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Relationship of Creatinine and Cystatin C-based Estimated Glomerular Filtration rates with Measured Glomerular Filtration Rate in Healthy Kidney Donors from South Asia

S. Kakde, S. Alexander, V. G. David, S. Jacob, A. Mohapatra, A. T. Valson, B. Gopal,[1] C. K. Jacob,[2] J. Hephzibah,[3] V. Tamilarasi, and S. Varughese

Department of Nephrology, Christian Medical College, Vellore, Tamil Nadu, India
[1]*Department of Nephrology, Central Northern Adelaide Renal and Transplant Service, Adelaide, Australia*
[2]*Department of Nephrology, Bangalore Baptist Hospital, Bengaluru, Karnataka, India*
[3]*Department of Nuclear Medicine, Christian Medical College, Vellore, Tamil Nadu, India*
Address for correspondence: *Dr. S. Kakde, Department of Nephrology, Christian Medical College, Ida Scudder Road, Vellore - 632 004, Tamil Nadu, India. E-mail: dr.shaileshkakde@gmail.com*

Abstract

Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation is currently recommended for the estimation of glomerular filtration rate (GFR). This retrospective study aimed to evaluate the correlation between creatinine and cysC-based estimated GFRs and measured GFR in healthy adults. Consecutive healthy adults who were accepted as voluntary kidney donors at our center between January 2008 and December 2012 were included in the study. The 336 individuals who comprised the study population had a mean age of 41.6 ± 11.8 years, male:female ratio 1:1.7, mean creatinine 0.9 ± 0.1 mg/dl, and mean cysC 0.8 ± 0.1 mg/dl. Mean measured GFR by Tc-99m diethylenetriaminepentaacetic acid using Gates method was 98.4 ± 21.2 ml/min/1.73 m^2. The mean ± standard deviation of eGFRs by various formulae were as follows: Cockcroft–Gault (CG) = 88.1 ± 15.9 ml/min/1.73 m^2, Modification of Diet in Renal Disease (MDRD) = 78 ± 14.7 ml/min/1.73 m^2, CKD-EPI creatinine = 88.1 ± 15.5 ml/min/1.73 m^2, CKD-EPI cysC = 97 ± 19.9 ml/min/1.73 m^2, CKD-EPI creatinine-cysC (CKD-EPI cr-cysC) = 92.5 ± 14.1 ml/min/1.73 m^2. The CKD-EPI cr-cysC equation had the highest accuracy, with 43% and 72% of values lying within ±10% and ±20% of the measured GFR, respectively. Bland–Altman analyses for levels of agreement showed least bias with CKD-EPI cysC overall and among females, while among males, CKD-EPI creatinine equation had the least bias. The CKD-EPI equation showed a higher performance than the MDRD and CG equation in GFR estimation of a healthy population. Among CKD-EPI equations, CKD-EPI cr-cysC had the highest accuracy and CKD-EPI cysC the least bias.

Keywords: *Chronic Kidney Disease-Epidemiology Collaboration*, *cystatin C*, *diethylenetriaminepentaacetic acid*, *estimated glomerular filtration rate*, *Modification of Diet in Renal Disease*

Introduction

Glomerular filtration rate (GFR) is widely accepted as the best index for assessing kidney function. Only one study has established a normal reference range for measured GFR in Indian adults[1] and showed these to be much lower than those from a Western population.[2]

GFR should be assessed by a method, which is accurate, safe, simple, and cost-effective. Since GFR measurement by the ideal filtration marker inulin is both cumbersome and expensive, GFR is currently measured using other filtration markers such as iohexol, I-125 sodium iothalamate, Cr-51 ethylenediamine tetraacetic acid, and Tc-99m diethylenetriaminepentaacetic acid (DTPA), each of which has its own limitations.[3] To circumvent these drawbacks, a number of specifically designed prediction equations have been proposed as reliable alternatives for bedside assessment of GFR. The two commonly used equations are the Cockcroft–Gault (CG) formula and Modification of Diet in Renal Disease (MDRD) Study equation. The studies from which these equations were derived primarily included patients with impaired renal function, and thus there are major limitations to their use in the workup of voluntary kidney donors (VKDs).[4,5] The CKD-Epidemiology Collaboration (CKD-EPI) developed a new equation which has higher precision and does not underestimate high measured GFR values.[6] This equation was shown to be as accurate as the MDRD equation at GFRs <60 ml/min/1.73 m^2 and substantially more accurate at GFRs >60 ml/min/m^2.

More than 80% of clinical laboratories now report eGFR routinely when testing for creatinine. Despite standardization of the creatinine assay, eGFR estimation using creatinine-based equations remains relatively imprecise due to inter-individual variations in the determinants of serum creatinine, which may potentially lead to misclassification of GFR. Serum cystatin C (cysC) possesses many of the attributes required of an ideal GFR marker. It is a low-molecular-weight protein (13.3 kDa) produced by all nucleated cells,[7] almost completely filtered by the renal glomerulus and normally almost completely reabsorbed and degraded by proximal tubular cells. CysC-based estimated GFR (eGFR) therefore correlates closely with measured GFR and was studied and validated by Inker *et al*.[8] Thereafter, an equation that combined creatinine and cysC was found to be better than one using creatinine or cysC alone by Fan *et al*.[9] However, the performance of cysC-based eGFR has not been studied in the South Asian population till date.

This retrospective study aims to compare commonly used GFR estimation equations with the DTPA GFR measured in healthy South Asian adults. The equations that we studied were CG-Creatinine clearance corrected for GFR, MDRD equation, CKD-EPI creatinine, CKD-EPI creatinine cysC (CKD-EPI cr-cysC), and CKD-EPI cysC.

Materials and Methods

Three hundred and thirty-six consecutive healthy adults who were assessed for suitability and accepted as voluntary kidney donors (VKD) between January 2008 and December 2012 at our center were included. Data were archived electronically and accessed from the hospital information system.

As part of their routine workup, all donors at our center have blood urea, serum creatinine, total serum protein, serum albumin, serum cysC, and DTPA GFR measured. Measurement of creatinine is done in the same laboratory using kinetic compensated Jaffe assay traceable to an isotope dilution mass spectrometry determination in the Cobas 8000 analyzer (Roche Diagnostics GmbH Mannheim, Germany). CysC is measured by immunoturbidimetry on the Cobas 8000 analyzer.

For the measurement of Tc-99m DTPA GFR using the Gates protocol, after breakfast, VKDs are requested to drink 300–500 ml water before the procedure, following which bolus injection of about 185 MBq (Mega Becquerel) of Tc-99m-labeled DTPA is injected intravenously. Radioactivity is measured in the syringe containing Tc-99m DTPA before and after the injection and total injected dose of radioactivity calculated. The site of injection on the arm is scanned using a Gamma camera (GC) after scintigraphy images are taken for 30 min each at 2 min per frame. At the 20^{th} min, 0.5–1 mg/kg body weight (up to 40 mg) furosemide is injected intravenously and an immediate post-void image is acquired, followed by a delayed image 2 h later. A region of interest (ROI) is drawn manually using summed images obtained every 2–3 min. The infra-renal background ROI is assigned and Infinia Hawkeye GC software (General Electric, Waukesha, Wisconsin, United States of America) is used to automatically calculate the GFR. In all VKDs, there was minimal residual radioactivity (<0.1%) at the injection site and no adverse reactions were observed during and after the procedure.

The fractionated uptake (FU) of each kidney is assessed as follows:

FU = (renal count/$e^{-\mu y}$)/total injected dose counts × 100[10]

Renal count is calculated from the renal uptake between 2 and 3 min in the renogram, y = kidney depth (cm), and μ = attenuation. Dose counts are expressed in counts per minute (cpm). Coefficient of Tc-99m (0.153) is calculated using Tonnesen's formula.[11] GFR is calculated, in ml/min, as: 9.75621 × FU − 6.19843. Tc-99m DTPA is prepared in-house at our center, regular chromatography for labeling efficiency is performed, and radiochemical purity has consistently been shown to be over 95%.

For this study, an online calculator was used for calculating eGFRs by different equations.[12] Different eGFR equations used were CG, MDRD2, CKD-EPI creatinine, CKD-EPI cysC, and CKD-EPI cr-cysC. The level of agreement between eGFRs estimated by various methods and measured GFR by Tc-99m DTPA GC method was calculated.

Statistical analysis

Analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 15, SPSS Inc. Chicago. The mean ± standard deviation (SD) of the different studied variables was calculated. The performance of various prediction equations was assessed in terms of their accuracy, bias, and precision. Accuracy was defined as the proportion of eGFR values that fell within ±10% (P10) and ±20% (P20) of the measured Tc-99m DTPA GFR for each eGFR equation. Bland–Altman approach was used to evaluate the agreement between the values estimated by the equations and the measured value. Bias was defined as mean difference between measured and eGFR for each eGFR equation. The differences were expressed in absolute values with confidence limits of 95%. Precision was defined as the limits of agreement or the limits between which 95% of values fall on either side of the bias (±1.96 times the SD around the bias) expressed in ml/min/1.73 m^2. Receiver operating characteristics (ROC) curve was used to assess the diagnostic performance of the equations for detecting GFR <90 ml/min.

Results

Characteristics of the studied population are shown in Table 1. Serum creatinine and serum albumin concentrations were lower in females compared to males; however, serum cysC concentrations were similar. Mean of the measured GFR by various methods are shown in Table 2. Females had higher measured GFR compared to males. Various prediction equations showed no significant difference between eGFR for males and females, with the exception of the CKD-EPI creatinine equation, which showed females to have a lower GFR [Table 2]. Accuracy of various GFR prediction equations within the range of ±10% and ±20% of the DTPA GFR was calculated and CKD-EPI (creatinine-cysC) equation was found to have the highest accuracy [Table 3]. Both overall [Table 4], and in females [Table 5], the CKD-EPI cysC equation had the least bias, while among males [Table 6], the CKD-EPI creatinine equation had the least bias.

Evaluation of agreement using Bland–Altman analysis is shown in Figure 1. Values with lower levels of agreement and with a tendency to negative values were obtained with MDRD. We also evaluated the diagnostic performance of the formulas to discriminate values of GFR below 90 ml/min, wherein the number of individuals improperly classified with respect to measured DTPA GFR was evaluated. Only 1.1% of the individuals were misclassified with CKD-EPI (creatinine-cysC) compared to 6.6% using MDRD, showing that the CKD-EPI (cr-cysC) equation performed best. The diagnostic sensitivity evaluated by ROC curve [Table 7] showed a larger area under the curve for the detection of GFR <90 ml/min with CKD-EPI (cr-cysC) compared to MDRD: 0.68 versus 0.61 ($P < 0.001$), respectively [Figure 2].

Discussion

GFR is one of the most sensitive parameters of renal function and varies between different racial and ethnic groups.[13] The earliest data on this subject, published in 1946 by Shock,[14] found no difference between the GFRs of African-American and Caucasian individuals. This was again studied and confirmed by Poggio *et al*.[15] in a bigger cohort. However, in the Asian population, normal GFR values could be lower than that of Caucasians as has been suggested by studies from India, China, and, to a lesser extent, Pakistan.[1,16,17] This lower value could either be due to low dietary protein intake (most Indians are

vegetarian)[17] or the high prevalence of low birth weight, which is a surrogate marker for low nephron endowment and has been shown to be associated with lower measured GFR.[18] Our study found the GFR of healthy VKDs as measured by GC-based Gates method to be comparable to DTPA GFR values in Caucasian donors[19] and higher than that reported previously from India.[1,20] However, these studies used the two-plasma sample method for measuring DTPA GFR, and the Gates protocol has been shown to slightly overestimate GFR compared to the plasma clearance method.[19,20]

Kidney Disease Improving Global Outcomes guidelines do not recommend different cutoffs for normal GFR for males and females. Like Poggio *et al*.,[15] we too found females to have a higher measured GFR than males, unlike data from a healthy Pakistani population, which did not show a difference in inulin clearance between the two genders.[17] Because DTPA binding to plasma proteins is in the range of 5%–10%, females, who had a lower serum albumin in this study, may have a higher measured GFR when DTPA is used as the filtration marker.[21] There is no evidence to suggest that the difference in measured GFR is due to gender differences in kidney morphology, since glomerular number and volume have been shown to be similar in males and females.[22] All eGFR equations showed females to have lower GFRs than males, though this difference was significant only for the CKD-EPI creatinine equation. This difference can largely be attributed to the fact that females had a lower mean serum creatinine owing to lower muscle mass and it highlights the drawbacks of using a solely creatinine-based prediction equation to estimate GFR in the subcontinent.

This study investigated the performance of various prediction equations for estimating GFR in healthy adults compared to DTPA GFR measured by the Gates protocol, which was used as the reference method. When assessing the agreement between two measurement methods, the most important attributes are accuracy, bias, and precision. The lower accuracy and higher (negative) bias of the MDRD equation confirms that the MDRD equation systematically underestimates GFR in healthy South Asians. ROC curve analysis further confirmed that the use of MDRD eGFR in the evaluation of South Asian VKDs might lead to inappropriate exclusion of at least 6.6% of donors due to the incorrect diagnosis of CKD G1, owing primarily to limitations of the equation rather than the subject.

Our data showed that the CKD-EPI cr-cysC equation had the highest accuracy among all equations studied, with 43% and 72% of values lying within ±10% and ±20% of the measured GFR, respectively. Both overall, and in females, the CKD-EPI cysC equation had the least bias, while among males, the CKD-EPI creatinine equation had the least bias. The lower bias for cysC-based equations is not surprising, as cysC is less subject to the effects of age, sex, and race as has been shown by Inker *et al*.[8,23] Our data also showed that the mean cysC concentration in our VKDs was similar to that reported in a Caucasian population with measured GFR ≥90 ml/min/1.73 m^2 and did not vary by gender. All these findings put together would argue for cysC-based prediction equations to be systematically adopted for the estimation of GFR in healthy adult South Asians, especially prospective VKDs.

Strengths and limitations

Our study has certain limitations. Our reference method, the measurement of DTPA GFR using the Gates protocol, has been shown to both overestimate[19] and underestimate[24] GFR compared to the two-plasma sample method. The strengths of this study are the large sample of VKDs recruited for the study, thus establishing normative values for measured and eGFR in the South Asian population. While values for cysC-based GFR have been previously described in healthy Indian adults,[25] it has not been compared to a reference method of measured GFR till date.

Conclusions

Our data suggest that overall, the CKD-EPI cr-cysC equation has the highest accuracy and acceptable bias across both genders. If, however, a gender-specific equation is to be used, the CKD-EPI cysC equation should be preferred in females and the CKD-EPI creatinine equation in males.

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Conflicts of interest

There are no conflicts of interest.

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Figures and Tables

Table 1

Demographics, baseline characteristics, and renal function of the study population

Characteristics	Total (*n*=336)	Males (*n*=121)	Females (*n*=215)	*P*
Mean age (years)	41.6±11.3	39.6±12.6	42.7±10.3	<0.01
BMI (kg/m^2)	24.0±3.8	22.7±3.5	24.7±3.8	0.19
BSA (m^2)	1.5±0.1	1.7±0.1	1.54±0.1	<0.01
24 h proteinuria (mg/day)	60.4±28	63.9±30.8	57.4±27.2	0.61
Blood urea (mg/dl)	22.1±6.0	22.8±5.8	21.6±6.1	0.48
Serum creatinine (mg/dl)	0.9±0.1	1.0±0.1	0.85±0.1	<0.01
Serum albumin (mg/dl)	4.5±0.3	4.6±0.3	4.4±0.3	<0.01
Cystatin C (mg/dl)	0.8±0.1	0.8±0.1	0.8±0.1	0.18

BMI: Body mass index, BSA: Body surface area

Table 2

Estimated and measured glomerular filtration rate in healthy individuals

GFR (ml/min/1.73 m^2)	Mean±SD	Males (n=121)	Females (n=215)	P
Cockcroft-Gault	88.1±15.9	92.9±15.7	85.5±15.5	0.85
MDRD	78.0±14.7	84.1±15.6	74.7±13.0	0.14
CKD-EPI creatinine equation	88.1±15.9	92.9±15.7	85.5±15.5	0.03
CKD-EPI cystatin C equation	97.8±19.9	99.4±20.2	97.0±19.8	0.26
CKD-EPI creatinine-cystatin C equation	92.5±16.9	95.4±17.3	90.9±16.5	0.21
DTPA (gates method)	98.4±21.2	92.3±17.4	101.9±22.3	0.01

GFR: Glomerular filtration rate, MDRD: Modification of Diet in Renal Diseases, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration, DTPA: Tc-99m diethylenetriaminepentaacetic acid, SD: Standard deviation

Table 3

Accuracy of estimated glomerular filtration rate equations compared to Tc-99m diethylenetriaminepentaacetic acid glomerular filtration rate (reference method)

eGFR (ml/min/1.73 m^2)	P10, *n* (%)	P20, *n* (%)
Cockcroft-Gault	78 (23)	172 (52)
MDRD	55 (16)	154 (45)
CKD-EPI creatinine equation	120 (35)	222 (66)
CKD-EPI cystatin C equation	102 (31)	217 (67)
CKD-EPI creatinine-cystatin C equation	141 (43)	242 (72)

P10 and P20 represent the eGFR within the range of ±10% and ±20% of the DTPA GFR (measured GFR). MDRD: Modification of Diet in Renal Diseases, eGFR: Estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration, DTPA: Tc-99m diethylenetriaminepentaacetic acid

Table 4

Agreement between estimated glomerular filtration rate by various equations and glomerular filtration rate measured by Tc-99m diethylenetriaminepentaacetic acid clearance among all individuals

eGFR (ml/min/1.73 m^2)	Bias	SD	Limits of agreement
MDRD	−20.4	22.2	−64.7, 24.0
CKD-EPI creatinine equation	−10.3	21.8	−53.8, 33.2
CKD-EPI cystatin C equation	−0.86	24.22	−49.3, 47.6
CKD-EPI creatinine-cystatin C equation	−6.15	21.71	−49.5, 37.2

SD: Standard deviation, MDRD: Modification of Diet in Renal Diseases, eGFR: Estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration

Table 5

Agreement between estimated glomerular filtration rate and glomerular filtration rate measured by Tc-99m diethylenetriaminepentaacetic acid clearance among females

eGFR (ml/min/1.73 m^2)	Bias	SD	Limits of agreement
MDRD	27.5	20.7	−13.9, 68.8
CKD-EPI creatinine equation	16.6	20.8	−24.9, 58.1
CKD-EPI cystatin C equation	5.4	24.5	−42.5, 53.3
CKD-EPI creatinine-cystatin C equation	11.4	21.4	−30.5, 53.5

SD: Standard deviation, MDRD: Modification of Diet in Renal Diseases, eGFR: Estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration

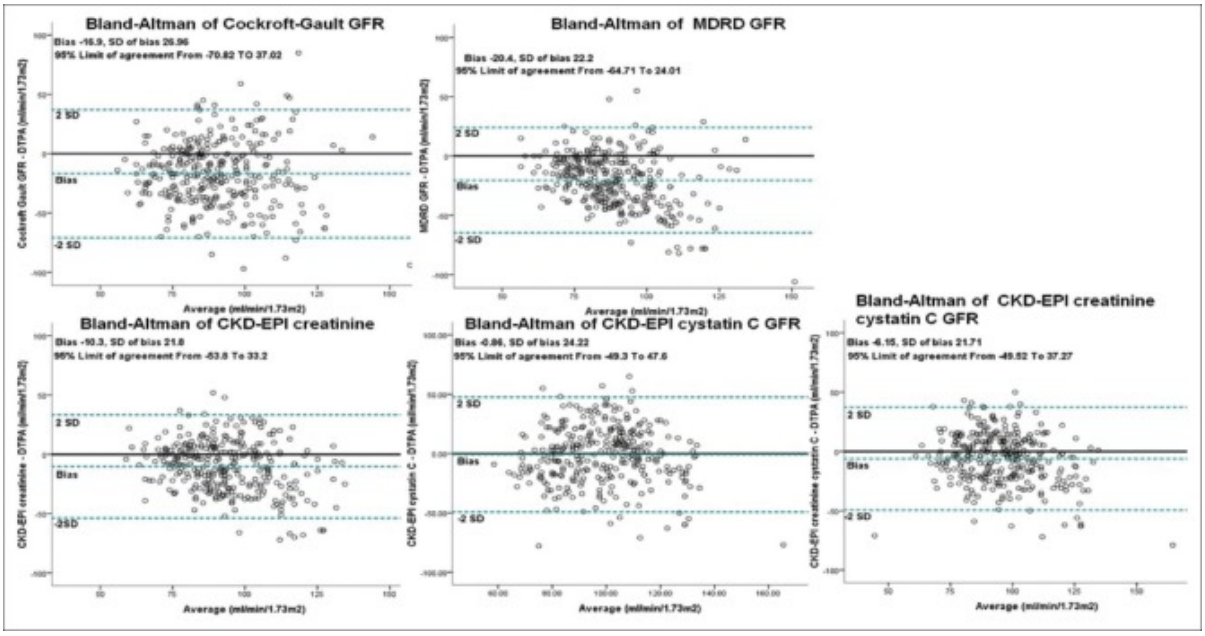
Table 6

Agreement between estimated glomerular filtration rate and glomerular filtration rate measured by Tc-99m diethylenetriaminepentaacetic acid clearance among males

eGFR (ml/min/1.73 m^2)	Bias	SD	Limits of agreement
MDRD	8.2	19.18	−30.2, 47.4
CKD-EPI creatinine equation	0.9	18.95	−37.2, 37.07
CKD-EPI cystatin C equation	7.1	21.6	−49.5, 35.31
CKD-EPI creatinine-cystatin C equation	3.1	17.4	−38.56, 30.97

SD: Standard deviation, MDRD: Modification of Diet in Renal Diseases, eGFR: Estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration

Figure 1



Bland–Altman plot showing the agreement between DTPA clearance and estimated GFR with CG, MDRD, and CKD-EPI equations. GFR: Glomerular filtration rate, MDRD: Modification of Diet in Renal Disease, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration, DTPA: 99m Tc-diethylenetriaminepentaacetic acid

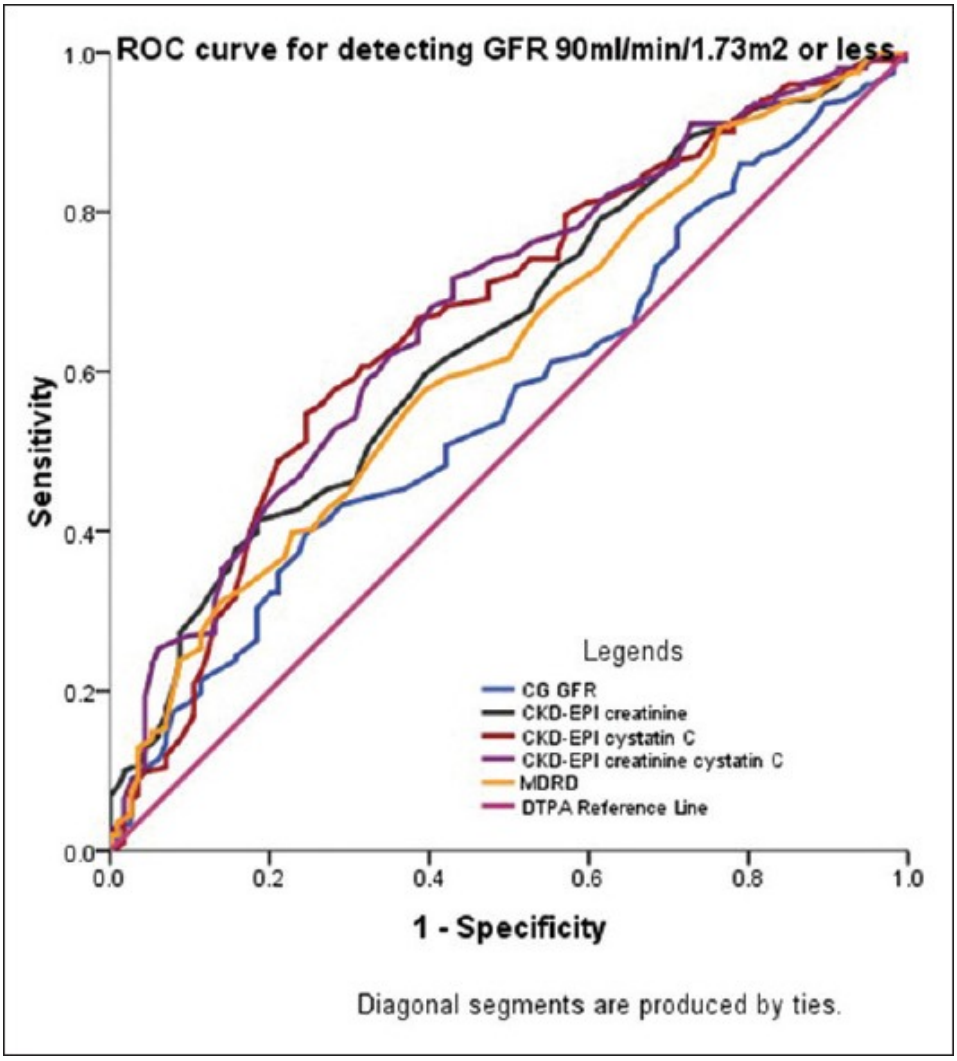
Table 7

Receiver operating characteristic curves comparing areas under the curves of the Cockcroft-Gault, Modification of Diet in Renal Diseases, and the Chronic Kidney Disease-Epidemiology Collaboration equation for the detection of glomerular filtration rate ≤90 ml/min/1.73 m^2

ROC characteristics	CG	MDRD	CKD-EPI		
			Creatinine	Cystatin C	Creatinine-cystatin C
AUC	0.56	0.62	0.64	0.66	0.68
95% CI	0.49-0.62	0.55-0.68	0.58-0.70	0.60-0.73	0.61-0.79
P	0.06	<0.01	<0.01	<0.01	<0.01

CG: Cockcroft-Gault, MDRD: Modification of Diet in Renal Diseases, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration, CI: Confidence interval, AUC: Areas under the curve

Figure 2



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Receiver operating characteristic curves comparing AUCs of the CG, MDRD, and CKD-EPI equation for the detection of GFR ≤ 90 ml/min/1.73 m^2. GFR: Glomerular filtration rate, CG: Cockcroft–Gault, MDRD: Modification of Diet in Renal Disease, CKD-EPI: Chronic Kidney Disease-Epidemiology Collaboration, DTPA: 99m Tc-diethylenetriaminepentaacetic acid, AUCs: Areas under the curve