

The role of serum Cystatin C as early marker of acute kidney injury in subjects undergoing percutaneous transluminal coronary angiogram with normal serum creatinine levels

Shylaja T.V.^{1,*}, Asmabi Makandar², Raghavendra D.S.³, Patnaik A.N.⁴, Shanthi Naidu⁵

^{1,2}Assistant Professor, ³Professor, East Point College of Medical Sciences, Bengaluru, Karnataka, ⁴Professor, Nizam's Institute of Medical Sciences, Hyderabad, ⁵Professor & HOD, Dept. of Biochemistry, Care hospitals, Hyderabad

***Corresponding Author:**

Email: shylajavishal@gmail.com

Abstract

Background: Despite more than half a century of investigations, Acute kidney injury (AKI) remains a major healthcare issue in medicine today, reported to occur in 1–32% of all hospital admissions and 10–90% of intensive care unit admissions and the diagnosis of AKI is usually based on changes in Serum Creatinine (SCr) which is a poor marker of early renal dysfunction.

Objectives: To study the role of Serum Cystatin C (CysC) as early marker of AKI and to correlate the Serum Cys C with conventional marker like SCr in early detection of Contrast induced acute kidney injury (CIAKI) in subjects undergoing Percutaneous transluminal coronary angiogram (PTCA).

Materials and Method: Prospective cohort study was conducted, where SCr and Serum CysC were serially measured in a heterogeneous group of subjects (n=60) presenting to cardiology department and the primary outcome was CIAKI.

Results: The study population consisted of 60 subjects. All subjects were divided into 2 groups: "CIAKI group" and "no-CIAKI group" according to predefined definition. The Serum Cys C increased at 4 hours and reached the peak at 24 hours, whereas SCr increased at 24 hours and reached peak at 48 hours respectively ($P < 0.001$) and there was no significant correlation between Serum Cys C with SCr at 0 hours, 4 hours, 24 hours and 48 hours.

Conclusion: Serum CysC is an early marker for detecting CIAKI compare to conventional marker SCr. So earlier detection of CIAKI by this novel biomarker can reduce morbidity and mortality and also hospital stay.

Keywords: Serum Creatinine, Estimated glomerular filtration rate, Cystatin C, Percutaneous Trans luminal Coronary angiogram, Contrast induced acute kidney injury.

Introduction

AKI presents a continuum of morbidity that can vary from subclinical injury, in which SCr changes minimally, to severe oliguric renal dysfunction associated with tubular necrosis and failure of the kidney to function.⁽¹⁾ In developed countries, AKI is common in elderly and hospital-acquired causes dominate. Where as in developing countries, AKI is the disease of younger subjects and community-acquired cases are common. Acute diarrheal diseases, acute glomerulonephritis, environmental agents and snake bite are the common causes of AKI⁽²⁾ and the acute radio contrast nephropathy is an another important cause of AKI in hospitalized patients undergoing contrast-based procedures.⁽³⁾ SCr has been the predominant marker of renal function in clinical practice for more than half a century and its limitations are well documented.^(4,5) Because of the limitations regarding use of SCr for the early detection of AKI, the role of serum biomarker, Serum Cys C that would allow earlier detection of AKI has been conducted and numerous studies also evaluated the use of Cys C level as an endogenous marker of kidney function in populations at risk with Chronic kidney disease (CKD), showing that Cys C performs comparably or superior to the diagnostic accuracy of SCr level in the discrimination of normal from impaired kidney function.⁽⁶⁾ Since there are only few Indian studies with regards Serum Cys C as early marker of AKI and also it is difficult to study these

markers in relation to entire etiology of AKI. So in this study we selected subjects undergoing contrast related procedure as a cause of AKI as the baseline values were also available in all these subjects.

Materials and Method

Study design and patient population: Prospective observational cohort study of 30 cases and 30 controls conducted in the department of Biochemistry, Nizam's Institute of Medical Sciences, and Hyderabad, Andhra Pradesh.

Study material: Subjects admitted in the department of Cardiology to undergo emergency or elective PTCA

Inclusion criteria: Subjects aged between 18-70 yrs of either gender who were undergoing elective and emergency PTCA by using Iohexol contrast, with normal SCr levels and normal eGFR $>60 \text{ ml/min/1.73 m}^2$ (MDRD formula) attended the hospital during the study period and willing to participate in this study after obtaining informed consent and ethical committee approval has been included.

Exclusion criteria:

1. Subjects with non-availability of normal SCr levels and normal eGFR $>60 \text{ ml/min/1.73 m}^2$ (MDRD) at admission.
2. Subjects with documented evidence of pre-existing CKD or ESRD requiring dialysis.

3. Subjects underwent contrast related procedure within 1 week or less from the index procedure.
4. If subjects had concomitant inflammatory conditions (such as active infection, inflammatory arthritis or connective tissue disease) or malignancies or had recent (<4 months) surgery or major trauma.

Sample Size calculated: Based on baseline data available on Serum Cys C the anticipated difference in mean between two groups is 147 and anticipated standard deviation (SD) is 100, to obtain a power of 99% with type 1 error of 0.001, the required sample size is 21. In this study a total of 30 cases and 30 controls are enrolled.

Sample collection: Venous blood samples were drawn from all participants after 12-hour overnight fast, before PTCA and other samples were taken at 4, 24 and 48 hours after the procedure. Blood samples were centrifuged at 2000 ×g for 10 minutes, and serum separated. SCr, fasting plasma glucose were estimated in these samples and in after procedure samples taken at 4hours, 24hours and 48 hours same centrifugation procedure repeated. Only SCr concentrations estimated in all these samples. Remaining serum was stored at -20°C, and the subjects were observed for primary outcome variable which was the development of CIAKI, defined as an absolute increase in SCr of >25% or 0.5mg/dl from baseline values occurring within 24 to 48 hours after the coronary procedure. Finally by looking at the primary outcome, we included about 30 cases (an increase in SCr by >0.5 mg/dl or >25% within 3 days after intravascular administration of contrast medium (CM) without an alternative aetiology) and 30 controls (who did not showed >25% increase in the SCr at end of

48 hours). All the data were analysed after completion of study period.

Methods: eGFR is Calculated by applying the MDRD formula,⁽⁷⁾ SCr estimated by Jaffe method(IDMS-traceable version),⁽⁸⁾ Glucose estimated by HK (hexokinase) method⁽⁹⁾ and Serum Cys C measured by Automated particle-enhanced turbid metric method.⁽¹⁰⁾ All the parameters were analysed by using fully automated analyzer (Hitachi-912).

Statistical analysis: Continuous variables were presented as mean ± SD and tested with student's *t* test and categorical variables were presented as frequencies and compared with the use of the Pearson chi-square test (Fisher's exact test was applied if the number of observations per cell was fewer than five). The changing trends of Serum Cys C was analysed by taking repeated measures at 0 hours, 4 hours, 24 hours and 48 hours before and after procedure. Spearman rank correlation coefficient were used to know the correlation between Cys C and SCr at different time points. All the analysis was performed using the SPSS 12.0 windows.

Results

The study population consisted of 60 subjects. All subjects were divided into 2 groups: "CIAKI group" and "no-CIAKI group" according to predefined definition.

The "no-CIAKI group" included a total of 30 controls, 27 males (90%) and 3 females (10%) with average age 57.60 ± 05.4 years, the CIAKI group included a total 30 cases, 26 males (86.7%) and 4 females (23.3%) with average age 60.20 ± 05.73 years. (Table 1)

Table 1: The main clinical characteristics of the patient population

Main Characteristics between "CIAKI" group and no "CIAKI" group			
Variables	No CIAKI (n=30)	CIAKI (n=30)	P value
Demographic Characteristics			
Mean age	57.60±05.4	60.20±05.73	<0.081
Male n (%)	27(90%)	26(86.7%)	1.0000
Clinical Characteristics n (%)			
Diabetes Mellitus(DM)	06(20.0%)	28(93.3%)	<0.0001
eGFR ml/min/1.73 m ²	64.90±03.07	62.80±02.68	0.0049
Type of contrast media (CM) n (%)			
Iohexol	30(100%)	30(100%)	-
Dose of contrast (ml)	117.70±33.70	280.00±64.17	<0.0001

Continuous values are expressed as mean ± SD; categorical values are expressed as total number and percentage of the global population (in parentheses).

As depicted in Table, that subjects with CIAKI group were found no difference in age than controls 60.20 ± 5.73 vs 57.60 ± 05.4 years respectively ($P<0.081$), DM ($P<0.0001$), eGFR ($P=0.004$) compared with subjects of "no-CIAKI group" and in subjects who developed CIAKI, a higher dose of CM was administered ($P<0.0001$), while the compared analysis of the subject in relation to the molecule type chosen to make coronaries opaque (Iohexol) did not show any significant statistical difference.

Table 2: Changing trends of novel biomarker Serum Cys C after CM exposure in all the 60 subjects (0, 4, 24 and 48 hours)

Changing trends of Serum Cys C after CM exposure		
Time(Hours)	Cys C (ng/ml) No CIAKI	Cys C (ng/ml) CIAKI
0	80.8	81.7
4	84.0	87.7
24	85.5	133.7
48	84.7	98.3

Table 3: Changing trends of Conventional marker SCr after CM exposure in all the 60 subjects (0, 4, 24 and 48 hours)

Changing trends of SCr after CM exposure		
Time(Hours)	SCr (mmol/L) No CIAKI	SCr(mmol/L) CIAKI
0	94.7	95.3
4	94.0	92.7
24	92.7	93.6
48	93.0	177.0

The changing trends of Serum Cys C and SCr were showed in Table 2, 3 and Fig. 1, 2 respectively. The Serum Cys C increased at 4 hours and reached the peak at 24 hours, while the SCr increased at 24 hours and reached peak at 48 hours respectively. Thus 24 hours after CM administration were considered to be appropriate time point for Cys C measurement.

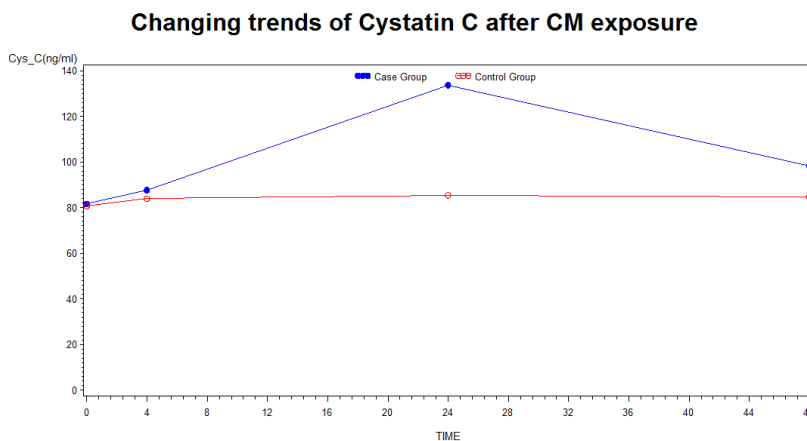


Fig. 1: Shows changing trends of Serum Cy C after CM exposure

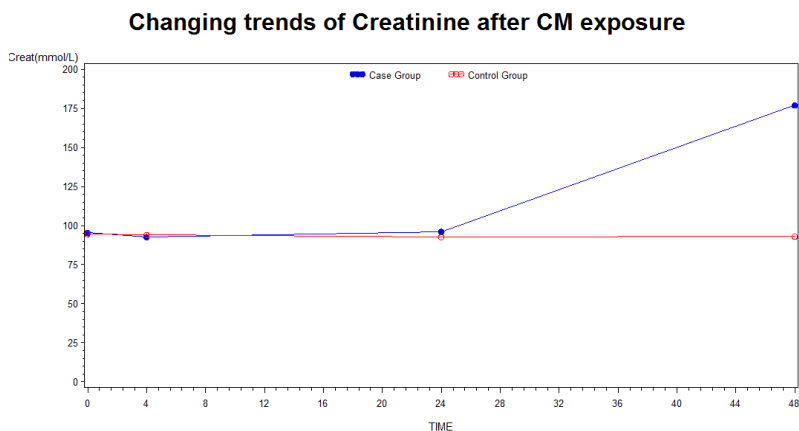


Fig. 2: Shows changing trends of SCr after CM exposure

Table 4: Changes in Serum Cys C and SCr before and after PTCA in CIAKI group

Changes in serum Cys C (ng/ml) and SCr (mmol/l) in CIAKI group					
Variables	Before PTCA:	4hours after PTCA	24 hours after PTCA	48 hours after PTCA	P value
Serum CysC(ng/ml)	80.8 ± 9.6	87.6 ± 8.1	133.6 ± 7.6 a	98.3 ± 23.7 b	<0.0001 a, b
SCr(mmol/l)	94.6 ± 5.7	92.6 ± 4.4	93.6 ± 4.3	177 ± 16.8 c	<0.0001 c

Results expressed as Mean ± SD a,b=P<0.0001: CysC – Baseline vs 24 hrs and 48 hrs
c=P<0.0001:SCr- Baseline vs 48 hrs

Table 4, Shows that in CIAKI the mean Serum Cys C level increased significantly at 24 hours after PTCA compared to the baseline value (133.6 ± 7.6 vs 80.8 ± 9.6ng/ml(P<0.001), while there was no significant difference between 4 hours after PTCA and baseline. Whereas in the mean SCr level, there was no significant difference between 0, 4, and 24 hours after PTCA but SCr showed significant difference compared to baseline (177 ± 16.8 vs 94.6 ± 5.7 mmol/l (P<0.001) at 48 hours.

Table 5: Pearson's correlation analysis

Correlation between Serum Cys C and SCr at different time line				
	0hours	4hours	24hours	48hours
r	0.1216	-0.2042	0.0499	-0.1081
P	0.5220	0.2789	0.7933	0.5696

Table 5, Performed to evaluate the correlation between SCr and Serum Cys C at 0 hours, 4 hours, 24 hours and 48 hours. The result indicated no significant correlation between Serum Cys C with SCr at 0 hours, 4 hours, 24 hours and 48 hours.

Discussion

CIAKI is a complication of radiological procedures that expose patients to CM. It may be very high in selected patient subsets, especially in cardiac procedures such as PCI (Percutaneous Coronary Intervention) and coronary angiography. Not only is this a leading cause of morbidity and mortality, but it also adds to increased costs in high risk patients undergoing PCI. DM is one of the strongest predictors of AKI after coronary intervention. In our study, we found that occurrence of CIAKI is significantly higher among subjects with DM compared to other subjects. This is in accordance with other study Toprak O et al.⁽¹¹⁾ eGFR found to have significant difference (P<0.004) in CIAKI subjects compared to no CIAKI subjects. Renal function deterioration after exposure to radiographic contrast agents is common in patients with impaired renal function. Volume of contrast showed that the CIAKI risk increases proportionally to the dose of CM like in CIAKI than no CIAKI this is in accordance with other study Kane GC et al.⁽¹²⁾

The discrepancies in the above findings may be due to contributions from several systemic factors like prolonged vasoconstriction, alterations in nitric oxide metabolism that lead to renal vasoconstriction, and impaired auto regulation induced by CM predisposing to

medullary hypoxia, in combination with direct cytotoxicity to the renal tubular epithelium.⁽¹³⁾

Finding new biomarker is very important for the early diagnosis of CIAKI. So we investigated Serum CysC changing trends after CM exposure and evaluated their characteristics as early biomarker for CIAKI.

Serum Cys C, cysteine proteinase inhibitor has many features of an ideal glomerular filtration marker. It is synthesized and released into plasma by all nucleated cells at a constant rate. It has small size (13kDa) with no positive charge at physiologic pH and no significant protein binding therefore, it is 99% freely filtered at the glomerulus. It is neither secreted nor reabsorbed by renal tubules but undergoes almost complete catabolism by proximal tubular cells, and thus little appears in the urine, with a half-life of about 2 hours. Thus Serum Cys C reflects GFR better than SCr.⁽¹⁴⁾

This study showed Serum Cys C increased at 4hours and reached the peak at 24 hours, whereas SCr increased at 24 hours and reached the peak at 48 hours. Bajorzewska-Gajewska H et al in their cohort study found a significant rise in CysC 24 hours after the procedure. Whereas SCr and Creatinine clearance remained unchanged after procedure.⁽¹⁵⁾ Herget-Rosenthal S et al reported that Serum Cys C is a useful detection marker to detect ARF 1 or 2 days earlier than Creatinine.⁽¹⁶⁾ Rickli H et al observed that the rise in Cys C was achieved a maximum at 24 hours after the application of the contrast agent and within 48 hours Cys C decreased to the same level as before angiography.⁽¹⁷⁾

In our study, we have done correlation between SCr and Serum CysC at 0, 4, 24, and at 48 hours. Serum Cys C and SCr showed no correlation among them at baseline and also after exposure to CM. The results might attribute to different time of production and elimination of these biomarker. LIU Xiao-li et al showed that plasma Cys C significantly correlated with SCr (r=0.340, P<0.001).⁽¹⁸⁾ Shaker OG et al in their study concluded that CysC as predictive biomarker for AKI after PCI.⁽¹⁹⁾ This is the study to my knowledge, which examined prospectively a novel marker (Serum CysC) of AKI in subjects undergoing PTCA at 0, 4, 24, and 48 hours, as well as correlations between Serum CysC and SCr in subject with normal SCr and with eGFR >60ml/min/1.73m².

Conclusion

Serum CysC increased earlier than SCr, hence Serum Cys C can be used as early marker for detecting CIAKI compare to conventional marker SCr. Application of Serum CysC as novel biomarker in routine can reduce morbidity and mortality and also hospital stay.

References

- Liagos O, Wald R, O'Bell JW, Price L, Pereira BJ, Jaber BL et al. Epidemiology and outcomes of acute renal failure in hospitalized patients: a national survey. *Clin J Am Soc Nephrol* 2006;1:43.
- Prakash J, Gupta A, Kumar O, Rout SB, Malhotra V, Srivastava PK et al. Acute renal failure in falciparum malaria Increasing prevalence in some areas of India a need for awareness. *Nephrol Dial Transplant* 1996;11:2414–2416.
- Wang A, Holcslaw T, Bashore TM et al. Exacerbation of radio contrast nephrotoxicity by endothelin receptor antagonism. *Kidney Int* 2000;57:1675–1680.
- Soni SS, Ronco C, Katz N, Cruz DN. Early diagnosis of acute kidney injury,+ the promise of novel biomarkers. *Blood Purif* 2009;28:165–174.
- Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the Rife criteria for early acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008;23:1203–1210.
- Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function. *Am J Kidney Dis* 2002;40:221-226.
- Poggio E, Wang X, Greene T, Van Lente F, Hall P. Performance of the Modification of Diet in Renal Disease and Cockcroft-Gault Equations in the Estimation of GFR in Health and in Chronic Kidney Disease. *J Am Soc Nephrol*, 2005;16:459-66.
- Lamb E, Path F, Price C. Creatinine, Urea and Uric acid. In: Burtis C, Ashwood E, Bruns D, editors. *Teitz fundamentals of clinical chemistry*. 6th ed. USA: Elsevier,2008:p.363-6.
- Kricka L. Principles of immunochemical techniques. In: *Teitz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th edn., Burtis CA, Ashwood ER, Bruns DA (Eds.). New Delhi: Elsevier,2006. pp. 219–44.
- Kyhe-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V, Grubb A: Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem*. 1994;40:1921-1926.
- Toprak O, Cirit M, Yesil M. Impact of diabetic and prediabetic state on development of contrast-induced nephropathy in patients with chronic kidney disease. *Nephrol Dial Transplant* 2007;22:819-826.
- Kane GC, Doyle BJ, Ierman A, Barsness GW, Best PJ, Rihal CS. et al Ultra low contrast volumes reduce rates contrast induced nephropathy in patients with chronic kidney disease undergoing coronary angiography. *J am coll cardiol*. 2008;51:89-90.
- Wellman TL, Jenkins J, Penar PL, Tranmer B, Zahr R, Lounsbury KM et al. Nitric oxide and reactive oxygen species exert opposing effects on the stability of hypoxia-inducible factor-1alpha (HIF-1alpha) in explants of human pial arteries. *FASEB J* 2004;18:379-381.
- Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A et al. Cystatin C as a marker of GFR history, indications, and future research. *Clin Biochem* 2005;38:1-8.
- Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophil gelatinase-associated lipocalin and renal function after percutaneous coronary angiography. *Am J Nephrol* 2006;26(3):287-292.
- Herget-Rosenthal S, Marggraf G, Hu'sing J. Early detection of acute renal failure by serum cystatin C. *Kidney Int* 2004;66(3):1115-1122.
- Rickli H, Benou K, Ammann P. Time course of serial cystatin C levels in comparison with serum creatinine after application of radio contrast media. *Clin Nephrol* 2004;61(2):98-102.
- LIU Xiao-li, WANG Zhi-jian, YANG Qing, YU Miao, SHEN Hua, NIE Bin et al. Plasma neutrophil-gelatinase-associated lipocalin and cystatin C could early diagnose contrast-induced acute kidney injury in patients with renal insufficiency undergoing an elective percutaneous coronary intervention. *Chin Med J* 2012;125(6):1051-1056.
- Shaker OG, El-Shehab yA, El-Khatib M. Early diagnostic markers for contrast nephropathy in patients undergoing coronary angiography. *Angiology* 2010;61:731-6.