Original Article

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Biochemistry Section

Clinical Utility of Serum Cystatin C in Comparison with Serum Creatinine, Urea and Uric Acid in Patients with Chronic Kidney Disease

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ABSTRACT

Introduction: Estimation of serum creatinine is the gold standard to diagnose Chronic Kidney Disease (CKD) and estimation of cystatin C could also be an important biomarker for the same.

Aim: To assess the correlation between serum cystatin C and conventional serum markers like creatinine, urea and uric acid in CKD.

Materials and Methods: A cross-sectional study was done between January 2012 to June 2013 in a tertiary care hospital which involved 40 patients with CKD and 20 healthy controls. Blood sample (10 mL) was collected from each patient before the initiation of dialysis and used for assay of serum cystatin C, serum creatinine, urea and uric acid. Serum cystatin C was analysed by using Diagnostic kit (SensIT) using latex enhanced immunoturbidimetry method. Serum creatinine, urea and uric acid were estimated by enzymatic assay kits. The data obtained were statistically analysed using Statistical Package for the Social Sciences (SPSS) software version 19. Descriptive statistics and Pearson correlation for these markers were determined. A p-value <0.05 was considered to be statistically significant.

Results: Majority of the patients were in the age group 55-65 years (57.5%). Majority (82.5%) of the patients were males. The age of patients showed significant positive correlation with serum cystatin C and serum creatinine with p-value<0.001. Mean duration of CKD in patients was 4.08 years with a standard deviation of 1.95 years. All the 40 patients were undergoing haemodialysis twice a week and for a mean duration of 2.12 years with a standard deviation of 1.02 years. Out of the 40 patients 50% were both diabetic and hypertensive. Comparison of serum cystatin C with serum creatinine, serum urea, and serum uric acid using Pearson correlation showed significant positive correlation with p-value <0.05.

Conclusion: This study shows that serum cystatin C can be used as a reliable marker in CKD to assess declining renal function like established markers.

Keywords: Correlation, Estimation, Renal injury, Serum biomarker

INTRODUCTION

Current evaluation of CKD depends on routine estimation of serum creatinine and determination of Glomerular Filtration Rate (GFR), and is considered to be the gold standard for the diagnosis. Many factors like age, gender, muscle mass and its metabolism, diet, drugs, fluid balance and tubular function affect serum creatinine level [1,2]. So Cockcroft and Gault formula and Modification of Diet in Renal Disease (MDRD) formula is being used in determination of GFR.

In CKD, serum creatinine and other parameters may rise only when 50% of renal function is lost. So, the demand for reliable biomarkers to detect early CKD always exists [3]. A biomarker is considered to be ideal when it has high sensitivity and specificity. It should be easily measured and reliable. It should help in early diagnosis and should have prognostic value.

There are several emerging biomarkers that have been proposed for early kidney damage out of which one biomarker is cystatin C [3]. It is a small 13kD protein. It belongs to cysteine protease inhibitor family. All nucleated cells produce it at a constant rate. It is unaffected by age, muscle mass, gender and tubular secretion [4].

It was found to rise in the blood when the kidney function declines and also more useful in predicting complications and end stage kidney disease than other established markers [5]. The limitations of the previous studies are that the comparison between serum cystatin C and other markers like serum creatinine, urea and uric acid was not assessed in a single study to show the comparison and hence, this study was undertaken to evaluate the same. Cystatin C may serve as useful biomarker to indicate early renal injury before chronicity when compared to other markers like serum creatinine, urea and uric acid which becomes evident only after chronic renal injury. Hence, evaluation of cystatin C can help in early diagnosis of CKD and any delay may lead to severity of kidney injury especially in chronicity irrespective of the primary cause and hence this study was undertaken as it is the need of the hour in order to diagnose as early as possible. The aim of this study was to know the level of serum cystatin C in comparison with serum serum creatinine, urea and uric acid in CKD and its role as an early biomarker of CKD for its reliability.

MATERIALS AND METHODS

This cross-sectional study was done from January 2012 to June 2013 in Department of Biochemistry and Department of Nephrology, Sree Gokulam Medical College Hospital, Venjaramoodu, Kerala, India. The study was carried out after approval from the Institutional Research Committee and Ethics Committee of Sree Gokulam Medical College Ref. No.SGMC/IEC/92/11. Blood sample (10 mL) was collected from each patient before the initiation of dialysis and used for assay of serum cystatin C, serum creatinine, urea and uric acid.

Sample Size

A sample size of 30 was sufficient for this study without type II error to estimate serum biomarker as per statistician's

suggestion, but a sample size of 40 was kept to increase the accuracy of the results.

Inclusion criteria: Forty patients with kidney disease for ≥3 months and GFR<60 mL/min/1.73m^2 (stage 3 and above CKD- national kidney foundation guidelines) in the age group of 18 years and above, attending the nephrology outpatient department and on maintenance dialysis were selected as study subjects. Twenty healthy subjects were included as controls. All the 40 patients were undergoing haemodialysis twice a week and for a mean duration of 2.12 years with a standard deviation of 1.02 years. Haemodialysis was done with low flux polysiphone membrane for duration of four hours. The samples were taken for all the assays before the initiation of dialysis.

Exclusion criteria: Critically ill and patients on immunosuppressive drugs were not included. Patients with renal transplantation, end stage liver disease, thyroid dysfuction, cardiovascular disease, neurological disorders and malignancy were also excluded.

Informed written consent was taken from the study subjects. Once included, socio-demographic and clinical data were collected from the study subjects using profoma.

Analysis of the sample

Blood (10 mL) was collected by venipuncture from patients before initiation of dialysis and serum was separated by centrifugation at room temperature. Haemolytic, icteric or lipaemic specimens were not used and samples were stored at -20°C. Serum cystatin C assay was done using SensIT Cystatin C diagnostic kit based on Latex enhanced immunoturbidimetry in Bayer RA 50 semi-automated clinical biochemistry analyser. (Reference range 0.55 to 1.44mg/L). Serum creatinine, urea and uric acid were estimated by enzymatic assay kits in automated clinical chemistry analyser Beckman Coulter Au 680.

STATISTICAL ANALYSIS

The data obtained after estimation of the analytes were statistically analysed using Microsoft Excel and SPSS software version 19. Descriptive statistics and Pearson correlation for different parameters were determined for data analysis. A p-value <0.05 was considered statistically significant. Mann whitney-U test (independent non -parametric test) was used to compare difference between serum cystatin C in 40 CKD patients and 20 controls.

RESULTS

Age Distribution

Majority of the patients were in the age group 55-65 years (57.5%) and mean age of CKD patients was 56.72 years.

Sex Distribution

Majority (82.5%) of the patients were males in this study. The percentage of females was 17.5%. Mean serum cystatin C value was same in both gender (3.28 mg/l with standard deviation 0.94mg/l in males and 1.01mg/l in females).

The age of patients showed significant positive correlation with serum cystatin C, serum creatinine with p-value <0.001. The correlation coefficient obtained when age was correlated with each of the two markers was highest for serum cystatin C [Table/Fig-1].

Variable	Correlation coefficient	p-value
Serum cystatin C (mg/L))	0.554	<0.001
Serum creatinine (mg/dL)	0.519	<0.001

[Table/Fig-1]: Comparison of serum cystatin C and serum, creatinine with age. Pearson correlation, N=40

Mean duration of CKD in patients was 4.08 years with a standard deviation of 1.95 years. All 40 patients were undergoing haemodialysis twice a week and for a mean duration of 2.12 years with a standard deviation of 1.02 years [Table/Fig-2].

Duration (years)				
	Minimum	Maximum	Mean	Standard deviation
CKD	1	8	4.08	1.95
Dialysis	1	4	2.12	1.02

[Table/Fig-2]: Distribution of patients according to duration of Chronic Kidney Disease (CKD) and dialysis in years.

Mean serum cystatin C value in control group (N=20) was 0.65mg/L (standard deviation 0.096) and for case group mean values for cystatin C and other markers is shown in [Table/Fig-3].

Blood marker	Minimum	Maximum	Mean	Standard deviation
Serum cystatin C (mg/L)	1.60	4.90	3.28	0.94
Serum creatinine (mg/dL)	1.80	5.40	3.74	1.08
Serum urea (mg/dL)	42	106	61.80	17.17
Serum uric acid (mg/dL)	7.80	11.20	9.35	1.07

[Table/Fig-3]: Mean values of serum cystatin C and other blood markers of CKD. N=40

Comparison of serum cystatin C with serum creatinine, serum urea, and serum uric acid using Pearson correlation showed significant positive correlation with p-value <0.05 [Table/Fig-4].

Blood markers	Pearson Correlation coefficient	p-value
Serum cystatin C (mg/L) Serum creatinine (mg/dL)	0.964	0.001
Serum cystatin C (mg/L) Serum urea (mg/dL)	0.939	0.001
Serum cystatin C (mg/L) Serum uric acid (mg/dL)	0.980	0.001

[Table/Fig-4]: Comparison of serum cystatin C and other biomarkers of CKD. N=40

Mann whitney-U test (independent non-parametric test) was used to compare difference between serum cystatin C (mg/l) (N=40) in CKD patients and controls (N=20). Serum cystatin C was significantly high in CKD patients compared to healthy controls with p-value <0.0001 and degree of freedom 58 [Table/Fig-5].

Comparison	Z-value	p-value
Serum cystatin C (mg/L) (N=40) in CKD patients and controls (N=20)	6.276	<0.0001

[Table/Fig-5]: Comparison of Serum cystatin C (mg/l) in CKD patients and controls.

In terms of estimated Glomerular Filtration Rate (eGFR) calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, serum cystatin C has shown significant results when compared to serum creatinine [Table/Fig-6].

eGFR	Mean±SD	Pearson correlation coefficient
Creatinine eGFR	18.72±8.85	0.989*
Cystatin C eGFR	17.77±8.63	

[Table/Fig-6]: eGFR comparing serum creatinine and cystatin C. Pearson correlation; eGFR- Estimated glomerular filtration rate; *p<0.0001: SD: Standard deviation

In this study group, 30% were smokers and 70% were non-smokers where as 50% were alcoholics and rest 50% non-alcoholics. Both, smoking and alcoholism were personal habits in 25%. Mean values of cystatin C were higher in smokers and alcoholics when compared to non-smokers and non-alcoholics.

Out of the 40 patients 95% were hypertensive, 55% were diabetic and 50% were having both diabetes and hypertension. The two main common causes for CKD in these 40 patients were hypertension and diabetes mellitus.

DISCUSSION

CKD is differentiated from an acute kidney disease, if it is present beyond three or more months. In this study, the age of patients showed significant positive correlation with serum cystatin C and serum creatinine. The Pearson correlation coefficient obtained when age was correlated with both the markers was highest for serum cystatin C. Similar findings were seen in other studies which considers both age of the patients and duration of illness. In one of the earlier study, cystatin C was shown as a better index of kidney function in elderly persons and therefore a better predictor of outcomes [5].

This is a clear proof that serum cystatin C estimation could help early diagnosis of kidney injury. Cystatin C and creatinine -based equations like MDRD equation are also age dependent. The two main common causes for CKD are hypertension and diabetes mellitus as evident from this study. Family history is very important as far as CKD is concerned. Family history of end stage renal disease, hereditary nephritis, cystic kidney disease and various tubular syndromes are important in CKD but in this study none of the patients had family history of kidney disease. Assessment of loss of kidney function is very difficult in initial stages as symptoms are not specific. Most of the cases present with complications and is diagnosed by blood levels of conventional marker, which is serum creatinine. Estimation of serum cystatin C can help early diagnosis of CKD [6].

The mean value obtained in this study for serum cystatin C was 3.28mg/l with a standard deviation of 0.94mg/L and for serum creatinine was 3.74mg/l with a standard deviation of 1.08mg/dl. Pearson correlation done for serum cystatin C and commonly used CKD marker serum creatinine showed significant correlation with correlation coefficient 0.964. This was found to be statistically significant with p-value <0.05. Therefore, serum cystatin C can be used as a marker in CKD to determine declining renal function like the established common marker serum creatinine. The result of this study is consistent with other earlier studies [7,8].

Mean values of cystatin C were higher in smokers and alcoholics when compared to non-smokers and non-alcoholics in this study. Out of the 40 patients 95% were hypertensive and 55% were diabetic. Both diabetes and hypertension were present in 50%. The epidemiological literature shows mixed observation as far as these personal habits and these markers are concerned [9].

Serum urea value rises only after 50% of kidney function is affected. Serum cystatin C was compared with serum urea in this study with Pearson correlation. The study showed mean value of serum urea as 61.80 mg/dl with a standard deviation of 17.17 mg/dL. Pearson correlation coefficient of 0.939 indicated strong correlation between the two blood markers that is serum cystatin C is a reliable marker when compared with serum urea, an established marker for decreased renal function. One of earlier study has shown that cystatin C is a much better marker than creatinine and urea in cases where there is only slight to moderate decrease in GFR. Serum uric acid has relation to incidence and progression of CKD [10].

Serum cystatin C was also compared with serum uric acid in these patients. The mean value obtained for serum uric acid was 9.35 mg/dl with a standard deviation of 1.07 mg/dl. Pearson correlation (Correlation coefficient 0.980) was found to be statistically significant with p-value <0.05 which is similar to one of earlier studies [11,12].

According to a study, improved GFR estimation was shown with combined measurement of serum cystatin C and serum creatinine in CKD patients [13]. eGFR value calculated based on CKD-EPI formula with serum cystatin C and serum creatinine separately was compared with Pearson correlation (Correlation coefficient 0.989) and it showed significant correlation with p-value <0.0001 especially in these patients on haemodialysis which is similar to an earlier study, thus suggesting it can be used in CKD patients undergoing haemodialysis [14]. This study therefore strongly recommends to add this test in routine examination to evaluate CKD.

Limitation(s)

Only CKD patients on haemodialysis were included in this study and hence, comparison could not be made between patients on haemodialysis and those who were not on haemodialysis.

CONCLUSION(S)

Positive correlation was obtained between serum cystatin C and other serum markers like creatinine, urea and uric acid. This shows that serum cystatin C can be used as an early reliable serum bio marker in CKD to assess early detection of declining renal function when compared to established markers which shows evident rise in serum only at later stages. Since, the clinical usefulness of cystatin C assay has been proved by this study, further development of kits for routine clinical use will be beneficial for CKD patients. eGFR can be calculated using serum cystatin C for routine clinical evaluation of CKD.

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