VOLUME - 11, ISSUE - 09, SEPTEMBER - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Nephrology

COMPARISON OF RENAL FUNCTION ASSESSMENT BY CYSTATIN C AND CREATININE BASED E-GFR IN TYPE 2 DIABETES MELLITUS FOR EARLY DIAGNOSIS OF DIABETIC NEPHROPATHY

Chandrasekaran Kaliyaperumal — Department of Nephrology, Thanjavur Medical College, Tamil Nadu

Rajakumar Veerasamy — Department of Nephrology, Thanjavur Medical College, Tamil Nadu

Varun Kumar* — Department of Community Medicine, Government Medical College and ESI Hospital, Coimbatore, Tamil Nadu *Corresponding Author

ABSTRACT

Introduction: Cystatin-C has been proposed to be promising marker to detect early kidney injury. So, the present study was conducted with the objective of comparing the e-GFR estimated by creatinine and cystatin C based equations in type 2 diabetics in different stages of albuminuria. **Materials and Methods:** A cross sectional study was conducted from January 2019 to January 2020 in the patients admitted in the Department of General Medicine, Thanjavur Medical College, Tamil Nadu. Systemic random sampling was done to get the sample size of 100. **Results:** Among 100 study participants, 66 were males and 34 were females. About, 32 had normo-albuminuria, 49 had microalbuminuria and 19 had macro albuminuria. Correlation found using Pearson's correlation test between serum albumin levels and estimated glomerular filtration rate showed that there were significant negative correlation between estimated glomerular filtration rate with serum creatinine (r = -0.58, P = 0.001), and as well as with serum cystatin C (r = -0.69, P = 0.001). Similarly, micro-albuminuria and macro-albuminuria showed significant positive correlation with estimated glomerular filtration rate. **Conclusion:** From the results of our study, we can conclude that serum cystatin C based E-GFR can be used as an early independent marker of chronic kidney disease in diabetic patients.

KEYWORDS : Cystatin C, serum creatinine, diabetic nephropathy, biological marker

INTRODUCTION:

Diabetes mellitus is one of the most rapidly-growing epidemics around the world. Type 2 diabetes mellitus is one of the most common forms of chronic disease globally[1]. Among people aged 20 – 79 years, 6.6% have diabetes. India is the second most populous country in the world and has the highest number of people with diabetes with expected increase to 69.9 million by 2025and 642 million by 2040 with global prevalence of 10%.[2] This increasing number has influenced the rate of diabetic complications, including diabetic kidney disease.

Diabetic nephropathy is defined by the presence of proteinuria of more than 0.5 g/24 h. This stage is referred to as overt nephropathy, clinical nephropathy, proteinuria, or macroalbuminuria. Diabetic nephropathy is the single most common cause of end-stage kidney disease.[3] Even when diabetes is controlled, it can lead to chronic kidney disease (CKD) and kidney failure[4]. Diabetic nephropathy is the leading cause of chronic kidney disease in patients starting renal replacement therapy[5].

The diagnosis of CKD at an early detectable stage is important to delay the renal complications. At present, diagnosis of CKD relies upon the assessment of kidney function, by calculating estimated glomerular filtration rate (eGFR). Assessment of kidney damage can be done by checking urinary albumin-to-creatinine ratio [UACR, urine albumin (mg/L)/urine creatinine (mmol/L)] in random urine samples. Though these tests can be performed easily, they have certain limitations.[6]

Serum creatinine is the routinely used marker for the assessment of renal function. Serum creatinine is filtered by the Glomerulus therefore, is used as an indirect measure of glomerular filtration. As glomerular filtration rate (GFR) diminishes, there is a rise in plasma concentrations of serum creatinine and blood urea. Raised serum creatinine has become reliable indicators of kidney dysfunction and can be used as prognostic markers and predictors of renal damage in diabetic patients [7].The values of serum creatinine does not show increase until GFR levels are moderately decreased (40 mL/min/1.73 m2) and the levels are affected by factors such as age, diet, and muscle mass.

Among several biomarkers, Cystatin-C has been proposed to be promising marker to detect early kidney injury. Cystatin-C is a low molecular weight non glycosylated protein, produced by all nucleated cells in the body. It is removed from the blood stream and freely filtered by the glomerular membrane in the kidneys. The serum levels of are not influenced by infections, inflammation or neoplastic states, and also by body mass, or drugs.[8] Here we are comparing the creatinine, cystatin-c levels of serum and urine as marker of early renal impairment with different stages of albuminuria in patients with type2 diabetes mellitus. So, the present study was conducted with the objective of comparing the e-GFR estimated by creatinine and cystatin C based equations in type 2 diabetics in different stages of albuminuria.

MATERIALS AND METHODS

Study Design:

A cross sectional study was conducted from January 2019 to January 2020 in the patients admitted in the Department of General Medicine, Thanjavur Medical College, Tamil Nadu. Systematic random sampling method was followed and every fifth patient was included in the study till a minimum sample size of 100 was reached.

Study Participants:

Patients with type 2 diabetes mellitus and aged between 30 to 70 years of both sexes were included in this study. Patients of type 1 diabetes mellitus, chronic inflammatory disorders, uncontrolled hypertension, thyroid disease, chronic kidney disease, on lipid lowering drugs, steroids, ACE inhibitors and pregnant ladies and those who did not give consent to take partin this study were excluded.

The study participants were divided into three sub groups depending on their albumin creatinine ratio (ACR). Those having normal range ACR (<30 mg/g) were placed in the normo-albuminuria group, those having ACR in the range of

30-300 mg/g in micro albuminuria group and those having ACR more than 300 mg/g were placed in the macro albuminuria group.

Data Collection:
Blood sample and spot urine sample were obtained from the participants after an overnight fast. Serum was separated by centrifuging blood at 4000 rotations per minute (rpm) for 5 minutes and this serum was analyzed for creatinine and cystatin C levels. Serum creatinine was measured by Jeffe's kinetic method. Serum cystatin C was measured by quantitative turbidimetric test.

The following equations were used to calculate the expected glomerular filteration rate (E-GFR)
CKD-EPI-Serum Cystatin C Based Equation:
E-GFR = 127.7 x (Cystatin C in mg/l)-1.17x (age in years)-0.13 x (x 0.91 if female)

CKD-EPI-Creatinine Equation:
E-GFR = 141 x min(SCr/κ, 1)α xmax(SCr /κ, 1)-1.209 x0.993Age x1.018 [if female] x1.159 [if Black]
CKD-EPI-Creatinine-Cystatin C Equation
E-GFR = 135 × min(SCr/κ, 1)α ×max(SCr/κ, 1)-0.601 ×min(Scys/0.8, 1)-0.375
×max(Scys/0.8, 1)-0.711×0.995Age ×0.969 [if female] ×1.08 [if black]

Data analysis:
Data were entered in the excel spreadsheet and variables were coded accordingly. The statistical analyses were performed using Graph pad Prism version 5 software. Fisher's exact test was used to compare the frequency distribution of categorical parameters between the groups. One way ANOVA was used to compare the mean value between the three groups. Pearson's correlation test was used to find the direction and strength of association between the parameters. The P value of less than 0.05 was considered to be statistically significant.

Ethical issues:
The study protocol was approved by the Institutional Ethical Committee (IEC) of Thanjavur Medical College, Thanjavur. Informed written consent was also obtained from the study participants in the vernacular language before enrolling them in this study.

RESULTS:

Age and sex distribution:
The sample size of the study population was 100. Among them 66 were males and 34 were females. About 33 study participants were in the age group of 40-50 years, 38 were in the age group 51-60 years and 28 were in the age group 61-70 years. The mean age of the study participants were 55.1 years with a standard deviation of 8.7 years. The distribution of study participants according to their age and gender is given in the table 1.

Table 1: Distribution of study participants according to age and sex (n = 100)

S. No	Age category	Male (n=66)		Female (n=34)	
		n	%	n	%
1	40 – 50 years	24	36.4	9	26.5
2	51 – 60 years	25	37.9	13	38.2
3	61 – 70 years	17	25.8	12	35.3

Distribution according to albuminuria:
Among 100 study participants, 32 had normo/-albuminuria, 49 had microalbuminuria and 19 had macro albuminuria. Fishers exact test was used to determine the frequency distribution of albuminuria types with age category and sex of the study participants and it was found to be significant with a P value of 0.001 and 0.033 respectively (Table 2).

Table 2: Distribution albuminuria types with age and sex of the study population (n = 100)

S. No	Demographic character		Normo-albuminuia (n=32)		Micro-albuminuria (n=49)		Macro-albuminuria (n=19)		P value
			n	%	n	%	n	%	
1	Age category	40 – 50 years	19	59.4	13	26.5	1	5.3	0.001
		51 – 60 years	9	28.1	20	40.8	9	47.4	
		61 – 70 years	4	12.5	16	32.7	9	47.4	
2	Sex	Male	21	65.6	37	75.5	8	42.1	0.033
		Female	11	34.4	12	24.5	11	57.9	

The various clinical parameters like random blood sugar (RBS), serum creatinine and serum cystatin C were compared with different types of albuminuria. The results were given in table 3.

Table 3: Comparison of various parameters with respect to type of albuminuria between the groups in the study (n = 100)

S. No	Parameters	Normo-albuminuia (n=32)		Microalbuminuria (n=49)		Macro-albuminuria (n=19)		F value	P value	
		Mean	SD	Mean	SD	Mean	SD			
1	Age in years	49.6	8.64	56.5	7.8	60.5	5.9	13.1	No Vs Mi	0.001*
									No Vs Ma	0.172(NS)
									Mi Vs Ma	<0.001*
2	RBS (mg/dl)	178	57	198	53	271	49	18.7	No Vs Mi	0.308(NS)
									No Vs Ma	<0.001*
									Mi Vs Ma	<0.001*
3	Serum Creatinine (mg/dl)	0.6	0.08	1.04	0.3	2.8	1.1	127.2	No Vs Mi	0.001*
									No Vs Ma	<0.001*
									Mi Vs Ma	<0.001*
4	Cystatin C (mg/L)	0.7	0.1	1.3	0.3	2.7	0.5	237.6	No Vs Mi	<0.001*
									No Vs Ma	<0.001*
									Mi Vs Ma	<0.001*

Data are expressed as mean with Standard deviation. One Way ANOVA with Bonferroni posthoc test was used to compare the means between the groups.

*indicates p<0.05 and considered statistically significant.

NS-Not significant; No- Normal abuminuria; Mi-Micro albuminuria; Ma-Macro albuminuria

Distribution according to stages of chronic kidney disease: The expected glomerular filtration rates were calculated in the study participants using the equations that included serum creatinine and serum cystatin C separately and the results were given in the table 4.

Table 4: Distribution of study participants according to expected glomerular filtration rate

S. No	Stage of CKD	GFR range (ml/min/1.73m2)	eGFR-Creatinine (n=100)		eGFR-Cystatin (n=100)		P value
			n	%	n	%	
1	Stage 1	≥90	53	53	33	33	0.004
2	Stage 2	89.9 – 60	17	17	15	15	0.699
3	Stage 3a	59.9 – 45	9	9	22	22	0.011
4	Stage 3b	44.9 – 30	7	7	11	11	0.323
5	Stage 4	29.9 – 15	10	10	18	18	0.103
6	Stage 5	<15	4	4	1	1	0.174

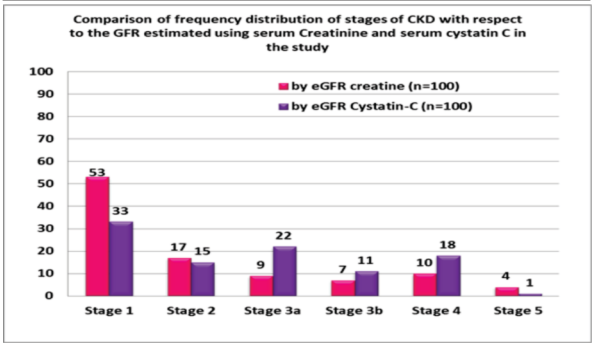

Figure 1:
Correlation was found using Pearson's correlation test between serum albumin levels and estimated glomerular filtration rate and it was found that there were significant negative correlation between estimated glomerular filtration rate with serum creatinine (r = -0.58, P = 0.001), and as well as with serum cystatin C (r = -0.69, P = 0.001). Similarly, micro-albuminuria and macro-albuminuria showed significant positive correlation with estimated glomerular filtration rate.

DISCUSSION:

The sample size was 100. Among them, 32% were innormoalbuminuria range, 49% in microalbuminuria range and 19.9% hadmacroalbuminuria. In normoalbuminuria patients, 59.4% were in 40-50 years age group, 28.1% were in 51-60 years age group, and 12.5% were in 61-70 years age group. In microalbuminuriagroup, 26.5% were in 40-50 years age group, 40.8% patients were in 50-60 years age group, and 32.7% patients were in 61-70 years age group. In macroalbuminuria group, 5.3% patients were in 40-50 years age group, 47.4% patients were in 51-60 years age group, and 47.4% patients were in 61-70 years age group.

In normoalbuminuria patients, 65.6% were male and 34.4 % were female. In microalbuminuria patients, 75.5% were male and 24.5% were female. In macroalbuminuria patients, 42.1% were male and 57.9% were female. In this study males and females in microalbuminuriastage and macroalbuminuria is more in females and microalbuminuria is more in males. P value is significant. Serum creatinine mean value for normoalbuminuria patients is 0.6, for microalbuminuria patients mean value is 1.04, for macroalbuminuria patients mean value is 2.8. Serum cystatin c mean value for normoalbuminuria patients is 0.7, for microalbuminuria patients mean value is 1.3, for macroalbuminuria patients mean value is 2.7.

Most number of patients was diagnosed in stage 2, stage 3 and stage 4 CKD. In this study, some of the patients with normal creatinine and normoalbuminuria with normal GFR were found to have elevated cystatin C. Therefore, cystatin C may be considered as an early marker for nephropathy in diabetic patients.

Amer AHet al conducted the study among diabetic subjects to find the correlation between serum creatinine, microalbuminuria and serum Cystatin C with diabetic nephropathy.[9]In that study Serum creatinine showed a significant correlation with the albuminuria and the reduced GFR groups. It was found that majority of subjects with lesser duration of diabetes had normal creatinine, normalbuminuria, normal to reduced GFR but elevated Cystatin C levels. Results of this study show that serum Cystatin C may be considered as an early marker for nephropathy in diabetic subjects. Similar results were also obtained in a study conducted by Shetty et al. [10]

In an another study conducted by Tian S et al[10], the results suggest that serum cystatin C measurement is an useful and practical tool for the evaluation of renal involvement in the course of diabetes and serum cystatin C based eGFR can be used as an early independent marker of diabetic kidney disease. Similar results were also found in the study conducted by Oddoze C et al.[12]

CONCLUSION:

From the results of our study, we can conclude that serum cystatin C based E-GFR can be used as an early independent marker of chronic kidney disease in diabetic patients.

Declarations:
Funding: None

Conflict of interest: None

Ethical approval: The study protocol was given approval by the institutional ethical committee (IEC), Thanjavur Medical College, Thanjavur, Tamil Nadu.

REFERENCES:

1. International Diabetes Federation. Diabetes Atlas, Fourth edition, International Diabetes Federation, 2009.
2. International Diabetes Federation. Diabetes Atlas, third edition International Diabetes Federation, 2006.
3. Ritz E, Zeng X. Diabetic nephropathy- Epidemiology in Asia and the current state of treatment. Indian J Nephrol. 2011;21:75-84.
4. Dabla PK. Renal function in Diabetic nephropathy. World J Diabetes 2010;1(2):48-56.
5. US Renal Data System: USRDS 2003 Annual Data Report: Atlas of End-Stage Renal Disease in the United States. Bethesda MD, National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2003.
6. Levey AS , Coresh J , Balk E , Kausz AT , Levin A , Steffes MW , et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification . Ann Intern Med 2003 ; 139 : 137 - 147.
7. Aldler AI, Stevens RJ, Manley SE. Development and progression of nephropathy in type 2 diabetes. Kideny Int. 2003; 63:225-232.
8. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat ClinPractEndocrinolMetab 2008; 4: 444–452.
9. Amer NH, Haridas N. Early Diagnostic Markers in Diabetic Nephropathy Patients. Journal of Clinical and Diagnostic Research, 2018, Nov, Vol-12(11): 59.
10. Shetty V, Jain H, Singh G, Parekh S, Shetty S. Plasma Cystatin C as marker of early renal impairment in diabetes mellitus. International Journal of Scientific Study. 2017;4(12):1-7.
11. Tian S, Kusano E, Ohara T, Tabei K, Itoh Y, Kawai T, et al. Cystatin C measurement and its practical use in patients with various renal diseases. Clinical Nephrology. 1997;48(2):104-08.
12. Oddoze C, Morange S, Portugal H, Berland Y, Dussol B. Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with diabetes. American Journal of Kidney Diseases. 2001;38(2):310-16.