Original Article

Agreement between Creatinine and Cystatin C-Based Equations in the Estimation of Glomerular Filtration Rate among Malaysian Patients with Renal Impairment

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ABSTRACT

Background: Cystatin C-based equations have been proposed as an alternative for creatinine-based equations in the estimation of Glomerular Filtration Rate (eGFR). Limited studies were available with regards to the Asian population to evaluate the agreement of eGFR measured based on both biomarkers. This study aimed to evaluate the agreement between various cystatin C- and creatinine-based eGFR equations. **Methods:** Patients were recruited from the Nephrology Clinic Universiti Kebangsaan Malaysia Medical Centre. Serum cystatin C and creatinine levels were analysed by particle-enhanced immunoturbidimetry assay and kinetic alkaline picrate method, respectively. Various cystatin C-based (Hoek, Larsson, Grubb, Flodin, cystatin C-based Chronic Kidney Disease Epidemiology (CKD-EPI)) and creatinine-based eGFR equations (Cockcroft-Gault, Modification of Diet in Renal Disease, creatinine-based CKD-EPI) were compared by using the Pearson's correlation and Bland-Altman plot. **Results:** A total of 118 patients included in this study have a mean age of 61 ± 15 years and 57.6% were female. The mean serum cystatin C and creatinine levels were 2.00 ± 1.06 mg/L and 2.21 ± 1.63 mg/dL, respectively. The correlation between serum cystatin C and creatinine level was significant ($r = 0.78$, $P < 0.01$). All cystatin C-based eGFR correlated significantly with the creatinine-based eGFR equations. The strongest correlation was between creatinine-based and cystatin C-based CKD-EPI equation ($r = 0.93$, $P < 0.01$). The Bland-Altman plot shows that cystatin C-based CKD-EPI equation had the least mean difference when compared with creatinine-based CKD-EPI equation. **Conclusion:** Significant correlation and good agreement were demonstrated between various cystatin C-based and creatinine-based eGFR equations. The cystatin C-based equations are appropriate alternative for GFR measurement among Malaysian patients with renal impairment.

Key words: Cystatin C, Creatinine, Estimated Glomerular Filtration Rate, Renal Function, Renal Elimination, Chronic Kidney Disease.

INTRODUCTION

Dosage of renally excreted drugs are commonly estimated based on glomerular filtration rate (eGFR). Creatinine clearance (CLcr) is often used for GFR estimation. A newer biomarker namely cystatin C has been suggested as an alternative biomarker to creatinine in renal function measurement. It is freely filtered by the glomerulus, reabsorbed and metabolized but not secreted by the proximal tubular cells.[1] It has a constant production rate by all nucleated cell types.[2] Serum cystatin C level is reported to be independent from other factors such as age, sex, diet, muscle mass and selected diseases.[3–6] Due to its short

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half-life (approximately 2 h),[7] it reaches steady-state faster than creatinine (half-life 6 h).[8] The fluctuation in serum cystatin C level is expected to be a more accurate reflection of the current kidney function, thus, avoiding the possibility of overestimation in glomerular filtration rate (GFR) values. This simple, accurate and rapid endogenous substance fulfil the criteria as an ideal biomarker for renal function measurement. A meta-analysis conducted by Dharnidharka *et al.* found cystatin C to be superior than creatinine as an endogenous biomarker in estimation of GFR.[9]

In clinical practice, the GFR is routinely estimated based on the clearance of creatinine. Examples of creatinine-based equations widely used in clinical practice are Cockcroft-Gault (CG),[10] Modification of Diet in Renal Disease (MDRD)[11] and creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation.[12] The usage of creatinine-based equations to estimate renal function often results in an overestimation of the GFR especially in elderly patients due to reduced muscle mass.[13] This condition could results in higher risk of drug dosing error especially amongst individuals with reduced renal function.[14] Several other factors such as age, gender, ethnicity, body mass and dietary intake have also been found to influence the level of serum creatinine.[3,10] It is also lacks in sensitivity in detecting small decrease in GFR and this is known as 'creatinine-blind range'.[15] Therefore, numerous studies recommended cystatin C, as a superior alternative biomarker than creatinine for the estimation of GFR.[9] Several equations have been developed by using serum cystatin C level to estimate GFR, either alone or in combination with serum creatinine. Most of the cystatin C-based equations have lesser covariates because it does not involve parameters such as age, sex, weight and height which is often found in creatinine-based eGFR equations. A cystatin C-based formula developed through a study conducted by Hoek *et al.* showed a greater accuracy and precision when compared to the CG equation.[16] Over the years, several other cystatin C-based eGFR equations were developed, such as Larsson,[17] Grubb[18] and Flodin[19] equations. These equations were useful for early detection of renal dysfunction and is a better risk predictor amongst patients with diabetic mellitus when compared to the creatinine-based eGFR equations.[20] Rapid detection of reduced renal function will allow earlier intervention and hence, prevent further deterioration of kidney problem.

Cystatin C-based eGFR showed a better accuracy in predicting prognosis when compared to creatinine-based eGFR, thus utilisation of cystatin C was suggested.[21] Both creatinine- and cystatin C-based equations were mostly developed based on the information from the Western population. The creatinine-based equations have been used and established in clinical settings for significant amount of time compared to cystatin C-based equations. Some modification towards creatinine-based equations have been done in some studies to include ethnicity coefficient for the non-western, non-African Americans or in other words, the Asian population.[21-22] The utilisation of cystatin C-based equations in Asian population has not been extensively explored. As such, validation of the cystatin C-based equations for renal function measurement in Asian population is required.

Another factor associated with the limited use of cystatin C include the lack of knowledge on the presence of other non-GFR determinants.[21] Ongoing search to investigate factors affecting cystatin C production or secretion should be conducted for better use of cystatin C-based equations in terms of GFR estimation, clinical interpretation and prognosis.[21] The relationship between non-GFR determinants of cystatin C and its serum concentration could be evaluated by using probable error of a coefficient correlation, developed by Fisher (1921).[24] The presence of numerous creatinine- and cystatin C-based equations indicated the need for further investigations on the most comparable pairs of equations. Therefore, this study aimed to investigate the agreement of eGFR measured by using cystatin C- and creatinine-based eGFR equations among multi-racial Malaysian patients with chronic kidney disease.

MATERIALS AND METHODS

Patients

Outpatients attending the Nephrology Clinic Universiti Kebangsaan Malaysia Medical Center (UKMMC), aged more than 18 years old were invited to participate in this observational and cross-sectional study. Patients were recruited from July 2017 until October 2017. These recruited patients were referred to the outpatient nephrology clinic either with general chronic kidney disease problem or renal transplant follow-up. Patients who were currently pregnant, on haemodialysis, diagnosed with Systemic Lupus Erythematosus (SLE) and critically ill were excluded from this study. The study was reviewed and approved by the Research Ethics Committee, Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2017-133) Written informed consent was obtained from all research participants included in the study.

Assay methods

Fasting blood samples were obtained to determine serum creatinine and cystatin C level. Serum creatinine

levels were measured by using the standardized-isotope dilution mass spectrometry (IDMS) traceable-kinetic alkaline picrate method on an Architect C System instruments (Abbott Laboratories, Illinois, USA).[25] The assays were performed in the Department of Diagnostics Laboratory Services, Universiti Kebangsaan Malaysia Medical Centre (UKMMC). The coefficient variation of this method was less than 6% for between day and within run imprecision. Serum creatinine levels reported as micromol per liter were converted to milligrams per deciliter. Conversion were made since creatinine-based equations used in this study is based on readings of serum creatinine level reported in milligram per deciliter. Serum cystatin C levels were assessed by particle-enhanced turbidimetric immunoassay (PETIA) on an ADVIA 1650/1800 Instrument (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) and reported as milligrams per liter.[26] This method has a coefficient of variation of less than 5% in the range of 0.1 to 8.3 mg/L. The assays were performed in the Pathology and Clinical Laboratory, Petaling Jaya, Selangor, Malaysia.

Estimation of GFR based on serum creatinine and serum cystatin C levels

Creatinine-based eGFR was calculated by using the Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and creatinine-based Chronic Kidney Disease Epidemiology Collaboration (Cr-CKD-EPI) equations. Cystatin C-based eGFR was calculated based on the Hoek, Larsson, Grubb, Flodin and cystatin C-based Chronic Kidney Disease Epidemiology Collaboration (CysC-CKD-EPI) equations. Table 1 shows all relevant equations for eGFR. CG and Larsson formula were expressed as ml/min/1.73m^2 after corrected to body surface area (BSA), based on the Dubois-Dubois formula.[27]

Table 1: Equations used to estimate GFR based on serum creatinine and serum cystatin C.

References	Equations
Cockcroft-Gault (CG)[10]*	$eGfR(ml/min) = \frac{(140 - age) \times BW}{72 \times SCr.}$
Modification of Diet in Renal Disease (MDRD)[11]†	eGFR (ml/min/1.73m^2) = 175 × $SCr^{-1.154}$ × $Age^{-0.203}$ × G × E G = 0.742 if female.1 if male E = 1.21 if African American,1 if non-African American
Creatinine-based Chronic Kidney Disease – Epidemiology (CKD-EPI)[12]†	eGFR (ml/min/1.73m^2) = $141 \times \min\left(\frac{SCr}{k},1\right)^{\alpha} \times \max\left(\frac{SCr}{k},1\right)^{-1.209} \times 0.993^{Age} \times G \times E$ α = −0.329 if female, −0.411 if male κ = 0.7 if female, and 0.9 if male min = the minimum of (SCr/κ) or 1 max = the maximum of (SCr/κ) or 1 G = 1.018 if female,1 if male E = 1.159 if African American,1 if non-African American
Hoek Equation[16]†	$eGfR(ml/min/1.73m^2) = \frac{80.35}{SCysC} - 4.32$
Larsson Equation[17]*	eGFR (ml/min) = 77.24 × $SCysC^{-1.2623}$
Grubb Equation[18]†	eGFR (ml/min/1.73m^2)=84.69 × $SCysC^{-1.680}$
Flodin Equation[19]†	eGFR (ml/min/1.73m^2)= 79.901 × $SCysC^{-1.4389}$
Cystatin C-based CKD-EPI [45]†	eGFR (ml/min/1.73m^2)= $133 \times \min\left(\frac{SCysC}{0.8},1\right)^{-0.499} \times \max\left(\frac{SCysC}{0.8},1\right)^{-1.328} \times 0.996^{Age} \times G$ min = the minimum of (SCysC/0.8) or 1 max = the maximum of (SCysC/0.8) or 1 G = 0.932 if female,1 if male

BW, body weight in kg; SCr, serum creatinine in mg/dL; G, gender factor; E, ethnicity factor; SCysC, serum cystatin C in mg/L.
*Corrected to body surface area (BSA, m^2) = 0.007184×height (cm)$^{0.725}$×weight (kg)$^{0.425}$
†Already expressed per body surface are (BSA)

Statistical analysis

Data were analyzed by using IBM Statistical Package for Social Science (SPSS) Statistics for Windows, Version 21 (IBM Corp, Armonk, NY) and MedCalc for Windows, Version 17.9.7 (MedCalc Software, Ostend, Belgium). Categorical variables were presented as frequency, n and percentages (%) while continuous variables were presented as mean±standard deviation (SD). The correlation and presence of any significant difference between mean serum cystatin C levels and cystatin C-based eGFR with mean serum creatinine levels and creatinine-based eGFR were investigated using Pearson's Correlation and Wilcoxon Signed-Rank test. The Bland-Altman plot was used to evaluate the agreement between eGFR values obtained by using the creatinine and cystatin C-based equations. Bias was defined as the systematic deviation of cystatin C-based eGFR from the creatinine-based eGFR. This systematic deviation was expressed as mean bias±95% confidence interval (CI) of the difference plots. The difference of eGFR values derived from the two equations on the y-axis were plotted against the means of the two measurements on the x-axis. The line of mean difference as well as 95% upper and lower limits of agreement (mean bias±1.96×SD) were plotted. The relationship between the differences and means of two measurements was assessed by using simple linear regression of the plots, of which slopes exhibiting±95% CI were recorded. *P*-value<0.05 were considered statistically significant in this study.

RESULTS

Baseline characteristics

A total of 118 patients were included in this study. The mean age was 61±15 years with 57.6% being female and majority were Malays (55.9%). The mean serum creatinine and cystatin C level were 2.21±1.63 mg/dL (0.61-9.08 mg/dL) and 2.00±1.06 mg/L (0.63-7.80 mg/L), respectively. Other baseline characteristics are summarised in Table 2.

Correlation of creatinine- and cystatin C-based eGFR values

The correlation between serum cystatin C and creatinine level was statistically significant (r=0.78, *P*<0.05) (Figure 1A). Meanwhile, a similar trend of inverse relationship was observed between serum creatinine and creatinine-based eGFR as well as serum cystatin C and cystatin C-based eGFR. The Pearson's Correlation analysis also shows a statistically significant relationship

Table 2: Patient demographic information and overall renal function data.

	n (%)
Gender Male Female	 50 (42.4) 68 (57.6)
Race Malay Chinese Indian Other	 66 (55.9) 44 (37.4) 7 (5.9) 1 (0.8)
	Mean±SD
Age (years)	61±15
Weight (kg)	70±15
Height (m)	1.61±0.08
BMI (kg/m^2)	27.21±7.79
Serum Creatinine (Cr) (mg/dL)	2.21±1.63
Serum Cystatin C (CysC) (mg/L)	2.00±1.06
eGFR_Cr-CG (ml/min/1.73m^2)*	48.44±34.66
eGFR_Cr-MDRD (ml/min/1.73m^2)†	41.98±28.36
eGFR_Cr-CKD-EPI (ml/min/1.73m^2)†	44.34±31.09
eGFR_CysC-Hoek (ml/min/1.73m^2)†	47.29±26.44
eGFR_CysC-Larsson (ml/min/1.73m^2)*	46.75±30.91
eGFR_CysC-Grubb (ml/min/1.73m^2)†	46.06±39.89
eGFR_CysC-Flodin (ml/min/1.73m^2)†	45.57±33.75
eGFR_CysC-CKD-EPI (ml/min/1.73m^2)†	43.52±29.81

Data are presented as number (%) or mean ± SD.
BMI, body mass index; eGFR, estimated glomerular filtration rate; CG, Cockcroft-Gault; MDRD, Modification of Diet in Renal Disease; Cr-CKD-EPI, Creatinine-based Chronic Kidney Disease Epidemiology Collaboration; CysC-CKD-EPI, Cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equations.
*Corrected to body surface area (BSA, m^2) = 0.007184×height (cm)$^{0.725}$×weight (kg)$^{0.425}$
†Already expressed as BSA

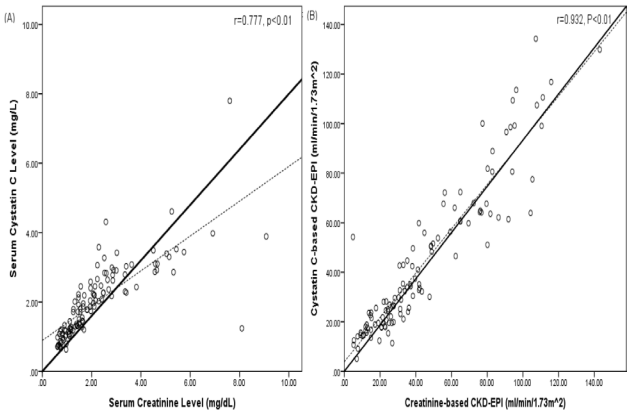


Figure 1: Comparison of serum cystatin C, cystatin C-based eGFR with serum creatinine and creatinine-based eGFR (A) Correlations between serum cystatin C and creatinine level. (B) Correlations of eGFR values derived from cystatin C-based CKD-EPI and creatinine-based CKD-EPI equation. The solid line is the line of identity. The dotted line is the trend line. eGFR, estimated Glomerular Filtration Rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Table 3: Correlation between creatinine- and cystatin C-based eGFR from various equations (n=118.)

		SCr	Cystatin C-based eGFR equations				
			Hoek	Larsson	Grubb	Flodin	CysC-CKD-EPI
SCysC		0.78*	-0.83*	-0.79*	-0.75*	-0.78*	-0.79*
Creatinine-based eGFR equations	CG	-0.66*	0.86*	0.81*	0.85*	0.86*	0.89*
	MDRD	-0.72*	0.90*	0.89*	0.89*	0.89*	0.92*
	Cr-CKD-EPI	-0.72*	0.92*	0.90*	0.90*	0.91*	0.93*

Data from Pearson's Correlation test. eGFR, estimated glomerular filtration rate; CG, Cockcroft-Gault; MDRD, Modification of Diet in Renal Disease; Cr-CKD-EPI, creatinine-based Chronic Kidney Disease Epidemiology Collaboration; CysC-CKD-EPI, cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equations.
*Significant at $p<0.05$

Table 4: Bias and imprecision of creatinine- and cystatin C-based eGFR equations.

Equations		Mean bias (95% CI) (ml/min/1.73m^2)	95% limits of agreement around the mean bias (ml/min/1.73m^2)	Slope of difference plot (95% CI)
Cystatin-C based	Creatinine-based			
Hoek	CG	-1.2 (-4.4, 2.1)	±35.1	-0.29 (-0.39, -0.19)†
	MDRD	5.3 (3.1, 7.5)*	±24.0	-0.07 (-0.16, 0.01)
	Cr-CKD-EPI	2.9 (0.7, 5.2)*	±24.5	-0.17 (-0.24, -0.09)†
Larsson	CG	-1.6 (-5.4, 2.1)	±40.1	-0.13 (-0.24, -0.01)†
	MDRD	4.9 (2.23, 7.52)*	±28.3	0.09 (0.00, 0.18)†
	Cr-CKD-EPI	2.5 (-0.1, 5.1)	±27.6	-0.01 (-0.09, 0.08)
Grubb	CG	-2.4 (-6.2, 1.5)	±41.5	0.15 (-0.05, 0.26)†
	MDRD	4.1 (0.5, 7.7)*	±38.8	0.36 (0.27, 0.45)†
	Cr-CKD-EPI	1.7 (-1.6, 5.0)	±35.4	0.26 (0.18, 0.34)†
Flodin	CG	-2.9 (-6.2, 0.5)	±36.1	-0.03 (-0.13, 0.07)
	MDRD	3.6 (0.8, 6.4)*	±29.8	0.18 (0.10, 0.27)†
	Cr-CKD-EPI	1.2 (-1.3, 3.8)	±27.5	0.09 (0.01, 0.17)†
CysC-CKD-EPI	CG	-4.9 (-7.8, -2.0)*	±31.1	-0.16 (-0.25, -0.07)†
	MDRD	1.5 (-0.6, 3.7)	±29.8	0.05 (-0.02, 0.13)
	Cr-CKD-EPI	-0.8 (-2.9, 1.2)	±22.1	-0.04 (-0.11, 0.03)

Data from Bland-Altman analysis. eGFR, estimated glomerular filtration rate; CG, Cockcroft-Gault; MDRD, Modification of Diet in Renal Disease; Cr-CKD-EPI, creatinine-based Chronic Kidney Disease Epidemiology Collaboration; CysC-CKD-EPI, cystatin C-based Chronic Kidney Disease Epidemiology Collaboration.
*Presence of statistically significant bias
†Presence of statistically significant proportional bias

between all creatinine- and cystatin C-based GFR estimates (Table 3). The strongest correlation was observed between cystatin C-based and creatinine-based CKD-EPI equations (r=0.93, $P<0.01$) (Figure 1B).

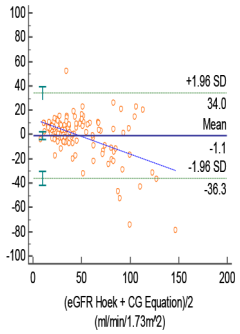
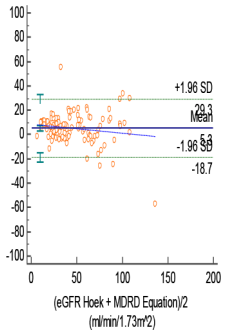
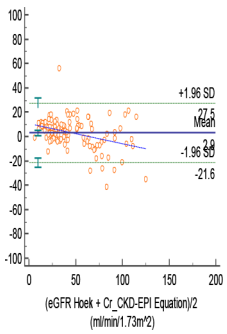
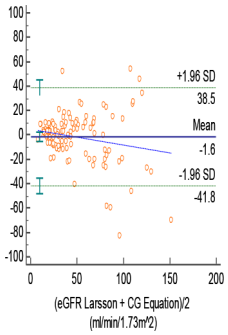
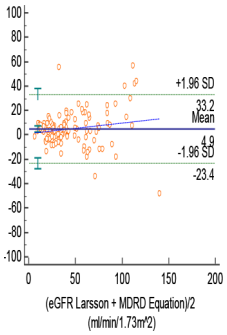
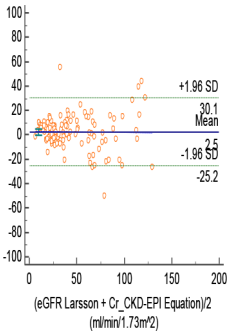
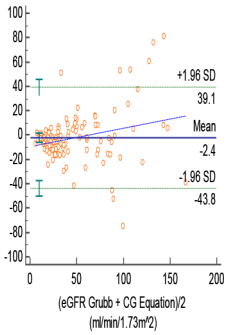
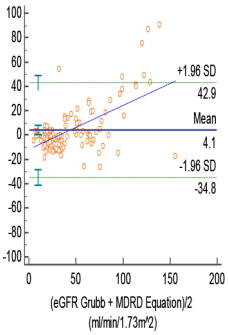
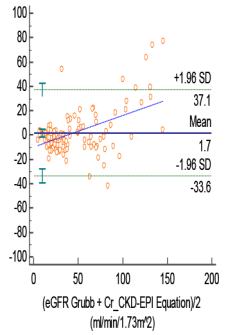
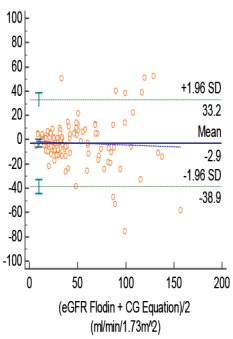
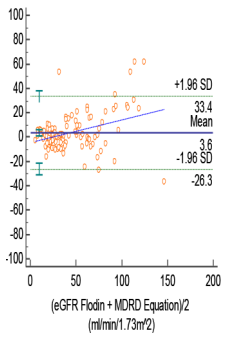
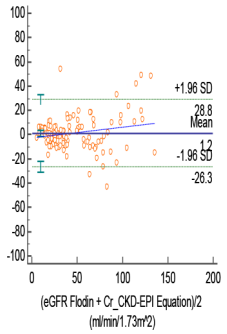
Presence of mean difference between creatinine- and cystatin C-based eGFR values

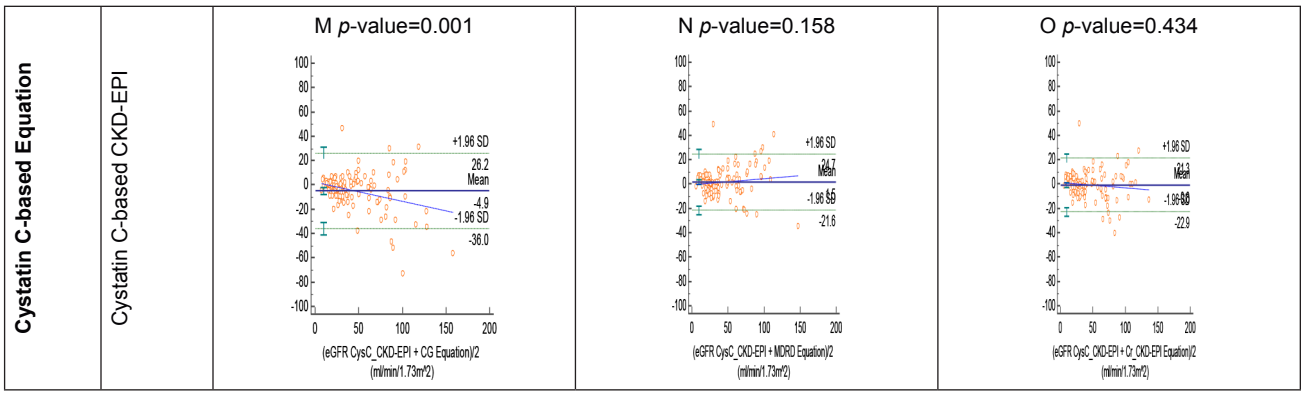
Wilcoxon Signed-Rank test demonstrated a presence of significant difference between seven pairs of eGFR values, calculated using cystatin C-based and creatinine-based eGFR equations (Grubb and CG, Z=-2.98, $P=0.003$; Flodin and CG, Z=-2.28, $P=0.023$; cystatin C-based CKD-EPI and CG, Z=-3.361, $P=0.01$; Hoek and MDRD, Z=-5.66, $P<0.01$; Larsson and MDRD, Z=-3.88, $P<0.01$; Hoek and creatinine-based CKD-EPI, Z=-3.52, $P<0.01$; and Larsson and creatinine-based CKD-EPI, Z=-2.46, $P=0.014$). Even though eGFR values calculated based on Hoek and creatinine-based CKD-EPI equations produced a significantly strong correlation (r=0.918, $P<0.01$), the Wilcoxon's Signed-Rank test shows the presence of a significant difference between the mean of eGFR values derived from these two equations ($P<0.01$).

Bias and imprecision between creatinine- and cystatin C-based eGFR values

The performances of the eight equations are presented in Table 4 and Figure 2 (A-O). The presence of bias between eGFR values obtained by using the following pairs of equations were not statistically significant. The paired cystatin C- and creatinine-based equations were Hoek and CG ($P=0.490$), Larsson and CG ($P=0.390$), Larsson and Cr-CKD-EPI ($P=0.059$), Grubb and CG ($P=0.226$), Grubb and Cr-CKD-EPI ($P=0.301$), Flodin and CG ($P=0.094$), Flodin and Cr-CKD-EPI ($P=0.343$), CysC-CKD-EPI and MDRD ($P=0.158$) and CysC-CKD-EPI and Cr-CKD-EPI ($P=0.434$) equations. In contrast, the rest of the paired measurements used in this study were found to have the presence of a statistically significant bias.

Figure 2: (A-O) Bland-Altman plots between each cystatin C-based and creatinine-based eGFR equations. The x-axis is the mean of creatinine- and cystatin C- based eGFR values. The y-axis is the difference between the two eGFR values. The solid line indicates the mean difference. The upper and lower horizontal dotted lines indicate the line for limits of agreement (mean ± 1.96SD). The dashed line indicates the regression line of differences.

		Creatinine-based Equation		
Cystatin C-based Equation		**CG Equation**	**MDRD Equation**	**Creatinine-based CKD-EPI Equation**
	Hoek Equation	A *p*-value=0.490 	B *p*-value<0.01 	C *p*-value=0.012 
	Larsson Equation	D *p*-value=0.390 	E *p*-value<0.001 	F *p*-value=0.059 
		Creatinine-based Equation		
Cystatin C-based Equation		CG Equation	MDRD Equation	Creatinine-based CKD-EPI Equation
	Grubb Equation	G *p*-value=0.226 	H *p*-value=0.027 	I *p*-value=0.301 
	Flodin Equation	J *p*-value=0.094 	K *p*-value=0.012 	L *p*-value=0.343 



eGFR, estimated glomerular filtration rate; CG, Cockcroft-Gault; MDRD, Modification of Diet in Renal Disease; Cr_CKD-EPI, Creatinine-based Chronic Kidney Disease Epidemiology Collaboration; CysC_CKD-EPI, Cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equations; SD, standard deviation.

DISCUSSION

Cystatin C is an ideal biofiltration marker due to its low molecular weight and constant production rate by all nucleated cells.[28] In a study involving handling of human cystatin C in rats, the clearance of cystatin C from the kidney is equivalent to 94% clearance of ^{51}-CR EDTA.[29] Meanwhile, Northern blot and Immunohistochemical studies conducted shows that 99% of the filtered cystatin C undergoes degradation in proximal tubule.[30] This indicates that cystatin C is freely filtered in the glomerulus and were then reabsorbed and catabolized at the tubular cells.[28,31] Besides, unlike creatinine, cystatin C is not influenced by age, height, sex or muscle mass.[32] These special features of cystatin C suggested that utilizing it as an endogenous biomarker will results in a more accurate renal function measurement.

Due to the significance of cystatin C as a potentially ideal biomarker, we evaluated the association between serum cystatin C- and serum creatinine-based equations to estimate GFR. In this study, we successfully demonstrated a strong correlation between all cystatin C- and creatinine-based eGFR equations among Malaysian patients with chronic kidney disease. Despite the good correlation shown, the mean eGFR values derived from some cystatin C- and creatinine-based eGFR shows a statistically significant difference. Therefore, further analysis of Bland-Altman plot was conducted to investigate which cystatin C-based eGFR demonstrated the least mean difference from creatinine-based eGFR equations.

All eGFR values obtained by using cystatin C-based equations shows a statistically significant positive correlation with creatinine-based equations, indicating the presence of general comparability between them. This result is consistent with findings from previously published study conducted by Lee *et al* involving 615 CKD patients.[33] Six cystatin C-based eGFR equations (Larsson, Hoek, Le Bricon, Filler, Orebro cystatin C (DAKO) and Orebro cystatin C (Gentian)) and Korean population based MDRD eGFR equation were compared.[33] In this current study, the strongest correlation found was between the creatinine-based and cystatin C-based CKD-EPI equation. However, high correlation coefficients do not automatically suggest these equations as the most accurate version of cystatin C- and creatinine-based formula for GFR estimation. Instead, correlation coefficient values should only be interpreted as presence of an association or relationship between the two variables.[34] Two sets of different methods of measurements for the same parameter will be expected to have a good correlation.[34] From this viewpoint, significant correlation will be achieved even if the two methods of measurements do not well agree.[34] Hence, the application of correlation coefficient alone is not always appropriate with respect to this matter.

Even though a strong correlation was previously demonstrated, the differences in the mean eGFR values derived from some creatinine and cystatin C-based eGFR equations were statistically significant. For example, the correlation of eGFR values obtained by using Hoek and CKD-EPI equations. The regression analysis of Hoek and CKD-EPI equation indicate presence of good agreement with a statistically significant correlation coefficient, $r=0.92$, $p<0.01$. However, analysis based on Wilcoxon Signed rank test shows the presence of a statistically significant mean difference in the values of eGFR derived from these equations. Thus, the unit of deviation between the two methods of measurement needs to be quantitatively measured. Analysis based on Bland-Altman plot were conducted to further investigate the agreement between the cystatin C- and creatinine-based eGFR equations.[34]

Comparing a new clinical measurement method with the established one is essential to determine whether the values calculated are in congruence with each other for

such replacement to take place.[34] In the Bland-Altman analysis, presence of non-significant bias was identified in nine pairs of cystatin C- and creatinine-based eGFR. The smallest mean difference found based on these pairs of eGFR equations is between cystatin C-based and creatinine-based CKD-EPI formula. Good agreement shown suggests that the cystatin C-based equations is comparable to creatinine-based equations. Further analysis on the forms of systematic difference existed was conducted by using simple linear regression slopes of the difference plots. Most pairs of equation have a positive bias at a lower eGFR values and negative bias at a higher eGFR values. As such, the creatinine-based eGFR values were higher amongst patient with high renal function and vice versa compared to cystatin C-based eGFR values. Previously published literature incorporating analyses of these selected creatinine and cystatin C-based eGFR equations were limited. Performance of cystatin C-based equations (Larsson, Hoek, Le Bricon, Filler and Orebro) has been compared with MDRD in Korean populations by using Bland-Altman plot whereby, the smallest mean difference from baseline was found in Filler equation (0.39 ml/min/1.73m^2).[33] Meanwhile, Xirouchakis *et al.* compared ^{51}Cr-EDTA GFR with MDRD, Larsson and Hoek equations.[35] Since comparison was made based on the reference GFR, the conclusion made based on the study is that Hoek and MDRD is the most accurate and precise equation, respectively.[35] In another recent study conducted among Malaysian elderly patients, cystatin C-based CKD-EPI were found to have a greater degree of biasness, lower imprecision and lesser accuracy compared to creatinine-based CKD-EPI and MDRD equation.[36] Different standardization of the cystatin C value used in the original CKD-EPI equation, small sample size and imbalanced number of patients in each CKD stage could possibly results in this unexpected finding. On the contrary, a study conducted by Marwyne *et al.* (2011) among obese patients found cystatin C-based eGFR to be superior than creatinine-based eGFR in terms of accuracy, sensitivity and specificity.[37] The association of serum creatinine with weight and muscle mass could be one of the contributing factors that led to poor performance of creatinine-based eGFR when compared with cystatin C-based eGFR.

The present study provides evidence that the cystatin C-based equations are comparable to the creatinine-based equations for estimating GFR. Prediction equations based on serum cystatin C level only, without any or even with less covariates is able to give a good estimate of GFR.[16] Some studies also revealed a higher accuracy and precision of eGFR values obtained by using cystatin C-based equations than the CG and simplified MDRD equation.[16,18] Cystatin C-based equations also demonstrated a better performance compared to creatinine-based equations in estimating GFR for certain group of people such as patients with liver cirrhosis. Since this group of patients have poor prognosis of renal impairment, employing cystatin C-based approach such as Hoek and Larsson formula could result in significant improvement of the GFR estimation.[38] Besides, eGFR equations based on cystatin C may also be appropriate for drug dosing. This is supported by several studies conducted in the recent years to evaluate the usefulness of cystatin C as a biomarker for dose prediction of renally excreted drugs such as vancomycin, gentamicin and amikacin. It was found that cystatin C demonstrated a better correlation with clearance or trough level of selected drugs being evaluated when compared with creatinine.[39–41]

An important limitation of this study was the absence of a gold standard reference GFR (rGFR) for comparison, such as inulin, iohexol or radioactive isotopes (i.e. ^{51}Cr-EDTA, ^{99m}-TC-DTPA, ^{25}I-Iothalamate). This limits the ability of this study to determine which equation has the best predictive performance in terms of estimating the measured GFR. Clearance measurement of these exogeneous substances were relatively expensive, invasive and labour intensive.[42] This is the same case in real clinical practice. Even though utilising the gold standard GFR measurement is very ideal and preferable, applying it in a big study population would be very costly and could results in a lot of complexities.[43,44]

Our future work will involve investigating factors affecting serum cystatin C level and cystatin C-based eGFR equations. Therefore, identification of the specific cystatin C-based equations which complement creatinine-based equations is required. Based on the positive correlation and good agreement shown between creatinine-based and cystatin C-based CKD-EPI equations, other factors such as genetic influence, drug and clinical factors that might affect cystatin C-based eGFR measurement could be investigated in our population. A better understanding on factors affecting serum cystatin C levels independent of GFR is highly crucial for better utilisation of cystatin C-based equations in clinical practice.[21]

CONCLUSION

Cystatin C-based equations are comparable with the creatinine-based equations for eGFR measurement among multi-racial Malaysian patients with CKD. Cystatin C was able to give a good estimate of GFR even with lesser covariates compared to creatinine-based

equations. There was a good agreement shown between cystatin C- and creatinine- based equations to estimate GFR. eGFR values derived from cystatin C-based CKD-EPI equation shows a strong correlation and the least mean difference from baseline when compared with creatinine-based CKD-EPI equation.

ETHICAL APPROVAL

All procedures conducted in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was reviewed and approved by the Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2017-133).

INFORMED CONSENT

Written informed consent was obtained from all research participants included in the study.

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CONFLICT OF INTEREST

All authors declare that they did not have any conflict of interest.

ABBREVIATIONS

GFR: Glomerular Filtration Rate; **eGFR:** Estimated Glomerular Filtration Rate; **BW:** Body Weight; **BMI:** Body Mass Index; **Scr:** Serum Creatinine; **ScysC:** Serum Cystatin C; **G:** Gender; **E:** Ethnicity; **BSA:** Body Surface Area; **CG:** Cockcroft-Gault; **MDRD:** Modification of Diet in Renal Disease; **CKD:** Chronic Kidney Disease; **CKD-EPI:** Chronic Kidney Disease-Epidemiology Collaboration Equation.

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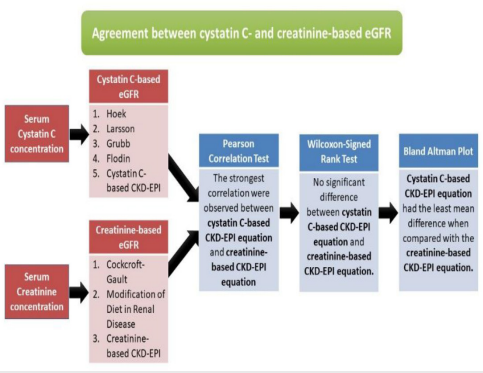
PICTORIAL ABSTRACT



Summary

- All cystatin C-based eGFR equations shows a statistically significant correlation with creatinine-based eGFR equations amongst Malaysian patients with CKD.
- eGFR values derived from cystatin C-based CKD-EPI equation shows the highest correlation and the least mean difference from baseline with creatinine-based CKD-EPI equation.
- Cystatin C-based eGFR equation will be a good alternative to creatinine-based eGFR equations in drug dose adjustment of renally excreted drugs.

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