Original Article

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Others Section

Estimation of Glomerular Filtration Rate and Predicting Diagnostic Accuracy of Cystatin C and Creatinine in Detection of Renal Function among Longstanding Type-2 Diabetics

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ABSTRACT

Introduction: Estimation of Glomerular Filtration Rate (GFR) is dependent on Serum Creatinine (SCr). Though, a sudden decrease in GFR would not result in rapid rise in concentrations of SCr, as they are imprecise but it leads to the over diagnosis of Chronic Kidney Disease (CKD).

Aim: To calculate GFR using Cystatin-C (Cys-C) based formulas to contrast its accuracy with SCr based formulas and to predict the diagnostic accuracy of Cys-C in patients with diabetes.

Materials and Methods: A total 48 type-2 diabetic patients were diagnosed with CKD and their GFR was assessed using the Cys-C and SCr. GFR was measured and estimated using three equations (Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CDK-EPI), that are based on SCr and three equations (LeBricon, Grubb and Hoek) based on Cys-C among the 48 CKD patients. The filtration rate was measured using labelled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) renal scan method as the standard for comparison. The Receiver Operating Characteristics (ROC) analysis was used to evaluate the diagnostic accuracy of the filtration rate.

Results: A significant association was observed (p=0.0001) among both the estimates which were equivocal when compared with measured iGFR, SCr and Cys-C based GFR estimate. A greater difference of Area Under the Curve (AUC) was observed between SCr (0.765±0.07) and Cys-C (0.569±0.09) (p=0.04) in the ROC analysis at a creatinine clearance <60 mL/min/m^2.

Conclusion: The SCr and Cys-C based formulae has equivocal performance in estimating GFR. SCr could be a better marker to estimate GFR among patients with Cr clearance <60 mL/min/m^2.

Keywords: Chronic kidney disease, Diabetes mellitus, Receiver operating characteristic curve

INTRODUCTION

Chronic kidney disease is a significant public health issue and is also associated with poor outcome and indulges high treatment costs [1]. GFR is studied with high precision by determining the plasma clearance rate of substances that are excreted exclusively through glomerular filtration. Some key substances that are injected to assess the GFR include inulin, 99mTc-diethylenetripentaaceticacid, 51Cr-EDTA or radiographic contrast media like iohexol and 125I-Iothalamate. These methods are time consuming and the patient is not completely risk-free. Therefore, for more than a century, the SCr has been used as a marker to estimate the filtration rate, respectively.

However, creatinine concentration is not an ideal marker for GFR because in addition to GFR other parameters such as muscle mass, diet, gender, age and tubular secretion significantly affect its concentration [2,3]. There are several successful attempts to develop GFR prediction equations, which include additional parameters to compensate for creatinine concentration's deficiencies as a GFR marker [4].

In the traditional clinical procedures, SCr has been commonly used as the marker of kidney function, but SCr concentration is indicated as a GFR biomarker and it may not be a perfect diagnostic marker to assess renal function. The reason is that creatinine in the blood does not increase until 50% of kidney function is damaged [5], though it is the conventional marker and it has frailty to perceive early stage of CKD [3,6,7]. The Cys-C is a probable alternative marker for the estimation of GFR in Type-2 diabetics [6]. There several commercial automated procedures are available for determining Cys-C [8-11].

However, prediction of decline of renal function with these markers are uncertain [12-15] in chronic diabetics. Hence, the present study aimed to evaluate eGFR using Cys-C and SCr based formula and compare with iGFR and to predict the diagnostic power of Cys-C and SCr in longstanding diabetics.

MATERIALS AND METHODS

This was a hospital-based, cohort study, conducted from November 2020 to December 2020. It constituted patients of longstanding type-2 diabetes that visited the health camp conducted at Endo-life hospital, Department of Endocrinology and Metabolism. Written informed consent was receved from all patients. The study procedure was approved by Institutional Ethical Committee (IEC No: ECR/647/INST/AP/2014), Endo-life Specialty Hospital, Guntur, India.

Inclusion criteria: The subjects with chronic diabetes with or without co-morbidities were included in the study.

Exclusion criteria: Subjects with known history of renal failure were excluded from the study.

Study Procedure

Creatinine and Cystatin C assay: All creatinine measurements were performed in the central laboratory of Endo-life hospital. The obtained samples were subjected to ROCHE COBAS c311 (auto analyser) to measure the SCr, adopting Jaffe's method [16].

Creatinine based estimation of GFR: The Cockcroft-Gault and MDRD formula [11,17] were used to predict GFR using SCr.

GFR-CG = {(140-age) multiply with weight (kg)}/72 × SCr (mg/dL) (for women, multiply with 0.8)

GFR-MDRD = 186 × (SCr in mg/dL) minus1.54 × age-0.203 (for women, multiply with 0.742)

CKD-EPI Formula [18]:

GFR=141 × min (S_{cr}/κ, 1)$^{\alpha}$ × max(S_{cr}/κ, 1)$^{-1.209}$ × 0.993^{Age} × 1.018 (if female) × 1.159 (if African American)

where:

S_{cr} is serum creatinine in mg/dL, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and max indicates the maximum of S_{cr}/κ or 1.

A correction for Body Surface Area (BSA) is essential for the CG formula. This was done with estimated BSA from Haycock's equation [10].

Cystatin C based estimation of GFR: GFR was estimated using three equations based on serum cystatin C that were proposed by Hoek, Lebricon and Grubb [19-21]:

GFR-Hoek=-4.32+(80.35×1/cystatin C)

GFR-LeBricon={(78)×(1/cystatin C)}+4

GFR-Grubb=89.12×cystatin C (mg/L)-1.1675

STATISTICAL ANALYSIS

The t-test was employed to compare the two means between creatinine clearance <60 and ≥60 mL/min/m^2. The medcalc 8.1 statistical software (Belgium) was used as a statistical tool. Correlation coefficients (r) and stepwise regression analysis was applied to compare measured and estimated GFRs among SCr and Cys-C formulas. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 48 patients (38 men and 10 women) with mean age of 59±7.2 years and age range from 41-74 years, with the mean±SD of SCr, Cys-C and measured GFR were 1.4±0.2, 1.9±0.5, and 47.7±9.5 [Table/Fig-1]. Based on the GFR levels, the patients were divided into two categories:

(1) GFR 30-59 mL/min/1.73 m^2 (47.7±9.5; n=34), according to National Kidney Foundation Stage-III CKD [22].

(2) GFR 60-89 mL/min/1.73 m^2 (61.7±3.75; n=14), stage-II CKD.

Parameters	Mean±SD	Median (Range)
Age (years)	59±7.2	59 (41-74)
Body Mass Index (kg/m^2)	26.2±4.9	27.3 (19.71-40.17)
Body Surface Area (kg/m^2)	3.1±0.6	3.2 (1.99-4.59)
Duration of diabetes (years)	12±7.4	11.5 (4-32)
Duration of hypertension (years)	6.4±8.2	3.5 (4-28)
Serum Creatinine (mg/dL)	1.4±0.2	1.34 (0.9-1.95)
Serum Cystatin-C (mg/L)	1.9±0.5	1.82 (1.34-3.06)
Measured GFR (mL/min/1.73 m^2)	47.7±9.5	52.50 (30-71)

[Table/Fig-1]: Demographic characteristic of patients.

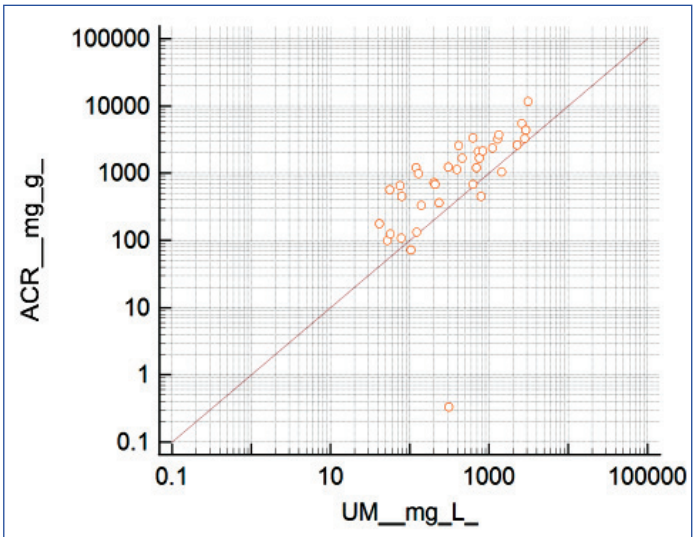
The SCr levels showed statistically significant association (p=0.002) among the patients with creatinine clearance <60 mL/min/1.73m^2 and less than 60 mL/min/1.73m^2 [Table/Fig-2]. The correlation between spot Albumin Creatinine Ratios (ACR) is illustrated in [Table/Fig-3]. The urine micro-albumin, Cys-C and duration of diabetes were significantly (r: 0.517, p=0.001; r: 0.314, p=0.029 and r: 0.296, p=0.040) correlated with ACR [Table/Fig-4-6]. ACR is also correlated with duration of hypertension (p=0.005). This represents, that these patients are at higher risk for kidney disease. Measuring of these parameters also helps to diagnose the disease in early stage and also assist to prevent further progression of disease. In this study, SCr and hs-CRP did not correlated with ACR (p<0.05).

Parameters	CrCl <60 (mL/min/1.73 m^2) N=34	CrCl ≥60 (mL/min/1.73 m^2) N=14	t-test p-value*
Serum creatinine (mg/dL)	1.4±0.2	1.2±0.1	0.002*
Serum cystatin C (mg/L)	1.9±0.5	2.0±0.5	0.76
Spot urine ACR (mg/g)	1395.98±1308.2	1527.6±1549.02	0.76

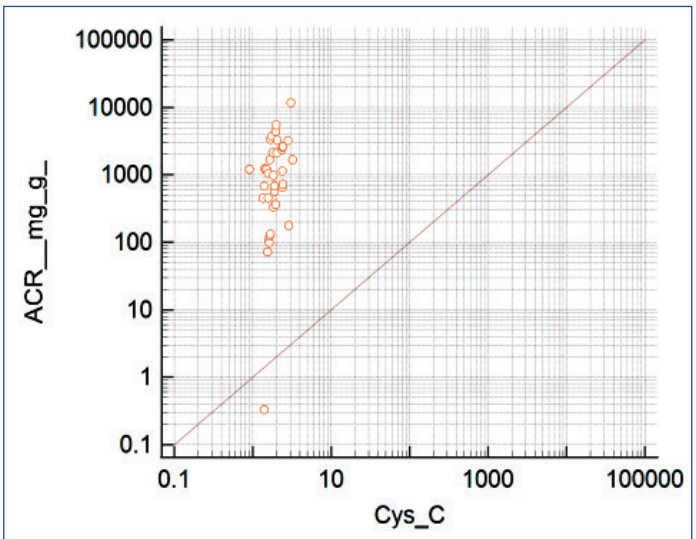
[Table/Fig-2]: Comparison of serum parameters between the creatinine clearance <60 and creatinine clearance ≥60 groups.
*The p-value <0.05 were considered statistically significant

Parameters	Correlation (r)	95% CI	p-value
ACR Vs urine micro albumin	0.517	0.273-0.698	0.001
ACR Vs Cys-C	0.314	0.033-0.549	0.029*
ACR Vs SCr	0.223	-0.064-0.477	0.126
ACR Vs duration of diabetes	0.296	0.013-0.535	0.040*
ACR Vs duration of hypertension	-0.395	-0.610-0.125	0.005*
ACR Vs hs-CRP	0.270	-0.014-0.514	0.063

[Table/Fig-3]: Correlation of ACR and other parameters.
*The p-value <0.05 were considered statistically significant

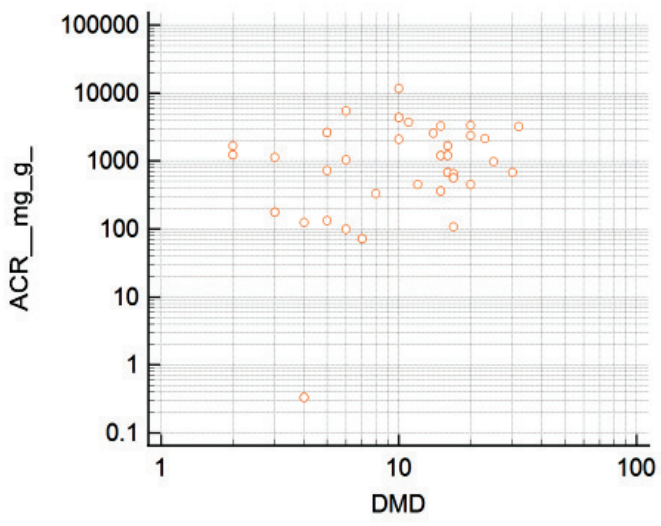


[Table/Fig-4]: Correlation between ACR and Urine Micro Albumin (r: 0.517; p=0.001).



[Table/Fig-5]: Correlation between ACR and Cys-C (r: 0.314; p=0.029).

In stage-III and II of CKD [Table/Fig-7,8], the measured filtration rate was compared to creatinine-based formulae and Cys-C based formulae. Of these formulae, both Cys-C and creatinine-based estimated GFRs showed significant correlation (p=0.0001). In the stage-II of CKD, only Cys-C based estimated formulae (p=0.0001) showed significant correlation. A significant direct correlation was established between stage-III CKD and measured SCr (p=0.0001),



[Table/Fig-6]: Correlation between ACR and duration of DM (r: 0.296; p=0.040).

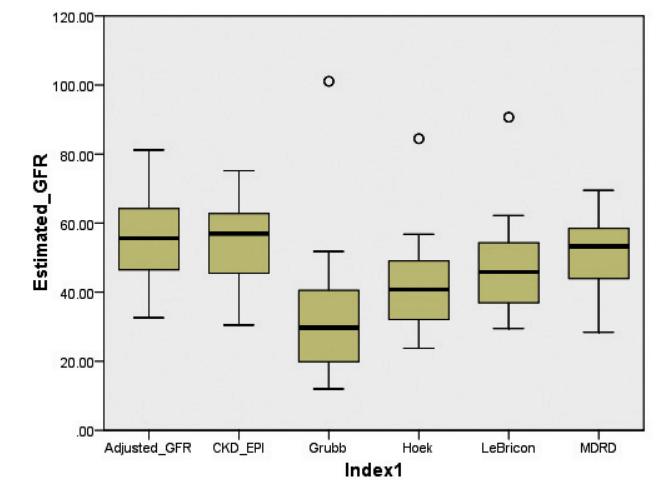
whereas there was an inverse correlation with measured Cys-C (p=0.09). The AUC showed a greater difference between SCr (0.765±0.07) and Cys-C (0.569±0.09).

must be identified using markers. SCr was considered specific but less sensitive because its level does not increase significantly until the GFR is reduced to <50% of normal [23]. Furthermore, many factors have a significant impact on SCr concentrations and which can interfere with assay. The factors includes, age, gender, muscle mass, dietary intake, changes in tubular secretions, various drugs and endogenous substances as well. However, serum Cys-C may be affected to a lesser extent by these factors and which may explain the possible superiority of serum CysC to SCr for predicting GFR [24].

Hence, this study assessed the eGFR using Cys-C and SCr based formula and compared with iGFR to predict the diagnostic power of Cys-C and SCr. The equations proposed by Filler G and Lepage N [25] and Le Bricon T colleagues and Grubby A et al., [20,21] provide a more accurate estimate of GFR among the different methods for Cys-C based GFR stimulations. The measured Cys-C was not statistically significant in relation to inulin clearance. Additionally, there was no correlation observed between measured SCr and estimated GFR with CG formulae (p<0.09). The results are supported by Mysliwic P et al., that conducted a study on morbidly obese subjects 26].

GFR level mL/min/m²	iGFR		Serum creatinine (SCr)				Serum cystatin C			
			Measured SCr	Estimated			Measured Cys-C	Estimated		
				CG	MDRD	CKD-EPI		Lebricon	Grubb	Hoek
30-59 (n=34)	Mean±SD	47.7±9.5	1.4± 0.2	47.7±7.8	46.8±10.6	49.6±11.9	1.9±0.5	46.04±9.9	30.9±11.8	40.9±9.6
		r	-0.7	0.82	-0.83	-0.86	-0.45	-0.977	-0.977	-0.95
		p-value	0.001*	0.001*	0.001*	0.001*	0.09	0.001*	0.001*	0.001*
60-89 (n=14)	Mean±SD	61.7±3.75	1.2±0.11	68.7±6.7	57.7±3.16	61.4±3.9	1.86±0.6	51.6±16.1	38.3±25.4	45.5± 16.8
		r	-0.53	-0.14	-0.47	-0.61	0.77	-0.82	-0.82	-0.89
		p-value	0.02*	0.95	0.05*	0.009*	0.001*	0.001*	0.001*	0.001*

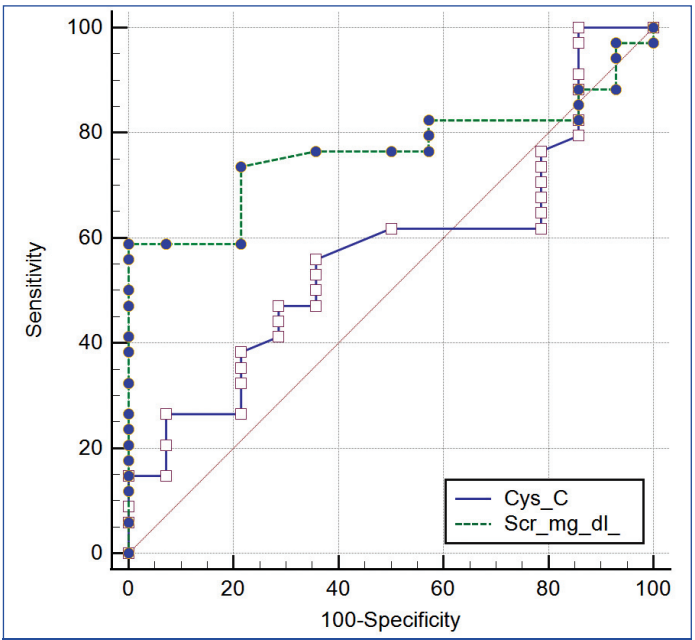
[Table/Fig-7]: Comparative representation of measured GFR Vs estimated GFRs using various formulae.
*p-value <0.05 were considered statistically significant



[Table/Fig-8]: Estimated GFR (eGFR) calculated using formulas based on creatinine (CG, MDRD and CKD-EPI) and cystatin C (Le Bricon, Grubb and Hoek) serum concentrations.

Marker	Area under the ROC curve	95% CI	t-test p-value
Cystatin C	0.569±0.09	0.418-0.711	0.04
Creatinine	0.765±0.07	0.620-0.875	

[Table/Fig-9]: The ROC analysis for prediction ability of SCr and Cys-C.
p-value <0.05 were considered statistically significant



[Table/Fig-10]: The ROC analysis of Serum Cystatin C and Serum creatinine.
AUC of Cys-C: 0.569 (p>0.05); AUC of SCr: 0.765 (p=0.04).

In the ROC analysis [Table/Fig-9,10], the mean (0.765±0.07) AUC of SCr was more as compared to Cys-C (0.569±0.09) AUC, when the cut-off value of GFR <60 mL/min/m². Hence, it indicates SCr showed better predictor than Cys-C.

DISCUSSION

Measurement of renal function is important in diabetic patients and early structural and functional changes in diabetic nephropathy

In the index study, no significant differences were shown between Cys-C based equations (p<0.09) and significant correlation was observed with SCr-based formulae, when compared with iGFR. This is because the amount of creatinine varies, because of muscle mass and tubular secretion, As a result, the test is susceptible to certain limitations. The Cys-C is produced endogenously at a constant rate and which was freely filtered completely through glomeruli and reabsorbed. Later, it was consolidated in renal tubules with the presence of renal cells and is not affected by severe disease, age, sex, height and obesity. There are many studies that support and undermine the results. The study conducted among a Korean population showed that Cys-C is more accurate than SCr in evaluating prognostic stage of diabetic nephropathy [12]. Another study reveals, SCr is a better indicator for assessing renal function than Cys-C. In addition, SCr based CKD-EPI is the best one for estimation of GFR among patients with type-2 diabetes having normal renal function [13]. Overall, a significant correlation was observed among all the formula based on both markers, when the CrCl value was 30-59 <60 mL/min/m^2. Moreover, there was no correlation with SCr based CG formula, when the CrCl value was 60-89 <60 mL/min/m^2. Hence, nearly all SCr and Cys-C based equations significantly correlated to each other.

In the ROC analysis, Cys-C was unable to predict renal function, at CrCl 60-89 <60 mL/min/m^2. Similarly, another study expressed that Cys-C may be an surrogate marker for early detection of renal function in subjects with slight reduction of GFR and also it may be a marker for early glomerular dysfunction in type 2 diabetes mellitus [6]. Hence, it is a reliable measure of kidney function. In kidney transplant recipients, the MDRD equation may provide a more accurate estimate of GFR than cystatin C-based equations or other creatinine-based GFR calculations [27]. In type-2 diabetes patients with GFR renal failure, Cys-C was observed to be better alternative than Scr and CG, when comparing the GFR < 80 mL/min per 1.73 m^2 and > 80 mL/min per 1.73 m^2 [28].

The ROC analysis demonstrates that SCr levels rise rapidly during the moderate renal damage (<60 mL/min/m^2). The predictive accuracy of SCr for individuals was shown in early stage of CKD as a better diagnostic biomarker, {AUC (0.765), (p<0.04)}. The mean of SCr levels was significantly increased in patients with renal impairment in comparison to Cys-C and ACR among those with CrCl <60 mL/min/m^2.

Limitation(s)

It was a pilot study with a limited sample size. The study was conducted among patients with only type-2 diabetes and with or without hypertension. It is better to include a larger population with different co-morbidities to predict the best marker in order to estimate GFR.

CONCLUSION(S)

The present study indicates that SCr is better than Cys-C. Therefore, SCr could be a better marker to estimate GFR among patients with CrCl <60 mL/min/m^2.

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