 12 h, and 18% at 24 h where as NGAL was 100% at 3 h and 50% at 6 h, at 12 h there was no change, at 24 h there was slight decrease and at 48 h there was little increase in levels of NGAL which was statistically not significant. Elevated levels of NGAL was observed at 3 ($p<0.02$) and 6 h ($p>0.03$) after the contrast induction, serum IL-18 was also found to be increased early at 3 h ($p<0.01$) and 6 h ($P<0.001$) which was statistically significant with t' test before and after the induction of contrast. The serum concentration levels of serum interleukin 18 (12.3 ± 0.8) showed an increase at 3 h when compare to that of NGAL at 3 h (6.7 ± 2.1) **(Figure 1 and Table 1)**.

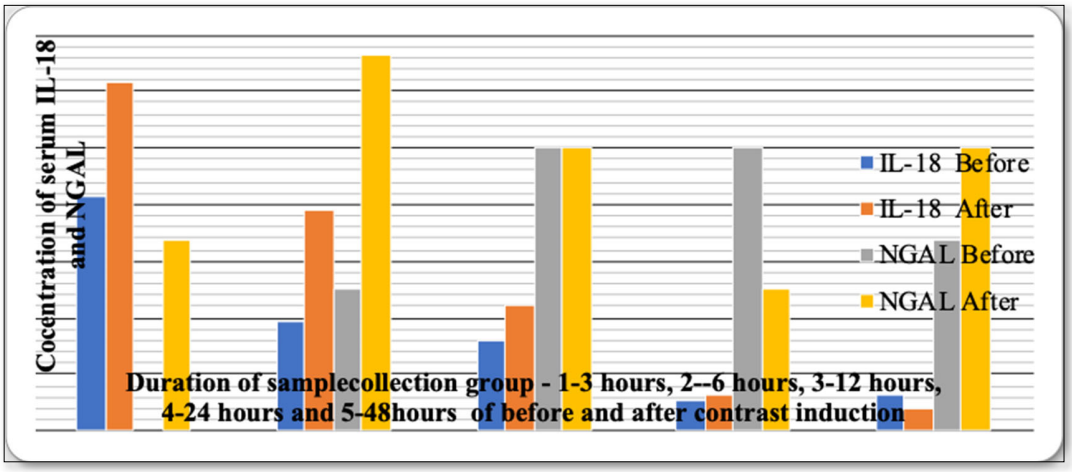

Figure 1. Serum levels of IL-18 and NGAL before and after induction of contrast.

Table 1. Serum levels of IL-18 and NGAL before and after induction of contrast.

Time Duration	IL-18		P value	NGAL	P value
Group 1	Before	8.3 ± 0.8	0.01	0.0±0.0	0.02
(3 h)	After	12.3± 4.5*		6.7±2.1*	
Group 2	Before	3.9 ±1.6	0.001	5.0±2.2	0.03
(6 h)	After	7.8 ±2.1*		13.3±7.6*	
Group 3	Before	3.2 ± 1.4	0.01	10±0.0	1.00
(12 h)	After	4.4 ± 0.9*		10±6.3	
Group 4	Before	1.1 ±0.3	0.01	10±2.6	0.07
(24 h)	After	1.3 ± 0.2*		5.0±3.4	
Group 5	Before	1.3 ± 0.2	0.07	6.7±3.3	0.17
(48 h)	After	0.8 ± 0.4		10±2.6	

Mean ± SEM levels of IL-18 and NGAL before and after induction of contrast Student t-test significant at p<0.05
**Indicates the significance with before induction of contrast*

DISCUSSION

In recent years, CIN has become a severe public health problem. The current study projects there was an increase in serum IL-18 levels at 3 h was 48%, at 6 h 100%, at 12 h and 24 h was 37% and 18%. There was a decrease of serum IL-18 levels after contrast induction at 48 h. The increase of serum IL-18 levels indicates the early ischemia of renal tubular tissue up to 24 h. The decrease of IL-18 levels at 48 h indicates the operation of auto regulatory response by the kidney to counter ischemia [6]. Potent vascular and tubular factors as well as inflammatory processes are involved in the pathogenesis of renal ischemia [7]. Earlier studies found that the macrophages are mediators of AKI in rats and mice, in a model of macrophages contribute to tissue damage during acute renal allograft rejection [8] and ischemic chemo attractants protein reduced macrophages infiltration and AKI [9]. In recent study in mice demonstrated that IL-18 derived from cells of bone marrow and contributes to the renal damage was observed during ischemic AKI in mice and these macrophages are the source of IL-18 in ischemic AKI [10]. IL-18 may be activated in the proximal tubules of nephron and directly contribute to tubular injury. The role of caspase-1 and IL-18 in hypoxia induces membrane injury of freshly isolated mouse proximal tubules invitro was studied [11]. All these studies support our current study.

Findings from the current study clearly indicate the early raise of NGAL at 3 h and 6 h and represent it, as a novel risk biomarker of CIN. NGAL is a small 25-kD protein, belongs to the family of "lipocalins", and is massively released in to blood and urine from injured tubular cells after various conditions potentially detrimental to the kidney in experimental and human clinical models [12]. 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. 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. A cohort study by Bolingano et al. [13], measured NGAL in patients affected by non-advanced CKD with stable renal function. Interestingly, apart from the already cited predictive value, a strict, independent and inverse correlation with estimated GFR was described for both serum NGAL and urine NGAL, suggesting that under these particular conditions this protein may also represent a surrogate index of residual renal function, similar to what has been previously described. Recently, Mori and Nakao proposed an interesting theory which might explain the relationship between NGAL and GFR, suggesting that the increase in NGAL is not just the passive consequence of a reduced renal clearance [14]. The study assumes that the increase in NGAL in chronic kidney disease is the consequence of a sustained production by damaged tubular cells or the results of a general loss of functional cells or nephrons. From this point of view, NGAL would represent as a real-time indicator of contrast induced AKI or CIN.

LIMITATIONS OF THE STUDY

Smaller sample size was the limitation of the study; more studies are warranted with a greater sample size in animals and humans.

CONCLUSION

An early rise of serum IL-18 and NGAL indicates an ischemic renal tubular injury and may probably help to diagnose the CIN at earliest. Current study demonstrates an early raise in serum interleukin-18 levels, suggests early ischemia of the renal tubular tissue and may give a good reflection towards the severity of damage caused due to contrast induction, may probably due to oxidative stress. These findings suggest that the IL-18 may be clinically useful, as an early diagnostic biomarker in CIN over NGAL. Detection of early rise of IL-18 helps nephrologists to adopt preventive measures that can further stop the renal tissue damage during the contrast dependent procedures.

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