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CORRELATION AND COMPARISON OF SERUM CYSTATIN C WITH SERUM CREATININE IN KIDNEY FUNCTION - ANIMAL MODEL

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ABSTRACT

Contrast Induced Nephropathy (CIN) is a complication that is underestimated in clinical practice after cardiac catheterization. Recently, Cystain C as a novel biomarker for the detection of acute kidney injury has been highlighted. The current study proved that serum Cystatin C is a better marker of GFR (Glomerular Filtration Rate) when compared to that of Creatinine in contrast based Acute Kidney Injury (AKI). 30 animals were randomly divided into 10 different cages having 3 animals each and cages were randomly divided into 5 different groups. Animals were injected iohexol (Contrast) 350 mg Iodine/kg bodyweight as per the weight of animal intraperitoneally. Blood samples were collected before and after inducing contrast and centrifuged. Serum was stored at -20^{0}Cfor further analysis. Results shown were that the Cystatin C is an earlier marker for AKI when compared to Creatinine. Cystatin C levels were observed to be elevated at 3Hours where as elevation in Creatinine was at 12 Hours and hence statistically significant P<0.05 with unpaired test. Current study evaluated that the raise of serum Cystatin C is an earlier marker of glomerular filtation rate (GFR) which can be probably correlated with contrast based AKI.

KEY WORDS: Contrast Induced Nephropathy (CIN), Acute Kidney Injury (AKI), Glomerular Filtration Rate (GFR), Cystatin C, Creatinine, Iohexol.

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INTRODUCTION

Contrast Induced Nephropathy (CIN) is a growing concern in the modern world. During many computed tomography and angiography procedures patients are administered special contrast agents orally, rectally or intravenously. These contrast materials are pharmaceutical agents. Contrast enables various tissues, blood vessels and organs to be identified more distinctly and specifically for any lesions. Early detection of acute kidney injury on contrast exposure is a clinical and research priority. Traditional measures contribute to delayed diagnosis of AKI. Recent bio markers, have promise for earlier detection and for research into novel interventions. The need for a simple, accurate and rapid endogenous marker of GFR has been a major limiting factor in clinical practice and research. Owing to inaccuracies associated with these methods, the measurement of endogenous blood substances are used to estimate GFR in common practice. Properties of an ideal endogenous blood substance to estimate GFR should include release into the blood stream at a constant rate and free filtration by the glomerulus, elimination via the kidneys. The early detection of acute kidney injury may allow for timely preventive or therapeutic measures. The review of literature discusses the role of traditional and novel biomarkers in early acute kidney injury and its diagnosis. Serum Creatinine is the most commonly used filtration marker in clinical practice but its accuracy is significantly hampered by assay interference, unreliability of urine collection and the confounding influence of diet, age, gender and muscle mass[1] and there are several well- reported difficulties concerning its analysis [2,3]. Cystatin C is a 122-amino acid 13 kDa protein that is a member of the family proteinase inhibitors. It is the product of a "housekeeping" gene expressed in all nucleated cells and is produced at a constant rate. Because of its small size and basic pH (9.0), Cystatin C is freely filtered by glomerulus. Cystatin C does not return to the blood stream and is not secreted by renal tubules; it has been suggested to be an "ideal" endogenous marker[4]. The early methods for the determination of serum Cystatin C were based on immunoelectrophorosis and single radial immunodiffusion from 1994 to 1997. Fully automated Assays were developed including Particle-Enhanced Turbidimetric Immunoassay (PETIA) and Particle-Enhanced Nephelometric Immunoassay (PENIA)[5,6] over the years till date. Aim of the current study was to correlate the serum Cystatin C and serum Creatinine to identify either of the two as the earliest

MATERIALS AND METHODS

Animals

30 male Wistar albino rats were procured from BRULAC, Saveetha University and maintained at Department of Research and Development, Saveetha Medical College, Chennai after obtaining the animal ethical clearance. (SU/BRULAC/RD/ 001/2014).

Methods

1. Cystatin C Turbidimetric Immunoassay (Auto Pure, Sphera system Pack) procured from Accurex Biomedical Pvt Ltd, India.
2. Creatinine – modified Jaffe's method Assay procured from ChemCHEK^{TM} AGAPPE.

Both the above chemistries were processed on RX imola (RANDOX) clinical chemistry analyser.

Experimental design

Animals were randomly divided into 10 different cages having 3 animals in each cage. These cages were randomly divided by using table of randomization into 5 different groups (Group 1 to Group 5). 6 animals in each group. The groups were divided according to the time duration of sampling after inducing contrast ranging from 3 to 48 hours.

Groups	Duration of sample collection
1	3 - Hours
2	6 - Hours
3	12 - Hours
4	24 - Hours
5	48 - Hours

All the groups of animals received contrast intraperitoneally, dose calculated as per the weight of animals. 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 of the groups 1 – 5 respectively. Blood was centrifuged and serum was stored at -20^{0}C for further analysis.

Statistical analysis

Analysis of data was done by using Graph Padprism 6, (USA). Values were given as Mean±SE. ANOVA was performed to calculate the F value and P value, multiple comparison was done by using Tuke's multiple comparison for correlation and significance of before and after levels of Cystain C and unpaired t test was performed to correlate mean values of Cystain C and Creatinine. Significance was assumed at $P<0.05$.

RESULTS

Significant results were observed between the Cystatin C and Creatinine. The mean values of Cystatin C were compared with mean values of Creatinine by using an Unpaired t test with Welch's correction, $P < 0.05$. (Table 1) Comparison of before and after value were done by using oneway ANOVA. There was a significant correlation between all the groups by corrected Barlet's test $P <0.05$.(Table 2)

Table 1
Comparison of Cystatin C and Creatinine after Inducing Contrast (Mean±SE)

	Groups	Cystatin C (Mean ±SE)	Creatinine (Mean± SE)
1	3 Hours	0.39±0.20	0.44±0.03
2	6 Hours	0.15±0.14	0.44±0.05
3	12 Hours	0.45±0.23	0.61±0.07
4	24 Hours	0.10±0.04	0.36±0.03
5	48 Hours	0.11±0.05	0.40±0.08

Unpaired t test with Welch's correction, P < 0.05.

(Base line for Cystatin C before inducing contrast was 0.24±0.07 and for Creatinine was 0.45±0.04. Reference range of Creatinine is 0.2-0.8 ml/dl referred at Rat Fan Club. Exotic animal companion medicine hand book for veterinarians, Johnson-Delaney, C., 1996, Zoological education network)

Table 2
Levels of Cystatin C (Mean±SE) before and after inducing contrast

Groups	Cystatin C		Mean ± SE	F Value	P Value
1	3Hours	before	0.21±0.21	37.42	<0.0001
		after	0.39±0.20		
2	6Hours	before	0.15±0.09	37.42	<0.0001
		after	0.15±0.14		
3	12Hours	before	0.05±0.03	37.42	<0.0001
		after	0.45±0.23		
4	24Hours	before	0.06±0.03	37.42	<0.0001
		after	0.10±0.04		
5	48Hours	before	0.20±0.09	37.42	<0.0001
		after	0.11±0.05		

ANOVA ordinary - * statistically significant with Bartlet's test (corrected), P<0.05.***

Figure 1
Comparison of Cystatin C and Creatinine

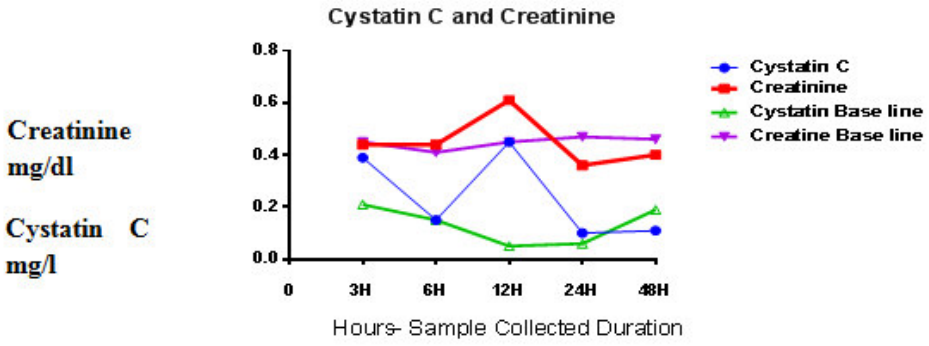
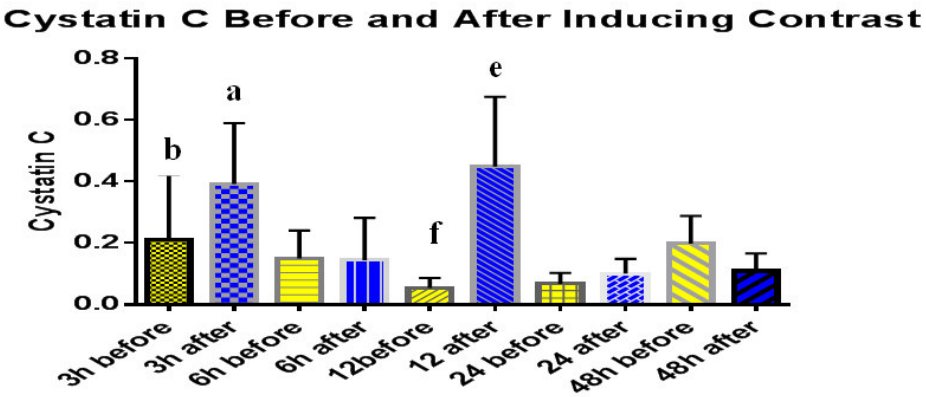


Figure 2
Cystatin C Before and After Inducing Contrast



(Letters a, b, e and f indicate the significance with that group p<0.05 for 3 Hours and 12 Hours before and after inducing contrast)

DISCUSSION

Despite all its disadvantages, serum Creatinine is considered to be a marker of GFR, as it is cheaper and simpler to Assay. In chronic kidney diseases GFR decreases as serum Creatinine level increases. Previous studies have proved that Cystatin C might be an early and better indicator of GFR when compared with serum Creatinine levels[7]. In this current study the correlation between serum levels of Cystatin C and Creatinine were compared. Cystatin C was an earlier and better indicator of GFR. It was observed in the current study that the levels of Cystatin C was increased in group 1 (3 Hours) after inducing contrast when compared to that of same group before inducing contrast. Serum levels of Creatinine showed an increase in group 3 (12 Hours) after inducing contrast. According to the study serum Cystatin C levels are significantly higher than that of Creatinine levels in same group (Table 1, figure 1). Khyse-Andersen et al included 27 healthy controls and 24 patients with reduced GFR and found a significantly better correlation between serum Cystatin C to GFR determined by clearance of iohexol than serum Creatinine. The diagnostic accuracy of Cystatin C for reduced GFR was superior to serum Creatinine[5]. Newman et al concluded that Cystatin C was a better marker of GFR than Creatinine and more sensitive to small changes in GFR. The current study also supports the earlier findings[8].

CONCLUSION

The current study has confirmed that serum Cystatin C is found to be more sensitive and better marker of impaired kidney functions when compared to that of Creatinine and may probably be considered for clinical practice to evaluate the AKI (Acute Kidney Injury) which helps in timely preventive measures to be taken to reduce the damage of kidney and is thought to be cheaper than the other AKI markers like NGAL, KIM, IL-18 to name a few.

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