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Performance and Determinants of Serum Creatinine and Cystatin C–Based GFR Estimating Equations in South Asians

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Introduction: The creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) estimated glomerular filtration rate (eGFR) equation was calibrated for the general Pakistan population (eGFRcr-PK) to eliminate bias and improve accuracy. Cystatin C–based CKD-EPI equations (eGFRcys and eGFRcr-cys) have not been assessed in this population, and non-GFR determinants of cystatin C are unknown.

Methods: We assessed eGFRcys, eGFRcr-cys, and non-GFR determinants of cystatin C in a cross-sectional study of 557 participants (≥40 years of age) from Pakistan. We compared bias (median difference in measured GFR [mGFR] and eGFR), precision (interquartile range [IQR] of differences), accuracy (percentage of eGFR within 30% of mGFR), root mean square error (RMSE), and classification of mGFR <60 ml/min/1.73 m^2 (area under the receiver operating characteristic curve [AUC] and net reclassification index [NRI]) among eGFR equations.

Results: We found that eGFRcys underestimated mGFR (bias, 12.7 ml/min/1.73 m^2 [95% confidence interval {CI} 10.7–15.2]). eGFRcr-cys did not improve performance over eGFRcr-PK in precision ($P = 0.52$), accuracy ($P = 0.58$), or RMSE ($P = 0.49$). Results were consistent among subgroups by age, sex, smoking, body mass index (BMI), and eGFR. NRI was 7.31% (95% CI 1.52%–13.1%; $P < 0.001$) for eGFRcr-cys versus eGFRcr-PK, but AUC was not improved (0.92 [95% CI 0.87–0.96] vs. 0.90 [95% CI 0.86–0.95]; $P = 0.056$). Non-GFR determinants of higher cystatin C included male sex, smoking, higher BMI and total body fat, and lower lean body mass.

Conclusion: eGFRcys underestimated mGFR in South Asians and eGFRcr-cys did not offer substantial advantage compared with eGFRcr-PK. Future studies are warranted to better understand the large bias in eGFRcys and non-GFR determinants of cystatin C in South Asians.

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Chronic kidney disease (CKD) is a critical public health challenge around the world.[1] The Global Burden of Disease Study 2015 showed that CKD ranked 12th for leading causes of death (1.1 million) globally.[2] The burden has profound impact in low- and middle-income countries[3] and is magnified in countries of South Asia, such as Pakistan, India, Bangladesh, and Sri Lanka, that have markedly elevated risks of hypertension, diabetes, and cardiovascular disease compared with other ethnicities.[4–6] Therefore, accurate ascertainment of CKD is critical for the prevention of CKD development, reduction of associated complications, and improvement of quality of life.

The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommends that clinicians use the creatinine-based CKD-EPI eGFRcr as an initial

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test and CKD-EPI eGFRcys or eGFRcr-cys or measured clearances (creatinine clearance [mClcr] or mGFR) as confirmatory tests for the detection of CKD.[7] The mClcr test is prone to error[8] and mGFR is invasive, inconvenient, and expensive for routine clinical practice.[9,10] The serum concentration of creatinine is affected by factors other than GFR (non-GFR determinants), including generation by diet or muscle, extrarenal elimination, and tubular secretion of creatinine; therefore, eGFRcr based on age, gender, and race is more accurate than the serum creatinine alone, and eGFRcr is reported in >90% of clinical laboratories in the United States when the serum creatinine measurement is available.[7,11] However, eGFRcr has been shown to be inaccurate among people with differences in muscle mass or diet independent of age, gender, and race.[12,13] Although the serum levels of cystatin C has been found to be less affected by non-GFR determinants,[14–18] the large-scale CKD-EPI consortium among diverse populations of 18 studies conducted predominantly among European and black populations found that the overall performance (bias, precision, and accuracy) of eGFRcys was not different from that of eGFRcr, and suggested that unknown and unmeasured non-GFR determinants of the filtration markers affect eGFRcys as much as eGFRcr.[11] In addition, the consortium study also found that eGFRcr-cys had improved accuracy over eGFRcr and eGFRcys alone, and proposed that non-GFR determinants of creatinine and cystatin C were likely to be independent, thus reducing their impact on accuracy in an equation using both markers rather than a single marker.[11] Compared with European counterparts, populations in South Asia have lower muscle mass and lower dietary protein intakes.[19] We previously validated the CKD-EPI eGFRcr equation in a general population from Pakistan, and found that it significantly overestimated mGFR levels; therefore, we calibrated the equation for Pakistan (eGFRcr-PK) to eliminate bias and improve accuracy.[19] To date, performances of eGFRcys and eGFRcr-cys have not been evaluated in a general population among South Asians, and non-GFR determinants of serum cystatin C have not been explored. This information is critical to examine whether cystatin C is useful to improve the GFR estimation accuracy for detection of CKD for South Asians.

In a cross-sectional population-based study in Karachi Pakistan, we aimed to examine the performance of CKD-EPI cystatin C–based eGFR equations (eGFRcys and eGFRcr-cys) compared with eGFRcr-PK using mGFR as the reference, and to explore non-GFR determinants of serum cystatin C compared with those of serum creatinine. We also explored the usefulness of eGFRcr-cys as a confirmatory test for detection of CKD in South Asians. Our working hypothesis was that performances of cystatin C–based eGFR equations would be superior to eGFRcr-PK in South Asians with improved performance and classification of mGFR, and that non-GFR determinants of serum cystatin C and serum creatinine are independent and different.

METHODS

Study Design

In this cross-sectional study, participants were randomly selected from 10 communities in Karachi. Few people were expected to have decreased kidney function among the general population, and therefore we enriched our sample with 40 participants with stage 3 CKD or worse (GFR <60 mL/min/1.73m^2). The detailed description of recruitment and stratification have been published previously.[20] In brief, 581 participants were enrolled in the current study, and the flowchart of the study design is shown in Supplementary Figure S1. The study approval was obtained from the Ethics Review Committee, Aga Khan University, and consent was provided by all participants. The approval for analysis presented in this paper was obtained from the National University of Singapore Institutional Review Board.

Assessment of Levels of Serum Cystatin C, Creatinine, and Other Biomarkers

In the morning, all participants visited the research laboratory following an overnight fast with the completion of 24-hour urine collections. The collection of blood samples was used for measuring serum cystatin C (Siemens Pro-spec instrument particle-enhanced immuno-nephelometric assay; Siemens Healthcare Diagnostics, Tarrytown, NY]), serum creatinine (Beckman Coulter Inc, Brea, CA), serum albumin (Beckman Coulter Inc), and low-density lipoprotein (LDL) cholesterol (Roche Diagnostics; Rotkreuz, Switzerland). The detailed measurement methods of serum creatinine, serum albumin, and LDL cholesterol were described in detail previously.[20] The 24-hour urine collection was used for measuring urine creatinine, urine albumin (nephelometry method on an array instrument) and urine urea nitrogen (enzymatic method). Urine albumin-to-creatinine ratio (ACR) was calculated by dividing urine albumin concentration in milligrams by urine creatinine concentration in grams. Albuminuria was categorized to 3 categories: normal to mildly increased (ACR <30 mg/g), moderately increased (30–300 mg/g), and severely increased (>300 mg/g).[7] There were no missing data for any variable (age, sex, and serum creatinine levels) included in the CKD-EPI eGFR equations.

The measurement of serum cystatin C was conducted at the Advanced Research and Diagnostic Laboratory at

Table 1. Characteristics of study participants by tertile of serum cystatin C levels in the total population

Characteristics	Overall, $n = 557$ (0.53–6.02 mg/l)	Serum cystatin C levels			P value[a]
		Tertile 1, $n = 182$ (0.53–0.91 mg/l)	Tertile 2, $n = 193$ (0.92–1.10 mg/l)	Tertile 3, $n = 182$ (1.11–6.02 mg/l)	
Age, yr	50.5 ± 10.0	45.5 ± 6.58	48.6 ± 7.97	57.5 ± 10.9	<0.001
Male sex	277 (49.7)	58 (31.9)	98 (50.8)	121 (66.5)	<0.001
Ever smoked, yes	135 (24.2)	21 (11.5)	43 (22.3)	71 (39.0)	<0.001
Weight, kg	66.0 ± 13.1	63.9 ± 12.1	67.2 ± 12.7	67.0 ± 14.3	0.06
Height, cm	159.5 ± 9.05	157.9 ± 7.83	160.4 ± 9.95	160.1 ± 9.02	0.007
Body mass index, kg/m^2	26.0 ± 5.02	25.6 ± 4.65	26.2 ± 4.79	26.2 ± 5.60	0.023
Waist circumference, cm	93.4 ± 11.7	90.7 ± 10.9	93.5 ± 10.6	95.9 ± 12.9	0.012
Total body fat, kg[b]	23.1 ± 8.23	23.0 ± 7.30	23.1 ± 7.71	23.2 ± 9.58	<0.001
Lean body mass, kg[b]	42.9 ± 10.2	40.9 ± 9.02	44.1 ± 10.5	43.7 ± 10.7	0.043
History of heart disease, yes[b]	43 (7.73)	7 (3.63)	7 (3.63)	29 (16.0)	<0.001
Serum albumin, g/dl	3.68 ± 0.32	3.71 ± 0.27	3.72 ± 0.32	3.60 ± 0.36	0.001
LDL cholesterol, mmol/l	110.6 ± 29.4	114.1 ± 27.3	112.2 ± 27.3	105.4 ± 32.8	0.010
Urine creatinine, mg/kg/day[c]	13.9 ± 4.99	13.4 ± 4.89	14.2 ± 4.85	14.0 ± 5.21	0.56
Dietary protein intake, g/day	44.2 ± 20.2	42.0 ± 14.0	44.9 ± 15.4	45.8 ± 28.3	<0.001
mGFR, mL/min/1.73 m^2	91 (74–110)	108 (92–123)	94 (84–109)	67 (36–84)	<0.001
Participants with mGFR <60 mL/min/1.73 m^2	88 (15.7)	4 (2.08)	5 (2.73)	79 (43.4)	0.004
Serum creatinine	0.73 (0.54–0.93)	0.59 (0.54–0.73)	0.73 (0.54–0.83)	1.02 (0.83–1.50)	<0.001

LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate.
[a]P for differences across tertiles of serum cystatin C levels in the total population. The differences were compared using the 1-way analysis of variance test for means, the Kruskal-Wallis test for medians, and the χ^2 for proportions.
[b]One missing value (n =556).
[c]Three missing values (n =554).
Categorical variables presented as n (%); continuous variables are presented as mean ± SD or median (interquartile ratio).

the University of Minnesota (Minneapolis, MN). Serum cystatin C was traceable to the certified reference material ERM-DA471/IFCC from the Institute for Reference Materials and Measurements (Geel, Belgium).[21–23] Serum creatinine assays from Pakistan were calibrated using the Roche enzymatic method (Hoffman-La Roche Ltd, Kaiseraugst, Switzerland). Serum creatinine was traceable to the National Institute of Standards and Technology (Gaithersburg, MD) creatinine standard reference material 967 at Cleveland Clinic.[19,24] A calibration factor ([−0.1256] + 0.9557x) was applied to calibrate the serum creatinine assays from Pakistan.[19]

Measurement of GFR

The measurement of GFR has been described in detail previously.[19,20] In brief, urinary inulin clearance was used as the reference standard. Plasma and urinary inulin levels were assayed at the Renal Laboratories at the Saint-Etienne Hospital, University of Jean Monnet (Saint-Etienne, France). GFR was calculated as the average of ≥2 measurements of urinary inulin clearance and multiplied by 1.73/body surface area (height$^{0.725}$[cm] × weight$^{0.425}$[kg] × 0.007184).[25] The median coefficient of variation for participants with 2 and 3 urine collections during the inulin clearance measurement was 6.64% (95% CI 5.78%–7.50%) and 7.06% (95% CI 5.83%–8.39%), respectively, which was consistent with a previous study reporting approximately 7% for repeated measures of inulin clearance.[26] The coefficient of variation of inulin clearance was relatively low compared with the smallest reported coefficients of variation of other mGFR methods (approximately 5%–15%).[27] The mGFR indexed for body surface area served as the criterion standard for comparison.[19]

Assessment of Demographic and Clinical Factors

Trained research staff visited homes of consented participants and conducted face-to-face interviews using a standardized questionnaire.[20] Information on demographic and lifestyle factors were collected and included age, sex, and smoking status (yes/no). History of heart disease was defined as self-reported physician-diagnosed heart disease (yes/no). A physical examination was performed and anthropometry measurements (weight, height, and waist circumference) were taken. A bioimpedance device (QuadScan 4000; Bodyscan Ltd, London, United Kingdom) was used to estimate total body fat and lean body mass.[28,29] BMI was computed using weight (kg) divided by height (m) squared. Dietary protein intake (g/day) was calculated using urine urea nitrogen and weight (dietary protein intake = [urea nitrogen (g/day) + weight (kg) × 0.031] × 6.25).[30]

Statistical Analysis

Analyses were conducted using Stata software (version 14.0; Stata Corp LLC, College Station, TX) and SAS software (version 9.4; SAS Institute, Inc, Cary, NC),

Table 2. Comparison the performances of GFR estimating equations compared with measured GFR ($N = 557$)

Equation	Bias, median difference,[a] ml/min/1.73 m^2	Percent bias, median difference,[b] %	Precision, IQR,[c] ml/min/1.73 m^2	Accuracy, P_{30},[d] %	RMSE[e]	Total deviation index,[f] %
CKD-EPI eGFRcr[11,g]	−6.76 (−9.10 to −5.90)	−8.90 (−11.2 to −6.73)	22.6 (20.3–25.4)	76.1 (72.4–79.6)	0.289 (0.263–0.323)	37.5 (34.5–40.5)
CKD-EPI eGFRcr-PK[19,g]	NA	NA	22.7 (20.6–25.8)	82.4 (79.0–85.5)	0.265 (0.243–0.297)	35.9 (32.7–39.2)
CKD-EPI eGFRcys[11,g]	12.7 (10.7–15.2)	15.4 (13.5–17.5)	25.6 (23.2–28.3)	73.3 (69.4–76.9)	0.322 (0.303–0.349)	43.4 (40.3–46.4)
CKD-EPI eGFRcr-cys[11,g]	2.73 (1.16–4.58)	3.21 (1.66–5.80)	21.2 (18.6–24.3)	83.1 (79.8–86.1)	0.253 (0.231–0.285)	34.8 (31.9–37.8)

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFRcr, CKD-EPI equation estimating GFR using creatinine; eGFRcr-PK, CKD-EPI equation estimating GFR using creatinine modified for Pakistan; eGFRcr-cys, CKD-EPI equation estimating GFR using combined creatinine and cystatin C; eGFRcys, CKD-EPI equation estimating GFR using cystatin C; GFR, glomerular filtration rate; IQR, interquartile range; NA, not applicable because bias was expected to be 0 (the equation was developed in the study population); RMSE, root mean square error.

[a]Bias was expressed as the median difference in measured GFR minus estimated GFR (95% confidence interval). A negative bias indicates overestimation of the measured GFR; a positive bias indicates underestimation of the measured GFR.

[b]Percent bias was expressed as the median difference in measured GFR minus estimated GFR relative to measured GFR ([measured GFR − estimated GFR]/measured GFR) and the corresponding 95% confidence interval. A negative bias indicates overestimation of the measured GFR, and a positive bias indicates underestimation of the measured GFR.

[c]Precision was expressed as the IQR of differences between measured GFR and estimated GFR (95% confidence interval).

[d]Accuracy (P_{30}) was defined as the percentage of individuals with estimated GFRs within 30% of measured GFR (95% confidence interval). The 95% CI of P_{30} was calculated using the Clopper–Pearson (exact) method.

[e]RMSE was defined as the square root of the average squared different of measured GFR and estimated GFR on the logarithmic scale.

[f]Total deviation index measures the allowable difference between measured GFR and estimated GFR (95% confidence interval), where a lower value represents better concordance. A total deviation index of 60% means that 90% of estimated GFR values fall within ±60% of measured GFR. The significance of differences among equations was evaluated using the bootstrap method with 10,000 replications. *P* values for eGFRcr-cys compared with eGFRcr, eGFRcr-PK, and eGFRcys were $P = 0.21$, $P = 0.64$, and $P < 0.001$, respectively. *P* values for eGFRcys compared with eGFRcr and eGFRcr-PK were both $P < 0.001$.

[g]The significance of differences among equations was determined with the use of the McNemar test for P_{30}, and the bootstrap method for IQR and RMSE with 10,000 replications. *P* values for eGFRcr-cys compared with eGFRcr-PK were $P = 0.52$ for precision, $P = 0.58$ for accuracy, and $P = 0.49$ for RMSE. *P* values for eGFRcr-PK compared with eGFRcys were $P = 0.037$ for precision, $P < 0.001$ for accuracy, and $P < 0.001$ for RMSE. *P* values for eGFRcr-cys compared with eGFRcys were $P < 0.001$ for bias, $P = 0.001$ for precision, $P < 0.001$ for accuracy, and $P < 0.001$ for RMSE. *P* values for eGFRcr compared with eGFRcys were $P < 0.001$ for bias, $P = 0.08$ for precision, $P = 0.26$ for accuracy, and $P = 0.39$ for RMSE.

where the statistical significance was determined by a 2-sided *P* value < 0.05. Characteristics of participants were presented overall and compared across 3 categories of serum cystatin C and creatinine levels using 1-way analysis of variance (for means), Kruskal–Wallis test (for medians), and χ^2 test (for proportions). Performances of eGFR equations and non-GFR determinants of serum cystatin C and serum creatinine were assessed for the entire dataset of 557 participants.

eGFR Equations and Metrics for Equation Performance

The current study calculated and compared 4 CKD-EPI equations: 1) 2009 eGFRcr[11]; 2) 2014 eGFRcr-PK[19]; 3) 2012 eGFRcys[11]; and 4) 2012 eGFRcr-cys.[11] The eGFRcr-PK was calibrated previously using this population ($n = 581$).[19] A total of 24 participants had missing values of serum cystatin C levels and were excluded, leaving 557 participants for the current analysis. The metrics for comparing performances of estimating equations were bias, precision, accuracy, and RMSE with corresponding 95% CI. In addition, AUC and NRI were compared for eGFRcr-cys versus eGFRcr-PK for the classification of mGFR <60 and ≥120 ml/min/1.73 m^2. Bias was expressed as the median difference in mGFR minus eGFR, with positive values suggesting an underestimation of mGFR.[19] Precision was assessed using the IQR of the differences.[19] We also computed the percent bias expressed as the median difference in mGFR minus eGFR relative to mGFR ([mGFR − eGFR]/mGFR).[31] Accuracy (P_{30}) was defined as the percentage of participants with eGFR within 30% of mGFR.[19] RMSE was defined as the square root of the average squared difference of eGFR and mGFR on the logarithmic scale.[19] The 95% CIs for these metrics were computed using the bootstrap method[32] with 10,000 replications. Bias was expected to be near 0 for eGFRcr-PK because the equation was developed in the study population. However, bias among the other 3 CKD-EPI equations (eGFRcr, eGFRcys, and eGFRcr-cys) was compared. Improvement in bias, IQR, and RMSE was indicated by a smaller value, and improvement in P_{30} was indicated by a larger value. The differences among equations were assessed using the Wilcoxon signed-rank test for bias, the McNemar test for P_{30}, and the bootstrap method for IQR and RMSE with 10,000 replications.

Comparison of Various eGFR Equations by Subgroups

To evaluate generalizability among subgroups, we compared performances of bias, precision, and accuracy of all eGFR equations by age (<45, 45–<65, and ≥65 years), sex (men and women), smoking status (yes/no), BMI (<25, 25–<30, and ≥30 kg/m^2), eGFR levels (<60, 60–<90, and ≥90 mL/min/1.73 m^2), and albuminuria categories (ACR <30, 30–300, and >300 mg/g). The statistical differences of bias, P_{30}, and IQR among equations were assessed using the Wilcoxon signed-rank test, the McNemar test, and the bootstrap method, respectively.

Concordance Between eGFR and mGFR

The concordance between eGFR estimated by various eGFR equations and mGFR was examined by the total deviation index (TDI).[33] The TDI is a measure that

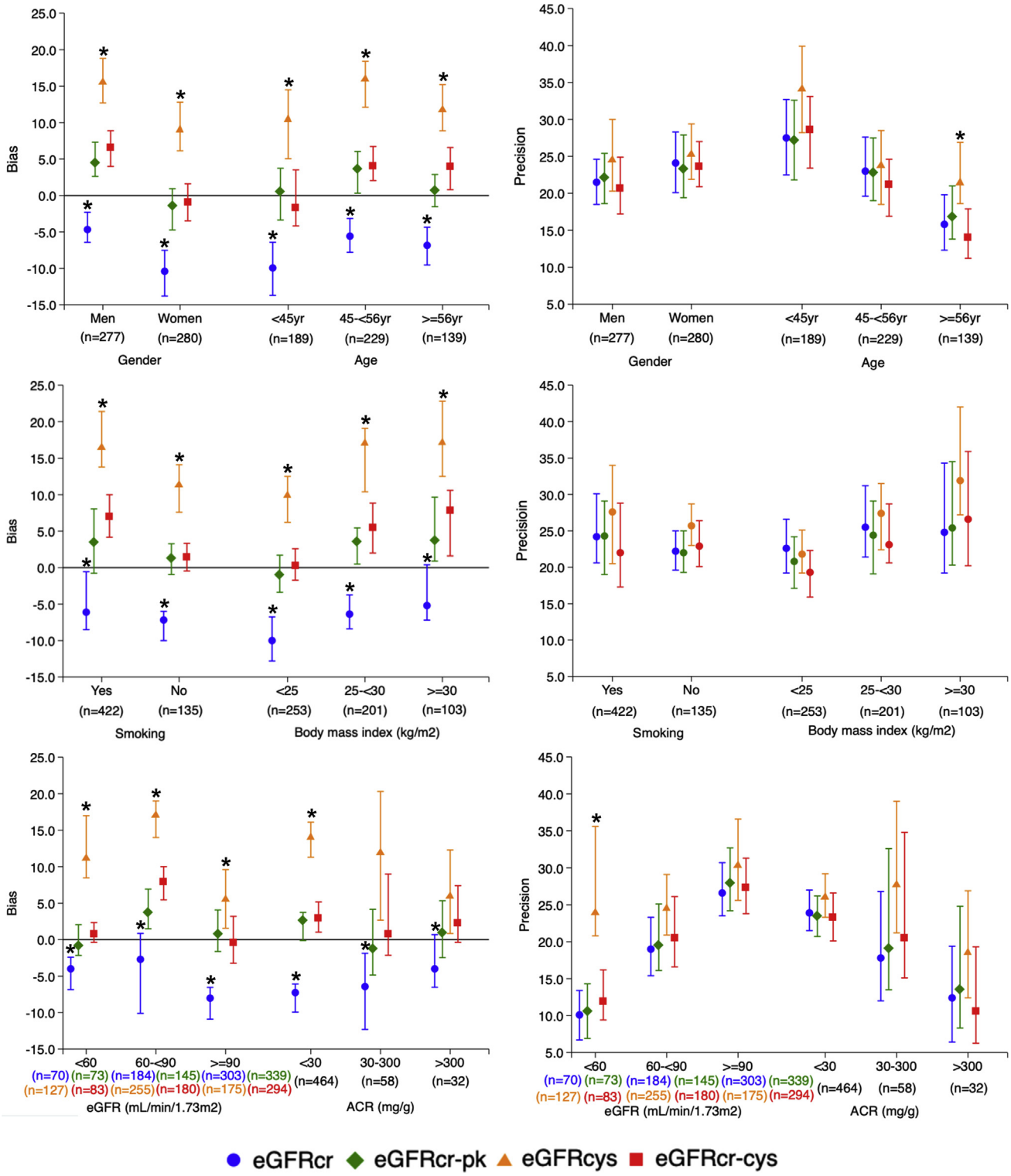

Figure 1. Performance of GFR estimating equations in subgroups. The 3 panels on the left show bias (median difference in measured GFR minus estimated GFR) and 3 panels on the right show precision (interquartile range of differences) in mL/min/1.73m^2. A positive value indicates an underestimation of measured GFR and a negative value indicates an overestimation of measured GFR. Sample size for subgroups were: men ($n = 277$), women ($n = 280$), age <45 years ($n = 189$), age 45–<56 years ($n = 229$), age ≥56 years ($n = 139$), body mass index <25 kg/m^2 ($n = 201$), body mass index 25–<30 kg/m^2 ($n = 103$), body mass index ≥30 kg/m^2 ($n = 83$), smoking (yes, $n = 422$; no, $n = 135$), eGFRcr <60 mL/min/m^2 ($n = 70$), eGFRcr 60–89 mL/min/m^2 ($n = 184$), eGFRcr ≥90 mL/min/m^2 ($n = 303$), eGFRcr-PK <60 mL/min/m^2 ($n = 73$), eGFRcr-PK 60–89 mL/min/m^2 ($n = 145$), eGFRcr-PK ≥90 mL/min/m^2 ($n = 339$), eGFRcys <60 mL/min/m^2 ($n = 127$), eGFRcys 60–89 mL/min/m^2 ($n = 255$), eGFRcys ≥90 mL/min/m^2 ($n = 175$), eGFRcr-cys <60 mL/min/m^2 ($n = 83$), eGFRcr-cys 60–89 mL/min/m^2 ($n = 180$), eGFRcr-cys ≥90 mL/min/m^2 ($n = 294$), ACR <30 mg/g ($n = 464$), ACR 30–300 mg/g ($n = 58$), and ACR >300 mg/g ($n = 32$). *$P < 0.001$ when comparing with eGFRcr-cys equation. ACR, albumin-to-creatinine ratio; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFRcr, CKD-EPI equation estimating GFR using (continued)

captures a large proportion of data within a boundary representing the allowable difference between 2 measurements (e.g., a TDI of 60% means that 90% of eGFR values fall within ±60% of mGFR), where a lower TDI represents a better concordance.[33,34] The 95% CI of TDI was computed using the bootstrap model with 10,000 replications. The statistical difference was assessed using the bootstrap method.

Classification Compared With mGFR <60 ml/min/1.73 m²

AUC was used to compare eGFR equations in identifying patients with mGFR <60 versus ≥60 mL/min/1.73 m². NRI statistics were used to compare the proportion of participants who were reclassified as having mGFR <60 or ≥60 ml/min/1.73 m². The increment in the AUC was tested for significance using the test proposed by DeLong *et al.*[35] NRI was tested for significance using the formulas developed by Pencina *et al.*[36]

Classification Compared With mGFR ≥120 ml/min/1.73 m²

We also compared the proportion of participants who were reclassified as having glomerular hyperfiltration (mGFR ≥120 mL/min/1.73 m²)[37] or <120 mL/min/1.73 m².

Non-GFR Determinants

mGFR and serum levels of cystatin C and creatinine were log-transformed. Linear regression models were applied to evaluate association between each potential predictor after standardization using respective IQRs (age, sex, weight, height, BMI, waist circumference, total body fat, lean body mass, history of heart disease, serum albumin, LDL cholesterol, urine creatinine, and dietary protein intake) and log-transformed levels of serum cystatin C and creatinine. Several models were established. Model 1 was the univariate model to examine the unadjusted association of each predictor. Model 2 adjusted for mGFR to examine the residual association of each predictor. Model 3 included mGFR, age, and sex as adjustments. In the sensitivity analyses, we further included all predictors for adjustment in the same model. We also tested potential interactions among significant predictors with serum levels of cystatin C and creatinine.

Regression coefficients relating serum cystatin C or serum creatinine levels to all potential predictors from linear regression models were transformed as $100 \times (e^{\text{coefficient}} - 1)$ so that they could be interpreted as the average percent difference in serum cystatin C or serum creatinine levels for an IQR difference in continuous predictors or a difference between categories for dichotomous predictors.[15,16] The strength of association for results with statistical significance (95% CI excludes 0) was defined as strong, intermediate, or weak if the absolute percent difference in serum cystatin C or serum creatinine levels was >10%, 5%–10% and <5%, respectively.[15]

RESULTS

Participants' Characteristics

Participants' characteristics are presented in Table 1 and Supplementary Table S1. Of 557 enrolled participants, the mean age was 50.5 (SD 10.0) years and 49.7% were men. The median value of mGFR levels was 91 (IQR 74–110) ml/min/1.73 m², with a range from 4 to 204 ml/min/1.73 m². Participants with higher serum levels of cystatin C were older and more likely to be men or smokers. In addition, they were also more likely to have higher adiposity, dietary protein intake, and serum creatinine levels, as well as lower levels of serum albumin, LDL cholesterol, and mGFR (Table 1).

Distribution of mGFR and eGFR

Compared with mGFR, the distribution of eGFRcr, eGFRcr-PK, eGFRcys, and eGFRcr-cys was shifted to higher levels of mGFR for eGFRcr, shifted to lower levels of mGFR for eGFRcys, and similar for eGFRcr-PK and eGFRcr-cys (Supplementary Figure S2).

Performances of Estimating Equations

eGFRcys underestimated mGFR levels with a large positive bias (12.7 [95% CI 10.7–15.2] ml/min/1.73 m²). eGFRcr overestimated mGFR with a large negative bias (−6.76 [95% CI −9.10 to −5.90]), but the magnitude of bias was significantly reduced relative to that of eGFRcys ($P < 0.001$). eGFRcr-cys had a small positive bias (2.73 [95% CI 1.16–4.58] ml/min/1.73 m²) and its performance was not better than eGFRcr-PK in precision (21.2 [95% CI 18.6–24.3] vs. 22.7 [95% CI 20.6–25.8]; $P = 0.52$), accuracy (83.1 [95% CI 79.8–86.1] vs. 82.4 [95% CI 79.0–85.5]; $P = 0.58$), and RMSE (0.253 [95% CI 0.231–0.285] vs. 0.265 [95% CI 0.243–0.297]; $P = 0.49$). eGFRcys had worse performance compared with eGFRcr-cys in bias (12.7 [95% CI 10.7–15.2] vs. 2.73 [95% CI 1.16–4.58]; $P < 0.001$), precision (25.6 [95% CI 23.2–28.3] vs. 21.2 [95% CI 18.6–24.3]; $P = 0.001$), accuracy (73.3 [95% CI 69.4–76.9] vs. 83.1 [95% CI 79.8–86.1]; $P < 0.001$), and RMSE (0.322 [95% CI 0.303–0.349] vs. 0.253 [95% CI 0.231–0.285]; $P < 0.001$; Table 2). Results were largely consistent among

Figure 1. (continued) creatinine; eGFRcr-cys, CKD-EPI equation estimating GFR using both creatinine and cystatin C; eGFRcr-PK, CKD-EPI equation estimating GFR using creatinine modified for Pakistan; eGFRcys, CKD-EPI equation estimating GFR using cystatin C; GFR, glomerular filtration rate.

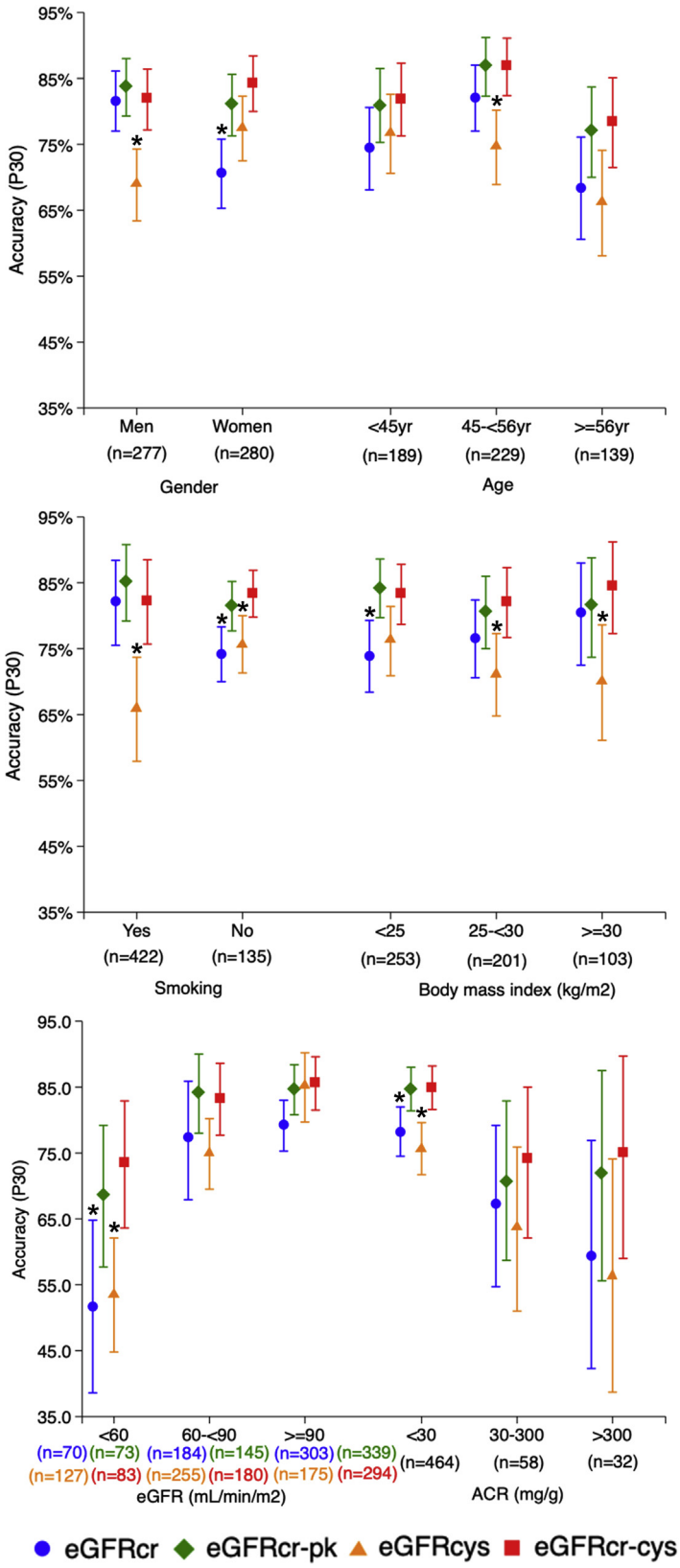

Figure 2. Accuracy (P_{30}) of GFR estimating equations in subgroups. The panels show accuracy (P_{30}; percentage of eGFR within 30% of measured GFR). Sample size for subgroups were: men ($n = 277$), women ($n = 280$), age <45 years ($n = 189$), age 45–<56 years ($n = 229$), age ≥56 years ($n = 139$), body mass index <25 kg/m^2 ($n = 201$), body mass index 25–<30 kg/m^2 ($n = 103$), body mass index ≥30 kg/m^2 ($n = 83$), smoking (yes, $n = 422$; no, $n = 135$), eGFRcr <60 mL/min/m^2 ($n = 70$), eGFRcr 60–89 mL/min/m^2 ($n = 184$), eGFRcr ≥90 mL/min/m^2 ($n = 303$), eGFRcr-PK <60 mL/min/m^2 ($n = 73$), eGFRcr-PK 60–89 mL/min/m^2 ($n = 145$), eGFRcr-PK ≥90 mL/min/m^2 ($n = 339$), eGFRcys <60 mL/min/m^2 ($n = 127$), eGFRcys 60–89 mL/min/m^2 ($n = 255$), eGFRcys ≥90 mL/min/m^2 ($n = 175$), eGFRcr-cys <60 mL/min/m^2 ($n = 83$), eGFRcr-cys 60–89 mL/min/m^2 ($n = 180$), eGFRcr-cys ≥90 mL/min/m^2 ($n = 294$), ACR <30 mg/g ($n = 464$), ACR 30–300 mg/g ($n = 58$), and ACR >300 mg/g ($n = 32$). (continued)

Table 3. Classification of participants of mGFR <60 ml/min/1.73 m^2 with the use of eGFRcr-cys versus eGFRcr-PK

GFR estimating equations	Total population		Subgroup with mGFR <60 mL/min/1.73 m^2				Subgroup with mGFR ≥60 mL/min/1.73 m^2				
	N	AUC (95% CI)	*N*	Correctly reclassified[a]	Incorrectly reclassified[a]	Net difference	*N*	Correctly reclassified[a]	Incorrectly reclassified[a]	Net difference	Overall NRI (95% CI)[c]
eGFRcr-PK	557	0.90 (0.86–0.95)[b]	—	—	—	—	—	—	—	—	—
eGFRcr-cys	557	0.92 (0.87–0.96)[b]	88	7 (7.95%)	0	7.95%	469	3 (0.64%)	6 (1.28%)	−0.64%	7.31% (1.52%–13.1%)

AUC, area under the receiver operating characteristic curve; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFRcr-cys, CKD-EPI equation estimating GFR using both creatinine and cystatin C; eGFRcr-PK, CKD-EPI equation estimating GFR using creatinine modified for Pakistan; mGFR, meausred glomerular filtration rate; NRI, net reclassification improvement.
[a]Number of cases (percentage of subgroup population).
[b]The AUC for eGFRcr-PK was 0.90 (95% CI 0.86–0.95) and for eGFRcr-cys was 0.92 (0.87–0.96) for detection of mGFR <60 vs. ≥60 ml/min/1.73 m^2. The difference between the 2 AUCs was not statistically significant ($P = 0.056$).
[c]Using a mGFR threshold of 60 mL/min/1.73 m^2, NRI for eGFRcr-cys compared with eGFRcr-PK was 7.31% (95% CI 1.52%–13.1%; $P < 0.001$).

subgroups stratified by age, sex, smoking status, BMI, eGFR levels, and albuminuria categories (Figures 1 and 2). eGFRcys substantially underestimated mGFR levels across all subgroups, while eGFRcr-PK and eGFRcr-cys were nearly unbiased in all subgroups (Figure 1). The worse bias of eGFRcys compared with eGFRcr-cys was statistically significant across almost all subgroups after accounting for possibility of multiple comparisons ($P < 0.001$; Figure 1).

Concordance Between eGFR and mGFR

The TDI ranged from 34.8% to 43.4%. eGFRcys had the highest TDI (43.4% [95% CI 40.3%–46.4%]), suggesting that 90% of eGFR calculated with eGFRcys fall within ±43.4% of mGFR (Table 2). Compared with eGFRcys, eGFRcr (37.5% [95% CI 34.5%–40.5%]), eGFRcr-PK (35.9% [95% CI 32.7%–39.2%]), and eGFRcr-cys (34.8% [95% CI 31.9%–37.8%]) all had significantly lower TDI with nonoverlapping 95% CIs ($P < 0.001$), indicating that eGFR calculated by eGFRcr, eGFRcr-PK, and eGFRcr-cys had better concordance with mGFR compared with that by eGFRcys (Table 2).

Classification of mGFR <60 ml/min/1.73 m^2

The difference of AUC between eGFRcr-cys and eGFRcr-PK did not achieve statistical significance (0.92 [95% CI 0.87–0.96] vs. 0.90 [95% CI 0.86–0.95]; $P = 0.056$; Table 3). Using an mGFR cutoff of <60 mL/min/1.73 m^2, NRI for eGFRcr-cys compared with eGFRcr-PK was 7.95% (95% CI 3.90%–15.5%; $P < 0.001$). For participants with mGFR ≥60 mL/min/1.73 m^2, NRI was −0.64% (95% CI −1.86% to −0.22%; $P < 0.001$). The overall NRI for eGFRcr-cys compared with eGFRcr-PK was 7.31% (95% CI 1.52%–13.1%; $P < 0.001$; Table 3). In terms of glomerular hyperfiltration, using an mGFR cutoff of ≥120 mL/min/1.73 m^2, NRI for eGFRcr-cys compared with eGFRcr-PK was 10.8% (95% CI 5.81%–19.3%; $P < 0.001$). For participants with mGFR <120 mL/min/1.73 m^2, NRI was −1.48% (95% CI −3.02% to −0.72%; $P < 0.001$). The overall NRI for eGFRcr-cys compared with eGFRcr-PK was 9.37% (95% CI 1.77%–17.0%; $P < 0.001$; Supplementary Table S2).

Associations With Non-GFR Determinants

Associations between non-GFR determinants and serum cystatin C and serum creatinine are shown in Tables 4 and 5 and Supplementary Tables S3 and S4. In the bivariate model adjusted for mGFR (model 2), we observed that older age (compared with younger age) had an intermediate association (absolute percent difference in serum cystatin C between 5%–10%) with higher serum cystatin C levels (7.25%), but had no association with serum creatinine. Male sex (compared with female sex) was strongly associated (absolute percent difference >10%) with higher levels of both serum cystatin C (12.5%) and serum creatinine (28.8%).

After adjustment of mGFR, age, and sex (model 3 of Tables 4 and 5), non-GFR determinants of higher serum cystatin C included male sex (strong), and smoking, higher BMI and total body fat, lower lean body mass, and history of heart disease (intermediate), and older age, higher weight, waist circumference, and dietary protein intake, and lower serum albumin and LDL cholesterol (weak; absolute percent difference <5%). Non-GFR determinants of higher serum creatinine included male sex and higher dietary protein intake (strong), and higher weight and BMI (intermediate), and younger age, higher waist circumference, and higher urine creatinine (weak).

Figure 2. (continued) *$P < 0.001$ when comparing with eGFRcr-cys equation. ACR, albumin-to-creatinine ratio; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFRcr, CKD-EPI equation estimating GFR using creatinine; eGFRcr-cys, CKD-EPI equation estimating GFR using both creatinine and cystatin C; eGFRcr-PK, CKD-EPI equation estimating GFR using creatinine modified for Pakistan; eGFRcys, CKD-EPI equation estimating GFR using cystatin C; GFR, glomerular filtration rate.

Table 4. Linear regression between baseline characteristics and log-transformed serum cystatin C levels ($N = 557$)

		Average percent difference (95% CI) in cystatin C levels[a]		
Factor of interest	IQR	Univariate model (model 1)	Bivariate model[b] (model 2)	Multivariate model[c] (model 3)
Age, yr	13.0	25.9 (20.9–31.0)[d]	7.25 (4.08–9.42)[e]	4.90 (2.55–7.31)[f]
Sex, men vs. women	—	12.4 (6.56–17.9)[d]	12.5 (9.74–15.3)[d]	11.1 (8.20–13.9)[d]
Smoking, yes vs. no	—	16.1 (7.56–25.3)[d]	14.6 (10.3–18.9)[d]	6.20 (1.63–11.0)[e]
Weight, kg	17.0	1.72 (−2.56 to 6.18)	5.61 (3.37–7.90)[e]	4.88 (2.76–7.03)[f]
Height, cm	13.3	1.01 (−3.79 to 6.06)	5.67 (3.11–8.28)[e]	−0.79 (−3.85 to 2.36)
Body mass index, kg/m^2	6.6	1.48 (−2.84 to 6.00)	3.17 (0.93–5.47)[f]	5.78 (3.61–7.99)[e]
Waist circumference, cm	15.0	8.51 (4.04–13.2)[e]	5.32 (3.10–7.58)[e]	4.68 (2.61–6.78)[f]
Total body fat, kg[g]	10.6	−0.93 (−4.69 to 2.97)	−3.68 (−5.53 to −1.78)[f]	5.20 (1.73–8.79)[e]
Lean body mass, kg[g]	14.7	−1.94 (−6.90 to 3.29)	5.21 (2.48–8.02)[e]	−6.36 (−10.6 to −1.89)[e]
History of heart disease[g]	—	47.5 (30.8–66.3)[d]	9.92 (3.07–17.2)[e]	9.75 (3.34–16.5)[e]
Serum albumin, g/dl	0.4	−11.0 (−14.5 to −7.46)[d]	−3.15 (−5.17 to −1.08)[f]	−3.93 (−5.81 to −2.00)[f]
LDL cholesterol, mmol/l	37.0	−9.42 (−13.0 to −5.64)[e]	−3.66 (−5.69 to −1.60)[f]	−2.99 (−4.90 to −1.04)[f]
Dietary protein intake, g/day	19.0	−11.9 (−22.3 to −0.19)[d]	9.90 (3.02–17.2)[e]	0.57 (0.06–1.08)[f]
Urine creatinine, mg/kg/day[h]	6.4	0.33 (-0.71 to 1.39)	1.06 (0.53–1.59)[f]	−1.11 (−7.55 to 5.78)

CI, confidence interval; GFR, glomerular filtration rate; IQR, interquartile range; LDL, low-density lipoprotein; RMSE, root mean square error.
[a]Average percent difference in serum cystatin C levels for an IQR (difference between the 25th and 75th percentiles) higher level in continuous variables was calculated as $100 \times (e^{\text{beta-coefficient}} - 1)$.
[b]Bivariate model (model 2) was adjusted for measured GFR (log-transformed).
[c]Multivariate model (model 3) was adjusted for age, sex, and measured GFR (log-transformed).
[d–f]Strength of association for statistically significant results. [d]Strong (absolute average percent difference in cystatin C levels >10%).
[e]Intermediate (absolute average percent difference in cystatin C levels 5%–10% inclusive).
[f]Weak (absolute average percent difference in cystatin C levels <5%). Cystatin C alone explained 76% of the variance of measured GFR.
[g]One missing value ($n = 556$).
[h]Three missing values ($n = 554$).

In the sensitivity analyses, we further included all variables in the same model, and the strong associations were largely unchanged (Supplementary Tables S3 and S4). A significant interaction has been observed between smoking and BMI (P for interaction = 0.005) with serum cystatin C levels (Supplementary Table S5). Among nonsmokers, higher BMI had an intermediate association with higher serum cystatin C levels (6.03%); among smokers, BMI had no association with cystatin C levels (Supplementary Table S6). No other interactions have been found with serum cystatin C or creatinine levels (Supplementary Tables S5 and S7). The significant non-GFR determinants accounted for a small percent of

Table 5. Linear regression between baseline characteristics and log-transformed serum creatinine levels ($N = 557$)

		Average percent difference (95% CI) in creatinine levels[a]		
Factor of interest	IQR	Univariate model (model 1)	Bivariate model[b] (model 2)	Multivariate model 1[c] (model 3)
Age, yr	13.0	23.4 (17.4–29.7)[d]	2.02 (-0.99 to 6.18)	−3.49 (−6.11 to −0.79)[e]
Sex, men vs. women	—	28.8 (23.6–34.2)[d]	28.8 (26.6–31.6)[d]	30.0 (27.2–32.7)[d]
Smoking, yes vs. no	—	25.9 (15.0–37.7)[d]	24.6 (17.4–31.0)[d]	−0.13 (−5.36 to 5.38)
Weight, kg	17.0	6.18 (0–11.6)[f]	10.5 (7.25–13.9)[d]	6.63 (4.03–9.29)[f]
Height, cm	13.3	13.9 (8.33–20.9)[d]	20.9 (16.2–24.6)[d]	2.24 (−1.57 to 6.20)
Body mass index, kg/m^2	6.6	−0.99 (−5.82 to 4.08)	1.01 (−1.98 to 4.08)	6.76 (4.10–9.49)[f]
Waist circumference, cm	15.0	9.42 (4.08–15.0)[f]	5.13 (2.02–8.33)[f]	4.54 (2.02–7.11)[e]
Total body fat, kg[g]	10.6	−12.1 (−15.6 to −7.68)[d]	−14.7 (−17.3 to −13.0)[d]	2.63 (−1.50 to 6.94)
Lean body mass, kg[g]	14.7	15.0 (8.33–22.1)[d]	24.6 (20.9–28.4)[d]	−2.41 (−7.83 to 3.32)
History of heart disease[g]	—	44.8 (25.9–68.2)[d]	4.08 (−4.87 to 13.9)	7.29 (−0.30 to 15.5)
Serum albumin, g/dl	0.4	−6.76 (−11.3 to −1.98)[f]	3.05 (0–6.18)[e]	−0.60 (−3.01 to 1.85)
LDL cholesterol, mmol/l	37.0	−9.51 (−13.9 to −4.87)[f]	−2.95 (−5.82 to 0)[e]	−1.72 (−4.08 to 0.69)
Dietary protein intake, g/day	19.0	15.0 (−1.98 to 33.6)	47.7 (36.3–61.6)[d]	13.1 (4.28–22.7)[d]
Urine creatinine, mg/kg/day[h]	6.4	1.01 (0–2.02)[e]	2.02 (1.01–3.05)[e]	0.81 (0.19–1.43)[e]

CI, confidence interval; GFR, glomerular filtration rate; IQR, interquartile range; RMSE, root mean square error.
[a]Average percent difference in serum creatinine levels for an IQR (difference between the 25th and 75th percentiles) higher level in continuous variables was calculated as $100 \times (e^{\text{beta-coefficient}} - 1)$.
[b]Bivariate model (model 2) was adjusted for measured GFR (log-transformed).
[c]Multivariate model (model 3) was adjusted for age, sex, and measured GFR (log-transformed).
[d–f]Strength of association for statistically significant results. [d]Strong (absolute average percent difference in creatinine levels >10%).
[e]Weak (absolute average percent difference in creatinine levels <5%).
[f]Intermediate (absolute average percent difference in creatinine levels 5%–10% inclusive). Creatinine alone explained 64% of the variance of measured GFR.
[g]One missing value ($n = 556$).
[h]Three missing values ($n = 554$).

variation in serum cystatin C levels (4%) and creatinine levels (4%–10%).

DISCUSSION

In this evaluation of cystatin C–based CKD-EPI equations and associated non-GFR determinants in a community-based population among South Asians, we observed that eGFRcys had a large positive bias (underestimation of mGFR) in the overall population as well as in all subgroups stratified by sex, age, smoking status, BMI, and eGFR levels. Moreover, eGFRcr-cys did not improve performance in any of the metrics (accuracy, precision, and RMSE) compared with eGFRcr-PK, nor did it consistently improve classification of mGFR <60 mL/min/1.73 m^2. Non-GFR determinants of higher serum cystatin C included male sex (strong), and smoking, higher BMI and total body fat, and lower lean body mass (intermediate). Overall, these results suggest that eGFRcys or eGFRcr-cys may not be a valuable confirmatory test in Pakistan. Future studies are warranted to better understand the large bias in eGFRcys and non-GFR determinants of serum cystatin C in South Asians globally.

Previous studies in South Asia evaluating GFR estimating equations were limited to a hospital-based, small-scale study among patients with low muscle mass and low dietary protein intake in India, which reported that eGFRcys overestimated mGFR with a small bias (−3.53 [95% CI −6.06 to −1.00] ml/min/1.73 m^2).[38] However, evidence on the performance of eGFRcys and eGFRcr-cys among South Asians, especially from the general population, is scarce. The large bias of eGFRcys observed in the current study (12.7 [95% CI 10.7–15.2] ml/min/1.73 m^2) was consistent with a study conducted among indigenous Australians (15.0 [95% CI 13.3–16.4] ml/min/1.73 m^2).[39] However, our findings were in contrast with the majority of other studies in the general population (from the United States, Canada, Sweden, Iceland, France, Singapore, China, and Africa) where eGFRcys had a smaller magnitude of bias (range −7.1 [95% CI −9.3 to −4.6] to 4.4 [95% CI N/A] ml/min/1.73 m^2)[11,40–48] and eGFRcr-cys had improved accuracy than that of eGFRcr.[11,40–46] A meta-analysis among populations relevant to primary health care also reported that eGFRcys and eGFRcr-cys were less biased and more accurate than eGFRcr.[49] The heterogenous findings in the performance of eGFRcys could be related to the higher prevalence of smoking or other clinical characteristics in our study population that differ from others.[50–53] Unlike our decision to develop eGFRcr-PK to account for bias in eGFRcr, presumably because of lower muscle mass and lower dietary protein intakes[19] in Pakistan compared with North American and European populations in which the CKD-EPI eGFRcr was developed, we elected not to develop a calibration factor for eGFRcys in Pakistan because the cause of the large bias of eGFRcys is not known, and therefore we were not confident that the calibrated equation would be robust across the country. Although eGFRcr-cys had good performance with little bias, high precision, high accuracy, and improved discrimination ability of those with high GFR, eGFRcr-cys uses both creatinine and cystatin C and the large bias of eGFRcys remains unknown. Therefore, we remain cautious and do not recommend the use of eGFRcr-cys equation in Pakistan despite its marginally improved performance compared with other existing equations. Further studies among populations in South Asia are warranted to investigate the cause of the bias of eGFRcys and demonstrate the validity and cost effectiveness of eGFRcr-cys for wide application.

Creatinine generated by muscle is thought to be the main cause of variation in serum levels, which is independent of GFR.[14] Muscle mass had more impact on creatinine than cystatin C,[54] and the latter has been found to be more associated with fat mass.[16] Consistent with previous findings,[14–16,55–57] we observed that cystatin C had a less strong association with sex than creatinine, and that cystatin C was associated with various adiposity measures (weight, BMI, waist circumference, total body fat, and lean body mass). The association between adiposity and serum cystatin C has been proposed as complex and shown to be mediated by metabolic dysregulation and inflammation in South Asian immigrants living in the United States.[58] In corroboration with this statement, we observed a significant interaction between BMI and smoking with serum cystatin C levels, where a positive association between higher BMI and higher cystatin C levels was observed only among nonsmokers but not among smokers. Our finding was consistent with an analysis of National Health and Nutrition Examination Survey III study, where the positive association between BMI and cystatin C levels was significant among former smokers, but not among current smokers.[59] The positive association of smoking with cystatin C in the current study, which corroborates results from most previous studies,[15,16,55,56] may support the contention that unmeasured inflammatory markers contribute substantially as a non-GFR determinant of cystatin C in native South Asians, thereby resulting in the underestimation of mGFR. Moreover, the association between smoking

and cystatin C may also reflect the higher metabolic rate in smokers compared with nonsmokers[15] or the decline in lung function.[60] A recent meta-analysis of 15 studies found that serum cystatin C levels had positive associations with chronic obstructive pulmonary disease and chronic obstructive pulmonary disease exacerbation.[60] Furthermore, consistent with previous findings, serum albumin (a marker for cardiovascular disease)[61] and a history of heart disease were associated with cystatin C,[14–16] suggesting that cystatin C may be associated with vascular diseases and complications. Some studies have shown that South Asians have a stronger predisposition to vascular disease than European origin populations.[51–53] Further studies are needed with broader measurements including inflammatory markers to validate and investigate the underlying mechanism.

Our findings have important clinical implications for using eGFR equations for South Asians. The KDIGO guideline recommends using eGFRcr as an initial test and eGFRcys, eGFRcr-cys, mClcr, and mGFR as confirmatory tests.[7] In addition, eGFRcys and eGFRcr-cys have been shown to have smaller bias at lower eGFR levels (<60 ml/min/1.73m^2) compared with higher levels (≥ 60 ml/min/1.73m^2).[11] However, our findings suggest that given its large bias across all levels of eGFR (<60 and ≥ 60 ml/min/1.73m^2), eGFRcys may not be a suitable confirmatory test for CKD in Pakistan. Although eGFRcr-cys was largely unbiased, the incremental benefits of eGFRcr-cys over eGFRcr-PK for CKD classification may be only marginal. Therefore, mClcr or mGFR would be the more suitable confirmatory test for CKD. However, clearance measurements for Clcr and GFR are not performed routinely, so they would be appropriate in special circumstances such as kidney donor evaluation or dosing of toxic drugs, as recommended by KDIGO guidelines, where more accurate GFR ascertainment will affect treatment decisions.[7] Of note, our conclusion depends on using eGFRcr-PK which is calibrated for the current population. It may not apply to populations where eGFRcr has not been calibrated or eGFRcr-PK is not accurate.

In addition, although cystatin C may not be accurate in estimating GFR levels, it has shown incremental benefits in predicting total and cardiovascular mortality over creatinine.[62–64] Possibly, eGFRcys could be useful for identifying patients with elevated risks for cardiovascular events and mortality among those with eGFRcr <60 mL/min/1.73 m^2.[65] However, this should be further evaluated in South Asian populations before cystatin C is widely measured in clinical laboratories in South Asia considering the higher cost of measurement compared with creatinine.[66]

The major strength of our study was the use of community-based sampling method, which may allow generalizability of our results to the general population in South Asia. We are not aware of other reports on cystatin C in community-based populations in South Asia. In addition, we enriched our samples with patients with CKD from clinics; therefore, our results could also be applicable to South Asians with advanced CKD. Furthermore, the criterion standard GFR reference was measured using optimal approaches (inulin clearance), and serum cystatin C and serum creatinine were measured using standardized assays. In addition, we used comprehensive statistical analyses to compare the performances among different GFR estimating equations, and the associated 95% CIs were computed using 10,000 bootstrapped replications. However, some limitations merit consideration. First, the current study did not measure inflammatory markers (e.g., C-reactive protein), and other residual confounding factors may exist; therefore, we could not ascertain the unmeasured residual associations that could account for the bias in eGFRcys. Second, the study population for the current study was relatively small, especially for those with GFR <60mL/min/1.73 m^2. Third, the current study was cross-sectional; therefore, the temporal relations between the non-GFR determinants and cystatin C could not be determined. Fourth, eGFRcr-PK has been calibrated in the same cohort, which could contribute to a reduced bias relative to eGFRcys. However, we compared the performance of the original 2009 CKD-EPI eGFRcr equation to that of eGFRcys in the current population, which is an external cohort for both equations. We found that eGFRcr also had a significantly reduced bias and better concordance with mGFR relative to eGFRcys ($P < 0.001$). Therefore, we believe our findings of a large bias of eGFRcys in South Asian population are robust. In addition, the calibrated eGFRcr-PK has been evaluated in a separate Pakistani cohort among 670 subjects (59% had eGFR <60 ml/min/m^2), and eGFRcr-PK has been found to have a high correlation ($r = 0.82$) and high agreement (88.7%) with the 24-hour urine creatinine clearance.[67] Future studies are warranted to validate the accuracy of eGFRcr-PK among South Asians globally.

In a general South Asian population, eGFRcys underestimated mGFR and eGFRcr-cys did not offer substantial advantage in classification of mGFR <60mL/min/1.73 m^2 compared with eGFRcr-PK. The large bias in eGFRcys and non-GFR determinants of serum cystatin C in South Asians warrants further exploration.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

THJ, ASL, and LAI were responsible for the research idea and study design. THJ and SJ performed data acquisition. YW, JA, and THJ were responsible for data analysis. YW and THJ were responsible for the interpretation and first draft. All authors submitted critical revisions and were responsible for approval of the final submission.

Data are available on reasonable request from THJ subject to approval by the institutional review board.

SUPPLEMENTARY MATERIAL

STROBE statement.

Figure S1. Flowchart of the study design.

Figure S2. Distribution of mGFR, eGFRcr, eGFRcr-PK, eGFRcys, and eGFRcr-cys.

Table S1. Characteristics of study participants by tertile of serum creatinine in the total population.

Table S2. Classification of participants of mGFR ≥120 ml/min/1.73 m^2 with the use of eGFRcr-cys versus eGFRcr-PK.

Table S3. Sensitivity analysis of linear regression between baseline characteristics and log-transformed serum cystatin C levels.

Table S4. Sensitivity analysis of linear regression between baseline characteristics and log-transformed serum creatinine levels.

Table S5. Potential interactions among significant baseline characteristics with log-transformed serum cystatin C levels.

Table S6. Linear regression between body mass index and log-transformed serum cystatin C levels by smoking status.

Table S7. Potential interactions among significant baseline characteristics with log-transformed serum creatinine levels.

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