Original article:

Serum cystatin C as an early biomarker of chronic kidney disease in diabetic patients

[1] DR. ARCOT KRISHNA KISHOR*, [2] DR.PRASUN SAGAR, [3] DR. SANDEEP RAI

[1]Jr. Resident, [2]Senior Resident, [3] Professor and Head of Unit
MGM Hospital, Kamothe / Medicine Department
Corresponding author*

Abstract:

According to SEEK, the prevalence of CKD in INDIA was estimated to be 17.4%. Cystatin C is being considered as a potential replacement for serum creatinine as a filtration marker. The present study is planned at a tertiary care centre to evaluate Cystatin C as an early biomarker of CKD in diabetic patients. The study showed that Cystatin C in comparison with Serum Creatinine can be a useful early marker in detecting renal impairment in Diabetic individuals. Thus, this study indicates that cystatin C can potentially offer a more efficient and early diagnostic tool than the traditional CKD markers in Diabetic patients with renal disease.

Keywords: Serum Cystatin, Chronic Kidney Disease.

INTRODUCTION

Diabetes is an increasing cause of morbidity and mortality in both the industrialized as well as the developing countries. In India approximately 77 Million people are Diabetic.[1] More than 90 % of them have Type 2 Diabetes; hence India is rightly called as the Diabetes capital of the world.[2] Chronic Kidney Disease is a worldwide public health problem both due to magnitude of patients and cost involved in treatment. The prevalence of CKD is 8 to 16% worldwide. According to first report published by the INDIAN CKD REGISTRY the most common cause of CKD in India is Diabetic Nephropathy (31%). As per the results of SEEK (Screening and Early Evaluation of Kidney Disease) study, prevalence of CKD was observed to be 17.2% with approximately 6 % having stage 3 or worse in India. According to SEEK, the prevalence of CKD in INDIA was estimated to be 17.4%. The definition of CKD as per the National Kidney Foundation (NKF) Kidney Disease Outcome Quality Initiative (K/DOQI) is:

1. Kidney damage for > 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, as manifested by either the pathologic abnormalities; or the markers of the kidney damage, including abnormalities in composition of blood or urine, or abnormalities in imaging tests.

2. GFR 3 months, with or without kidney damage.[3]

Cystatin C is being considered as a potential replacement for serum creatinine as a filtration marker. Most studies show that serum levels of Cystatin C (Scys) are more closely correlated with GFR than serum creatinine (Scr). The concentration of serum Cystatin C is almost completely dependent upon GFR. Some of the other studies done in the past have shown that serum Cystatin C is an early biomarker among the diabetic patients, but many studies have not been done so. [4,5]

The present study was conducted at a tertiary care teaching hospital to evaluate Cystatin C as an early biomarker of CKD in diabetic patients and to correlate parameters like Serum Creatinine, Urinary micralbumin-creatinine ratio (UACR) and Cystatin C as an early biomarker of Chronic Kidney Disease in Diabetic patients.

Objectives

1. To calculate grade of Chronic Kidney Disease by CKD-EPI method 141 x Min(SCr/Kappa,1)Alpha x Max(SCr/Kappa,1)-1.209 x 0.993Age x 1.018(Female) x 1.159(if black) Where kappa is 0.7 in females,0.9 in males, alpha is -0.329 for females and -0.411 in males.

2. To study and correlate all these parameters to evaluate Cystatin C as early biomarker in Chronic Kidney Disease in Diabetic patients.

Methods

A hospital based prospective, comparative study was conducted on 120 patients to evaluate Cystatin C as early biomarker of Chronic Kidney Disease in Diabetic patients. The patients were divided in the following three groups of 40 patients each:

☐ **Group I:** Healthy individuals

☐ **Group II:** Diabetic patients with UACR <30 mg/g

☐ **Group III:** Diabetic patients with UACR ≥30 mg/g

Study Groups

Total 120 individuals consisting of control and study subjects were enrolled in this study and shall be divided into following three groups.

Group I (n=40)

Healthy subjects.

Inclusion criteria:

☐ Healthy normal patients with no history of Diabetes were enrolled in this group.

☐ Age Group- 35- 60 yrs

Exclusion criteria:

☐ Patients with history of any other major illness like cancer, cardiovascular disease, HTN, Diabetes, liver disorder or HIV positive.

Group II (n=40)

This group included known patients of Diabetes.

Inclusion criteria:

☐ Known cases of Diabetes with UACR < 30 mg/g and a history of at least 5 yrs or more of Diabetes.

Exclusion criteria:

☐ Diabetic patients with UACR ≥ 30 mg/g or presenting with nephropathy due to any other cause other than Diabetes.

Group III (n=40)

Inclusion criteria:

☐ Known cases of Diabetes with UACR > 30 mg/g and history of at least 5 yrs or more of Diabetes.

Exclusion criteria:

☐ Diabetic patients with UACR ≤ 30 mg/g or having nephropathy due to any other cause other than Diabetes.

Investigations

Clinical biochemistry analyser (AU480 by Beckman coulter)

Consumables for estimation of fasting glucose, Serum creatinine, Cystatin. Kits for estimation of urinary microalbumin and creatinine from commercial sources and CKD-EPI were calculated with calculator available on computer.

10 ml of the blood sample was collected and the volumes were divided in to fluoride & EDTA bulb for further analysis.

The estimation of plasma sugar by Glucose oxidase & peroxidase (GOD POD) method.

Statistical Analysis

Basic Descriptive Statistics

Association between variables

Table 1 to 4 shows statistical analysis of the results obtained during study period.

Table 2 represents associations between variables of Group I. There is significant correlation between Serum Cystatin C and other variables, as marked with **(* and **)** 0.01 and 0.05.

Serum Cystatin C is statistical significant with Serum ceatinine with r = 0.801, Urinary Microalbumin r = 0.724, eGFR r = -0.402 respectively which is represented in Graph 4.1, Graph 4.2 and Graph 4.3. (Where 'r' is rank correlation coefficient)

Table 3 represents associations between variables of Group II. There is significant correlation between Serum Cystatin C and other variables, as marked with **(* and **)** 0.01 and 0.05.

Group II - Serum Cystatin C is statistical significant with Serum ceatinine with r = 0.514, Urinary Microalbumin r = 0.465, eGFR r = -0.470 respectively

Table 4 represents associations between variables of Group III. There is significant correlation between Serum Cystatin C and other variables, as marked with **(* and **)** 0.01 and 0.05.

Group III - Serum Cystatin C is statistical significant with Serum ceatinine with r = 0.393, eGFR r = -0.426 respectively.

Table 1

| | Group | | | | | |
| | Control Group | | Group II | | Group III | |
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Serum Cystatin C	.72	.09	1.58	.24	3.93	1.39
FBS	95.80	9.47	113.78	13.51	135.95	38.00
PPBS	126.83	15.36	152.53	15.16	188.95	50.79
Serum Creatinine	.71	.10	.88	.15	8.23	2.84
Urinary Microalbumin	19.60	2.96	24.55	4.28	5765.80	2697.33
UACR	27.66	1.42	27.69	1.69	703.33	252.70
eGFR (CKD- EPI Creatinine Equation)	121.48	14.14	106.05	21.79	8.30	5.38

Table 2

Group I-Correlations

			Serum Cystatin C	Age	FBS	PPBS	Serum Creatinine	Urinary Microalbumin	UACR	eGFR (CKD-EPI Creatinine Equation)
Spearman's rho	Serum Cystatin C	Correlation Coefficient	1.000							
		Sig. (2-tailed)								
		N	40							
	Age	Correlation Coefficient	-.047	1.000						
		Sig. (2-tailed)	.776							
		N	40	40						
	FBS	Correlation Coefficient	.295	.023	1.000					
		Sig. (2-tailed)	.065	.889						
		N	40	40	40					
	PPBS	Correlation Coefficient	.239	-.111	.731**	1.000				
		Sig. (2-tailed)	.137	.497	.000					
		N	40	40	40	40				
	Serum Creatinine	Correlation Coefficient	.801**	.036	.140	.183	1.000			
		Sig. (2-tailed)	.000	.824	.391	.259				
		N	40	40	40	40	40			
	Urinary Microalbumin	Correlation Coefficient	.724**	.058	.134	.222	.930**	1.000		
		Sig. (2-tailed)	.000	.722	.412	.168	.000			
		N	40	40	40	40	40	40		
	UACR	Correlation Coefficient	-.172	.197	-.040	.077	-.068	.256	1.000	
		Sig. (2-tailed)	.290	.223	.808	.639	.676	.111		
		N	40	40	40	40	40	40	40	
	eGFR (CKD-EPI Creatinine Equation)	Correlation Coefficient	-.402*	-.581**	-.140	.053	-.605**	-.559**	-.144	1.000
		Sig. (2-tailed)	.010	.000	.390	.746	.000	.000	.376	
		N	40	40	40	40	40	40	40	40

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 3

			Group II-Correlations							
			Serum Cystatin C	Age	FBS	PPBS	Serum Creatinine	Urinary Microalbumin	UACR	eGFR (CKD-EPI Creatinine Equation)
Spearman 's rho	Serum Cystatin C	Correlation Coefficient	1.000							
		Sig. (2-tailed)								
		N	40							
	Age	Correlation Coefficient	.175	1.000						
		Sig. (2-tailed)	.280							
		N	40	40						
	FBS	Correlation Coefficient	.109	-.082	1.000					
		Sig. (2-tailed)	.504	.613						
		N	40	40	40					
	PPBS	Correlation Coefficient	.042	-.222	.565**	1.000				
		Sig. (2-tailed)	.796	.168	.000					
		N	40	40	40	40				
	Serum Creatinine	Correlation Coefficient	.514**	.099	-.327*	-.141	1.000			
		Sig. (2-tailed)	.001	.544	.039	.384				
		N	40	40	40	40	40			
	Urinary Microalbumin	Correlation Coefficient	.465**	.017	-.317*	-.181	.925**	1.000		
		Sig. (2-tailed)	.002	.916	.046	.263	.000			
		N	40	40	40	40	40	40		
	UACR	Correlation Coefficient	-.067	.096	.145	.060	.089	.288	1.000	
		Sig. (2-tailed)	.683	.555	.373	.713	.585	.071		
		N	40	40	40	40	40	40	40	
	eGFR (CKD-EPI Creatinine Equation)	Correlation Coefficient	-.470**	-.478**	.397*	.202	-.775**	-.647**	.067	1.000
		Sig. (2-tailed)	.002	.002	.011	.212	.000	.000	.679	
		N	40	40	40	40	40	40	40	40

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4

			Serum Cystatin C	Age	FBS	PPBS	Serum Creatinine	Urinary Microalbumin	UACR	eGFR (CKD-EPI Creatinine Equation)
						Group III- Correlations				
Spearman 's rho	Serum Cystatin C	Correlation Coefficient	1.000							
		Sig. (2-tailed)								
		N	40							
	Age	Correlation Coefficient	.026	1.000						
		Sig. (2-tailed)	.876							
		N	40	40						
	FBS	Correlation Coefficient	.007	-.232	1.000					
		Sig. (2-tailed)	.964	.150						
		N	40	40	40					
	PPBS	Correlation Coefficient	-.116	-.088	.695**	1.000				
		Sig. (2-tailed)	.478	.591	.000					
		N	40	40	40	40				
	Serum Creatinine	Correlation Coefficient	.393*	-.200	.156	-.097	1.000			
		Sig. (2-tailed)	.012	.215	.335	.550				
		N	40	40	40	40	40			
	Urinary Microalbumin	Correlation Coefficient	.206	-.029	-.115	-.333*	.675**	1.000		
		Sig. (2-tailed)	.202	.861	.481	.036	.000			
		N	40	40	40	40	40	40		
	UACR	Correlation Coefficient	-.104	.149	-.130	-.271	-.029	.644**	1.000	
		Sig. (2-tailed)	.522	.359	.424	.091	.861	.000		
		N	40	40	40	40	40	40	40	
	eGFR (CKD-EPI Creatinine Equation)	Correlation Coefficient	-.426**	.077	-.058	.247	-.853**	-.668**	-.023	1.000
		Sig. (2-tailed)	.006	.635	.722	.124	.000	.000	.889	
		N	40	40	40	40	40	40	40	40

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

It was observed in the present study that the mean serum Creatinine values of patients in Group I was significantly lower compared to patients of Group II and Group III (0.71±0.10mg/dL vs. 0.88±0.15mg/dL vs. 8.23±2.84mg/dL). The mean urinary microalbumin values of patients in Group I was significantly lower compared to patients of Group II and Group III (19.60±2.96mg/l vs. 24.55±4.28mg/l vs. 5765.80±2697.33mg/l). The mean Urine Albumin Creatinine Ratio (UACR) values of patients in Group I was significantly lower compared to patients of Group II and Group III (27.66±1.42mg/g vs. 27.69±1.69mg/g vs. 703.33±252.70mg/g). This is similar to the study of Ashwin Kumar et al.[6] In the present study, the mean estimated Glomerular Filtration Rate (eGFR) values of patients in Group I was significantly higher compared to patients of Group II and Group III (121.48 ±14.14mL/min/1.73m^2 vs.106.05±21.79mL/min/1.73m^2 vs.8.30±5.38mL/min/1.73m^2). This result is similar to the studies of Yang et al[7] and Kyung-Jeon et al.[8] In the present study, there was significant association of Serum Cystatin C with estimated Glomerular Filtration Rate (eGFR) in Group II and Group III patients. This finding was consistent with the studies of Assal et al[9], Ogawa et al[10], Kedam et al[11].

The analysis shows association between blood investigations or markers of study in Group I (Healthy individuals) of Serum Cystatin C with serum creatinine (r = 0.801), urinary microalbumin (r = 0.724), and eGFR (r = -0.402) are linearly associated significantly with level of significance 0.01 & 0.05 respectively.

There is also an association between blood investigations or markers of study in Group II of Serum Cystatin C with serum creatinine (r = 0.514), urinary microalbumin (r = 0.465), and eGFR (r = -0.470) are linearly associated significantly with level of significance 0.01 & 0.05 respectively.

The analysis further shows association between blood investigations or markers of study in Group III of Serum Cystatin C with serum creatinine (r = 0.393), and eGFR (r = -0.426) are linearly associated significantly with level of significance 0.01 & 0.05 respectively. Similar observations were noted in the studies of Gupta et al[12], Jeon et al[13], Takir et al[14], Tan et al[15] and few others as well.

Conclusion

Serum Cystatin C showed a significant correlation with albuminuria and reduced eGFR. Hence Cystatin C in comparison with Serum Creatinine can be a useful early marker in detecting renal impairment in Diabetic individuals.

Microalbuminuria has been known as an earliest biomarker for detection of Diabetic Nephropathy. However, it has many limitations, like low sensitivity and large variability. Being increased in serum or urine, even before the appearance of albuminuria, raised creatinine and decrease in eGFR, Cystatin C may offer an additional advantage to traditional CKD markers with respect to early detection of Diabetic Nephropathy and its progression, that will allow for timely intervention and management of Diabetic Nephropathy.

Cystatin C and Cystatin C-based eGFR are also an important predictor of clinically important outcomes including all-cause and cardiovascular mortality in patients with Diabetes. Thus, this study indicates that cystatin C can potentially offer a more efficient and early diagnostic tool than the traditional CKD markers in Diabetic patients with renal disease. However this was a single centre study with limited patients. More prospective multicentric studies with large number of patients will be needed to further validate these results.

References

1. King H, Aubert RE, Herman WH. Global prevalence of Diabetes. Diab Care 1998;21:1414–31.

2. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1979;241:2035-8.

3. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation Classification and Stratification. Am J Kidney Dis 2002;39:1-266.

4. Rigalleau V, Beauvieux MC, Le Moigne F. Cystatin C improves the diagnosis and stratification of chronic kidney disease, and the estimation of glomerular filtration rate in Diabetes. Diabetes Metab 2008;34:482-9.

5. Oddoze C, Morange S, Portugal H. Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with Diabetes. Am J Kidney Dis 2001;38:310–6.

6. Ashwin Kumar AS, Anil Kumar AS. Serum Cystatin C and serum creatinine levels in Type 2 Diabetes mellitus. Int J Res Med Sci 2015;3:174-7.

7. Yang YH, He XJ, Chen SR. Changes of serum and urine neutrophil gelatinase-associated lipocalin in Type-2 diabetic patients with nephropathy: one year observational follow-up study. Endocrine 2009;3:45-51.

8. Jeon YK, Kim MR, Huh JE. Cystatin C as an early biomarker of nephropathy in patients with Type 2 Diabetes. J Korean Med Sci 2011;26:258–63.

9. Assal HS, Tawfeek S, Rasheed EA. Serum Cystatin C and tubular urinary enzymes as biomarkers of renal dysfunction in Type 2 Diabetes mellitus. Clin Med Insights Endocrinol Diab 2013;6:7-13.

10. Ogawa Y, Goto T, Tamasawa N. Serum Cystatin C in diabetic patients. Not only an indicator for renal dysfunction in patients with overt nephropathy but also a predictor for cardiovascular events in patients without nephropathy. Diabetes Res Clin Pract 2008;79:357-61.

11. Kedam DP, Polur H, Bandela PV. Cystatin-C as a Biomarker in Predicting Early Renal Impairment in Normo-Albuminuric Patients with Type 2 Diabetes Mellitus. J Pharm Sci Res 2015;7:913-6.

12. Gupta K, Nayyar SB, Sachdeva J. Cystatin C in the early diagnosis of diabetic nephropathy and its correlation with albuminuria. Int J Adv Med 2017;4:56-9.

13. Jeon YL, Kim MH, Lee WI. Cystatin C as an early marker of diabetic nephropathy in patients with Type 2 Diabetes. Clin Lab 2013;59:1221-9.

14. Takir M, Unal AD, Kostek O. Cystatin- C and TGF-β levels in patients with diabetic nephropathy. Nefrologia 2016;36:653-9.

15. Tan GD, Lewis AV, James TJ. Clinical usefulness of Cystatin C for the estimation of glomerular filtration rate in Type 1 Diabetes reproducibility and accuracy compared with standard measures and iohexol clearance. Diab Care 2002;25:2004-9.