Original Research Article

A comparative study of GFR estimated using serum cystatin C (Hoek equation) with that of serum creatinine (MDRD equation) in a diabetic patients

S Sindu Priya[1], R P Sathvika[2*]

[1]Assistant Professor, Department of Biochemistry, Annapoorana Medical College and Hospital, Salem, Tamil Nadu, INDIA.
[2]Associate Professor, Department of Biochemistry, Government Dharmapuri Medical College, Dharmapuri, Tamil Nadu, INDIA.
Email: sindukarthick@gmail.com, drrpsathvika@gmail.com

Abstract

Background: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is estimated that type 2 diabetes mellitus (T2DM) is the primary cause leading to kidney disease in 20-40% of people starting dialysis. Serum or plasma creatinine levels have become the most commonly used markers for GFR determination. In present study we compared GFR estimation using serum cystatin C (Hoek equation) with that of serum creatinine (MDRD equation) in a diabetic patient. **Material and Methods:** This was a cross-sectional study which included 90 known T2DM patients in the age group of 40-70 years and patients were categorized based on the urine albumin-creatinine ratio (UACR) into three groups. GFR was estimated using, MDRD formula and Hoek formula and findings were compared. **Results:** Study patients were categorized into three groups based on the urine albumin-creatinine ratio into Normoalbuminuria (Group A), Microalbuminuria (Group B) and Macroalbuminuria (Group C). No significant difference was observed in the mean of age, gender and BMI among the three groups. Statistically significant difference was observed in the mean duration of diabetes, SBP, DBP, total cholesterol, triglyceride levels, creatinine and cystatin C levels among the three groups ($p<0.05$). Statistically significant difference was observed in the mean of UACR, MDRD equation and Hoek equation among the three groups ($p<0.05$). Cystatin C has no association with age, duration, BMI, TC, TGL, Non-HDL and LDL. Statistically significant positive correlation was observed between cystatin C and blood pressure, FPS, UACR and creatinine. **Conclusion:** Cystatin C is more accurate and sensitive than creatinine for the detection of early renal impairment in type 2 DM. Cystatin C may be considered as a useful, non-invasive marker for early detection of renal injury in type 2 DM.

Keywords: GFR, cystatin C, serum creatinine, diabetic nephropathy.

[*]**Address for Correspondence:**
Dr R P Sathvika, Associate Professor, Department of Biochemistry, Government Dharmapuri Medical College, Dharmapuri, Tamil Nadu.
Email: drrpsathvika@gmail.com

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes has become a major global health problem. This disease affects 6.6% (285 million people) of the world's population in the 20-79 years age group.[1] According to the International Diabetic Federation (IDF), this number is expected to grow to 380 million by 2025.[2,3] Diabetes is a disease that is strongly associated with both microvascular and macrovascular complications, including retinopathy, nephropathy, and neuropathy (microvascular) and ischemic heart disease, peripheral vascular disease, and cerebrovascular disease (macrovascular), resulting in organ and tissue damage in approximately one third to one half of people with diabetes.[4] It is estimated that type 2 diabetes mellitus (T2DM) is the primary cause leading to kidney disease in 20-40% of people starting dialysis. During the present decade, 30% of the predicted $1.1 trillion medical costs of dialysis world-wide will result from diabetic

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nephropathy.[5] The glomerular filtration rate (GFR) is considered the best general index that reflects kidney function, both in health and in disease.[6] Serum or plasma creatinine levels have become the most commonly used markers for GFR determination because of the simplicity and lower costs of this method.[7,8] However, serum creatinine concentration does not increase until renal function decreases to less than 50% of the normal value.[9] Serum creatinine is an insensitive indicator of diminished GFR because its concentration is affected by meat intake, gender, muscle mass, malnutrition and ageing.[10] Serum cystatin C, a cysteine protease inhibitor, which is freely filtered by the renal glomeruli and metabolized by the proximal tubule has been identified as a promising marker of renal impairment. On this background, we compared GFR estimation using serum cystatin C (Hoek equation) with that of serum creatinine (MDRD equation) in a diabetic patient.

MATERIAL AND METHODS

This was a cross-sectional study which included 90 known cases of T2DM patients attending the Diabetic Outpatient Department of a tertiary care centre. This study was conducted after getting ethical committee clearance. Informed written consent was obtained from all the study participants. All study participants were age and sex matched.

INCLUSION CRITERIA

Known cases of type 2 diabetic patients in the age group of 40-70 years were selected. Further these patients were categorized based on the urine albumin-creatinine ratio (UACR) into,

1. Normoalbuminuria (UACR: < 30mg/g)
2. Microalbuminuria (UACR: 30mg/g – 300mg/g)
3. Macroalbuminuria (UACR: > 300mg/g)

EXCLUSION CRITERIA:

Type 2 diabetics with UTI, on treatment with ACE inhibitors or antihypertensive drugs, with thyroid disorders, under thyroid medications, steroid therapy, nephrotoxic drugs, other renal disorders, cardiovascular disease, chronic liver disease and cancer. Detailed medical history and clinical examination was done for all the study participants. Height, weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded. Body mass index (BMI) was calculated for all the study participants. After an overnight fasting of 12-14 hours, about 5 ml of venous blood was drawn under aseptic precautions for measuring plasma glucose (by glucose oxidase – peroxidase method), creatinine (MODIFIED JAFFE'S METHOD), cystatin C (Immunoturbidimetric assay) and lipid profile which includes total cholesterol (TC) (BY CHOD-PAP METHOD), triglyceride (TGL) (BY GPO-POD METHOD) and HDL by direct enzymatic method. Serum LDL is calculated using Friedewald equation. Non-HDL cholesterol is calculated as a difference between total cholesterol and HDL cholesterol. Under aseptic precautions, spot urine sample was collected. Urine albumin (Immunoturbidimetric assay) and urine creatinine were estimated and albumin creatinine ratio (milligram of albumin excreted per gram of urinary creatinine) was calculated in mg/g.

GFR was estimated using,

1. MDRD formula:

eGFR (ml/min/1.73m^2) = 186 X (creatinine/88.4) $^{-1.154}$ X (age) $^{-0.203}$ X 0.742 (if female)

2. Hoek formula: eGFR (ml/min/1.73m^2) = (80.35/cystatin C) – 4.32

Data analysis was done with the help of computer using Epidemiological Information Package (EPI 2010) and range, frequencies, percentages, means, standard deviations, chi square and 'p' values were calculated. Analysis of variance (ANOVA) test was used to test the significance of difference between quantitative variables. Yate's and Fisher's chi square tests were used for qualitative variables. A 'p' value less than 0.05 is taken to denote significant relationship.

RESULTS

The present study is a cross-sectional study which included 90 known T2DM patients. These patients were categorized into three groups based on the urine albumin-creatinine ratio into Normoalbuminuria (Group A), Microalbuminuria (Group B) and Macroalbuminuria (Group C). The mean age was 53.8±9.1, 53.4±7.9 and 57.4±8.1 years for normoalbuminuric, microalbuminuric and macroalbuminuric groups respectively. No significant difference was observed in the mean of age among the three groups. There is no significant association between gender and the normoalbuminuric, microalbuminuric and macroalbuminuric groups. The means of BMI were 24.3±3.0, 24.3±2.4, 24.0±2.5 Kg/m^2 for normoalbuminuric, microalbuminuric and macroalbuminuric groups respectively. No significant difference was found in the mean of BMI among the three groups. The mean duration was 4.43±2.43, 7.33±3.92 and 12.03±4.16 years for group A, B and C respectively. Statistically significant difference was observed in the mean of duration among the three groups ($p< 0.05$). The mean of systolic blood pressure was 119.7±7.2, 127.7±7.7 and 129.7±11.6 mm/Hg and mean of diastolic blood pressure was 80.0±6.4, 83.3±7.6 and 88.0±7.6 mm/Hg for normoalbuminuric, microalbuminuric and macroalbuminuric groups respectively. Statistically significant difference was found in the mean of SBP, mean of DBP in microalbuminuric and macroalbuminuric group when compared to normoalbuminuric group ($p<0.05$). Statistically significant difference was observed in the mean of TC in macroalbuminuric group when compared to

normoalbuminuric and microalbuminuric groups ($p<0.05$). Statistically significant difference was observed in the mean of TGL among the three groups ($p<0.05$). The mean of creatinine was 0.84±0.12, 0.99±0.18 and 1.23±0.41 mg/dL and mean of cystatin C was 0.94±0.23, 1.11±0.25 and 1.64±0.68 mg/L for the three groups respectively. Statistically significant difference was found in the mean of creatinine and cystatin C among the diabetic groups ($p<0.05$).

Table 1: Distribution of general characteristic

General characteristic	Group A No (%)	Group B No (%)	Group C No (%)	P value
Mean age (years)	53.8 ± 9.1	53.4 ± 7.9	57.4 ± 8.1	p>0.05
Gender				p>0.05
Male	14 (46.7%)	16 (53.3%)	16 (53.3%)	
Female	16 (53.3%)	14 (46.7%)	14 (46.7%)	
BMI (Kg/m^2)	24.3 ± 3.0	24.3 ± 2.4	24.0 ± 2.5	p>0.05
Mean duration of diabetes (years)	4.43 ± 2.43	7.33 ± 3.92	12.03 ± 4.16	p< 0.05
Systolic blood pressure	119.7 ± 7.2 mm/Hg	127.7 ± 7.7 mm/Hg	129.7 ± 11.6 mm/Hg	p<0.05
Diastolic blood pressure	80.0 ± 6.4 mm/Hg	83.3 ± 7.6 mm/Hg	88.0 ± 7.6 mm/Hg	p<0.05
Total Cholesterol (mg/dL)	195.3 ± 27.7	196 ± 24.6	233.8 ± 34.9	p<0.05
Triglyceride (mg/dL)	197.2 ± 28.7	262.7 ± 37.3	280.2 ± 49.3	p<0.05
Creatinine (mg/dL)	0.84±0.12	0.99±0.18	1.23 ± 0.41	p<0.05
Cystatin C (mg/dL)	0.94±0.23	1.11±0.25	1.64±0.68	p<0.05

(p value < 0.05 is significant)

Statistically significant difference was observed in the mean of UACR, MDRD equation and Hoek equation among the three groups ($p<0.05$).

Table 2: UACR, MDRD and HOEK'S eGFR among the diabetic groups

Group	UACR (mg/g)		MDRD (ml/min/1.73m^2)		Hoeks (ml/min/1.73m^2)	
	Mean	SD	Mean	SD	Mean	SD
Group A	15.4	7.3	90.3	21.0	86.2	24.0
Group B	96.8	48.3	76.9	19.2	71.2	15.2
Group C	505.9	158.2	65.1	25.2	52.3	20.7
	'p' value between					
Group A and Group B	<0.0001 S		0.012 S		0.0054 S	
Group A and Group C	<0.0001 S		<0.0001 S		<0.0001S	
Group B and Group C	<0.0001 S		<0.0457 S		0.0002 S	

Cystatin C has no association with age, duration, BMI, TC, TGL, Non-HDL and LDL. Statistically significant positive correlation was observed between cystatin C and blood pressure, FPS, UACR and creatinine. In contrast, strong negative correlation was achieved between cystatin C and HDL, MDRD and Hoeks equation.

Table 3: Correlation Coefficient of Cystatin C With Other Parameters

Parameter	Correlation coefficient with Cystatin C (r value)
Age	0.175
Duration of illness	0.444
BMI	0.1392
SBP	0.6127*
DBP	0.5637*
FPG	0.6824*
TC	0.4464
TGL	0.4378
HDL	-0.5048*
Non-HDL	0.491
LDL	0.4135
UACR	0.7834*
Creatinine	0.68*
MDRD	-0.5244*
Hoek formula	-0.8766*

A significant difference was observed between males and females in the mean value of creatinine whereas no significant difference between males and females in the mean cystatin C values.

Table 4: Association of Creatinine, Cystatin C With Sex

Sex	Normal No (%)	Abnormal No (%)	'p'
Creatinine			
Male	39 (79.6%)	10 (20.4%)	0.0437 Significant
Female	38 (92.7%)	3 (7.3%)	
Cystatin C			
Male	22 (45.8%)	26 (54.2%)	0.3415 Not significant
Female	22 (52.4%)	20 (47.6%)	

In present study, group A, group B and group C, the normal creatinine levels were 100%, 93.3% and 63.3% respectively whereas normal cystatin C levels were 70%, 56.7% and 20% respectively.

Table 5: Creatinine and Cystatin C In The Three Groups

Group	CREATININE No (%)		CYSTATIN C No (%)	
	Normal (0.7 – 1.2 mg/dL)	Abnormal (> 1.2 mg/dL)	Normal (0.51 – 1.05 mg/L)	Abnormal (>1.05 mg/L)
Group A	30 (100%)	-	21 (70%)	9 (30%)
Group B	28 (93.3%)	2 (6.7%)	17 (56.7%)	13 (43.3%)
Group C	19 (63.3%)	11 (36.7%)	6 (20.0%)	24 (80%)

The cut-off, lower and upper limit of 30–300 mg/gm of urine albumin creatinine ratio was selected as indicator of developing nephropathy and was used for calculations of sensitivity and specificity. The sensitivity, accuracy and negative predictive value of cystatin C is 62%, 64% and 48% respectively, whereas for creatinine it is 22%, 48% and 39% respectively.

Table 6: Diagnostic Validity of Cystatin C Compared With Creatinine

Parameter	CREATININE	CYSTATIN C
Sensitivity	22%	62%
Specificity	100%	70%
Accuracy	48%	64%
PPV	100%	80%
NPV	39%	48%

DISCUSSION

Diabetic nephropathy is a clinical hall mark of microangiopathy and is the most important single disorder that leads to renal failure in adults.[11] The earliest clinical evidence of nephropathy is the appearance of low but abnormal albumin levels in the urine and hence the early detection of nephropathy in diabetes mellitus patients has focused on the estimation of urinary albumin excretion rate. Impaired renal function may also present even in patients with normal urinary albumin excretion rate.[12] An ideal marker of GFR is defined as an endogenous molecule that, produced at a constant rate, is freely disposed by the kidney only by glomerular filtration, without being either secreted or reabsorbed by tubular cells.[13] Currently, the "gold standard" for GFR determination is to measure the clearance of exogenous substances, such as inulin, iohexol, 51Cr-EDTA, 99mTc-DTPA and 125I-iothalamate.[14] However, these measurements are not only time-consuming, labor-intensive and expensive but also require the administration of rare substances and hence these methods are not routinely used.[15] In our study, mean BMI in the three groups were 24.3±3.0, 24.3±2.4, 24.0±2.5 Kg/m^2 which was in the normal BMI range. This is in accordance with Assal *et al.*[16] and Jeon YK *et al.*[17] study. Even patients who are not obese as per the traditional weight criteria, may have an increased percentage of body fat distributed mostly in the abdominal region. These factors are thought to contribute to the higher insulin resistance and susceptibility to diabetes mellitus in South Asian Indians.[18] Longer the duration of DM, higher the frequency of developing diabetic nephropathy. Jiji Inassi *et al.*,[19] confirmed and extended the frequent occurrence of microproteinuria with increasing duration of diabetes. In our study also, there was an increase in the duration of diabetes from group A to group C. The mean duration was 4.43±2.43, 7.33±3.92 and 12.03±4.16 years for group A, B and C respectively. Dyslipidemia present in our patients might have contributed to the progression of renal damage. In a study conducted by Jha P *et al.*,[20] significant difference was seen in triglyceride levels between the normoalbuminuric, and macroalbuminuric group, but not in cholesterol, HDL and LDL.

Serum creatinine, in the present study was found to be significantly different among the three groups. This is in accordance with other studies. [17,20] Serum creatinine significantly increase when more than 50% of the GFR is reduced. Even though statistically significant difference was found among the three groups, it was found that in the group B, only (6.7%) and group C, only (36.7%) had abnormal creatinine values. Also a significant difference

between males and females in the mean value of creatinine was noted. Hence, Serum creatinine is an insensitive marker. Therefore, a need arises for new biomarkers to detect renal failure at earlier stages. Cystatin C is a new emerging and promising marker for detecting renal failure. Cystatin C, a cysteine protease inhibitor, which is freely filtered by the renal glomeruli and metabolized by the proximal tubule has been identified as a promising marker of renal failure. Cystatin C is produced at a constant rate by nucleated cells in the body. It is released into bloodstream with a half-life of 2 hours and its concentration is almost totally dependent on GFR. In our study, mean of cystatin C significantly differed between the diabetic groups. Hany S. Elbarbary[21] stated that, the major advantage of cystatin C over creatinine is its ability to detect mild reduction in GFR to which creatinine is insensitive. Early detection of impaired renal function is very crucial to prevent the progression of renal disease and to improve the patient outcome. In the present study, even in few normoalbuminuric patients, cystatin C levels were above normal value. This increment may be probably due to the tubular phase before glomerular manifestation.[17] In our study cystatin C is more sensitive than creatinine. The sensitivity to detect the renal impairment was 62% for cystatin C and 22% for creatinine, indicating that serum cystatin C is a better marker than serum creatinine for detecting early renal function decline in type 2 diabetes mellitus patients. Limitations of the study were Small sample size and serial estimation of cystatin C and creatinine could have been done. The present study could be extended by assessing the urine cystatin C levels along with serial monitoring of serum cystatin C levels in a larger population of diabetic nephropathy patients.

CONCLUSION

Diabetic nephropathy is a leading cause of end-stage renal disease. Cystatin C is more accurate and sensitive than creatinine for the detection of early renal impairment in type 2 DM. Cystatin C may be considered as a useful, non-invasive marker for early detection of renal injury in type 2 DM.

REFERENCES

1. International Diabetes Federation. The IDF Diabetes Atlas. 5th. Brussels: International Diabetes Federation: 2011. A summary of the figures and key findings.
2. Ramachandran A, Snehalathas C, Baskar AD, Mary S, Kumar CK, Selvam S, Catherine S, Vijay V. Temporal changes in prevalence of diabetes and impaired glucose tolerance associated with lifestyle transition occurring in the rural population in India. Diabetologia. 2004;47:860–5.
3. Wild S, Roglic G , Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
4. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. Diabetologia. 1991;34:877–890.
5. Novel Biomarkers of Diabetic Nephropathy. Pacific Biomarkers, Inc. 645 Elliott Ave W, Suite 300 Seattle, WA 98119 p: 206.298.0068 p: 800.767.915, Pacific Biomarkers.
6. Levey AS, Coresh J, Balk E, *et al.*; National Kidney Foundation. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med. 2003;139(2):137-147.
7. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, *et al.* (2012) CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 367: 20–9.
8. Slort PR, Ozden N, Pape L, Offner G, Tromp WF, *et al.* (2012) Comparing cystatin C and creatinine in the diagnosis of pediatric acute renal allograft dysfunction. Pediatr Nephrol 27: 843–9.
9. Viberti G, Walker JD. Natural history and pathogenesis of diabetic nephropathy. J Diabet Complications. 1991;5:72–75.
10. Raymond L. Heilman and Marec J. Mazur. Cystatin-C as a More Sensitive Indicator of Diminished Glomerulor Filtration Rate. Liver Transplantation 2005 Mar Vol-11, No.-3; p: 264- 266.
11. Ramachandran A, Snehalatha C, Latha E *et al.* Rising prevalence of NIDDM in an urban population in India. Diabetologia 1997; 40: 232-237.
12. Olivarius Nde F, Andreasen AH, Keiding N, Mogensen CE. Epidemiology of renal involvement in newly-diagnosed middle-aged and elderly diabetic patients. Cross-sectional data from the population-based study “diabetes care in general practice”, Denmark. Diabetologia 1993; 36:1007-16.
13. Donadio C, Lucchesi A, Ardini M, Giordani R. Cystatin C, beta 2-microglobulin, and retinol-binding protein as indicators of glomerular filtration rate: comparison with plasma creatinine. J Pharm Biomed Anal. 2001;24(5-6):835-842.
14. Florkowski CM, Chew-Harris JS (2011) Methods of Estimating GFR-Different Equations Including CKD-EPI. Clin Biochem Rev 32: 75–9.
15. Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L (2008) Serum cystatin C as an endogenous marker of renal function in patients with chronic kidney disease. Ren Fail 30: 181–6.
16. Heba S. Assal, *et al.*, Serum Cystatin Cand Tubular Urinary Enzymes as Biomarkers of Renal Dysfunction in Type 2 Diabetes Mellitus, Clinical Medicine Insights: Endocrinology and Diabetes 2013:6 7–13
17. Yun Kyung Jeon *et al.*,Cystatin C as an Early Biomarker of Nephropathy in Patients with Type 2 Diabetes, J Korean Med Sci 2011; 26: 258-263.
18. Yajinik CS Adiposity, inflammation and hyperglycemia in rural and urban Indian men: Coronary Risk of Insulin Sensitivity in Indian subjects (CRISIS) study. Diabetologia jan; 51(1) 6-7, 2008.
19. Jiji Inassi, Vijayalaksmi R, Role of duration of diabetes in the development of nephropathy in type 2 diabetic

patients,. National journal of medical research, 2013; 3(1):5-8.
20. Jha P, Das BKL, Shrestha S, Majhi S, Chandra L, Sharma S, Baral N., Glycemic status, lipid profile and proteinuria in diabetic nephropathy. J Nepal Med Assoc 2010; 49 (178): 143-6.
21. Hany S. Elbarbary,Nabil A. El-Kafrawy, Ahmed A. Shoaib, Samar M. Kamal El-deen., Serum cystatin C an early indicator of renal function decline in type 2 diabetes, American Journal of BioScience 2014; 2(3): 89-94.

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