RESEARCH PAPER

Cystatin C-based Glomerular Filtration Rate Estimating Equations in Early Chronic Kidney Disease

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Objective: To Compare performance of combined creatinine and cystatin C-based equation with equations based on either cystatin C or creatinine alone, in early chronic kidney disease.

Design: Diagnostic accuracy study.

Setting: Tertiary-care hospital.

Patients: One hundred children with chronic kidney disease who underwent 99mTc diethylenetriamine pentaacetic acid (DTPA) glomerular filtration rate measurement.

Methods: Estimating equations for glomerular filtration rate (GFR) based on serum cystatin C alone and in combination with serum creatinine were generated using regression analyses. These equations and the creatinine-based equation [0.42 x height/creatinine] were validated in 42 children with glomerular filteration rate between 60 and 90 mL/min/1.73 m^2. Bias, precision and accuracy of estimating equations using DTPA glomerular filteration rate as gold standard.

Results: Cystatin C-based equation (GFR=96.9 - 30.4 x cystatin) overestimated while the combined cystatin C-and creatinine-based equation [GFR=11.45 x (height/creatinine) 0.356 x (1/cystatin) 0.188] underestimated the measured GFR. Cystatin C-based equation had less bias (1.9 *vs.* 12.4 ml/min/1.73 m^2), and higher precision (13.1 *vs.* 25.6 mL/min/1.73 m^2) and accuracy (92.1% *vs.* 75.7%) than creatinine-based equation. The combined cystatin C and creatinine equation had bias (-1.4 mL/min/1.73 m^2) precision (15.2 mL/min/1.73 m^2) and accuracy (91.2%) similar to cystatin C-based equation.

Conclusions: Cystatin C-based equation has a better performance in estimating glomerular filtration rate than creatinine-based equation in children with early chronic kidney disease. Addition of creatinine equation does not improve the performance of the cystatin C-based equation.

Keywords: *Chronic renal insufficiency; Creatinine; Kidney function test; Tc-DTPA.*

An accurate and reliable method for assessment of renal function is essential for children with kidney diseases. Serum creatinine based equations for estimating glomercular filtration rate (GRF) are useful for diagnosis of chronic kidney disease (CKD) stage III or beyond (GFR <60 mL/min/1.73m^2) [1]. Cystatin C is an endogenous marker of renal function; its whole blood levels are not affected by age, sex and nutrition [2]. Serum cystatin C is considered to be a more sensitive marker than creatinine in patients with moderate decrease in GFR, 'the creatinine blind area' of initial impairment [3]. Of several cystatin-based GFR equations in children over a wide range of GFR, some are superior to creatinine-based equation in terms of accuracy and precision at lower renal function [4,5], while others are similar [6,7]. It is unclear whether cystatin C-based equation alone or in combination with creatinine, is superior to the latter in early renal impairment. Since inclusion of markers of renal function is likely to improve the GFR estimating equations, we hypothesized that addition of creatinine to cystatin C would improve the performance of the cystatin C-based GFR estimating equation in early chronic kidney disease. We generated GFR estimating equations, based on cystatin alone and with creatinine, in children with CKD, and validated these in children with CKD stage II (GFR between 60-90 mL/min/1.73 m^2).

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METHODS

Following informed parental consent, we screened consecutive children between 2-18 years of age with CKD undergoing GFR estimation using ^{99m}Tc-diethylenetriamine pentaacetic acid (DTPA) scan at All India Institute of Medical Sciences, New Delhi, India. Those receiving cotrimoxazole, corticosteroids or cephalosporins in the previous week, having jaundice or severe edema, or those undergoing dialysis, were excluded. Initial 100 consecutive children formed the index dataset for developing the GFR estimating

equations. Subsequent 42 children who had ^{99m}Tc-DTPA GFR between 60-90 mL/min/1.73 m^2 formed the validation dataset for evaluating the equations. The study was approved by the Institute Ethics Committee.

Weight and height were measured by standard techniques and the body surface area (BSA) was derived using Du Bois formula [8]. Radionuclide GFR estimation (dGFR) was performed by two-plasma-sample method with blood collected at 60 and 180 minutes after intravenous administration of 1mCi ^{99m}Tc-DTPA and normalized to body surface area [9]. Blood (2 mL) was drawn, sera separated and stored at –70°C. Cystatin C concentration was measured by particle enhanced immunoturbidimetry using the Cystatin PET kit (DAKO, Hamburg, Germany) within 3 months of collection [10]. Serum creatinine was measured on the same day as estimation of GFR by kinetic Jaffe method [11].

Ordinary least square regression was used to determine the coefficients of the GFR estimating equations. Logarithmic transformation of the continuous variables was done if it improved R^2 of the regression model. The general regression model was given as:

$$\log \text{dGFR (mL/min per 1.73 m}^2) = \log (k) + m\log (X) + nY$$

where k is constant, X represents continuous predictor variables and Y is the categorical variable examined. The eGFR ($eGFR = k\,[X]^m \exp\,[n]^Y$) was obtained using the expected values of the regression coefficients (k, m and n) along with specific values of the independent variables (X,Y) for each individual. We verified whether the data used to generate regression models met the assumption for ordinary least square regression using tests for homoscedasticity of residuals, linearity, collinearity between predictor variables and errors in model specification. The newly generated equations were compared for bias, precision and accuracy on a validation dataset. Bias was defined as mean or median of the differences between estimated and measured GFR. Precision was assessed by the standard deviation for the differences between estimated GFR and measured GFR. Accuracy, which is affected by both bias and imprecision, was expressed as the percentage of estimated GFR values within 10% and 30% of measured GFR. Bias and 95% limits of agreement were plotted using Bland and Altman analysis for comparing the prediction equations [12]. Measured (dGFR) was plotted on the *x*-axis instead of average of estimated and measured GFR because dGFR was the gold standard representing the 'true' GFR. We used bias on the raw scale because it is easier to interpret, although measures on the raw scale tend to emphazise errors at higher GFRs. The data for generation of the GFR equations were log transformed but exponentiated back to yield the final equations; hence we did not use bias on percentage or log scale. The STATA statistical software package (intercooled STATA 11, STATA Corporation, College Station, TX, USA) was used for analyses.

RESULTS

The chief causes of CKD were genitourinary tract anomalies in 83.1% and glomerular diseases in 10.2%. Twenty-two patients were underweight (weight-for-age <-2.0 SD) and 23 were stunted (height–for-age 2.0 SD) using WHO growth charts [13] (***Table* I**). The median serum creatinine was 0.8 mg/dl and dGFR ranged from 33-97.6 (median 74) mL/min/1.73 m^2. Inter- and intra-assay coefficients of variation for cystatin C and creatinine were less than 3% and 2%, respectively.

A univariate regression analysis of body size parameters, serum creatinine and cystatin with body surface area (BSA) adjusted dGFR was performed. Serum cystatin C (R^2=39%) explained greater proportion of variability in the dGFR as compared to 1/serum creatinine (R^2= 17.7%). Variables other than height and creatinine, including age, gender, weight, and body mass index did not have any additional predictive power to explain the variability in dGFR. The addition of height/serum creatinine to cystatin C-based model improved the R^2 from 39% to 58.9%, with decrease in root mean square error. Data used for generating the regression models were verified for the assumptions of ordinary least square regression. There was no departure from linearity and the variance of the residuals was homogenous.

The new cystatin C-based equation [GFR = 96.9 - 30.4 × cystatin] and the combined cystatin C-and creatinine-based equation [$GFR = 11.45 \times (\text{height/creatinine})^{0.356} \times (1/\text{cystatin})^{0.188}$] and the previously reported 'bedside' creatinine equation [0.42 *x* height/serum creatinine] [14] were compared on a validation dataset comprising of 42 new subjects who had dGFR between 60-90 mL/min/1.73 m^2. The baseline characteristics of the validation dataset were similar to the index dataset (***Table* I**). The new coefficient for height/creatinine equation in the index dataset in this study was 0.427. This was not very different from the previously published equation which was developed in a much larger cohort of patients whose baseline characteristics were similar to the patients in the present study. Therefore we chose to use the previously developed height/creatinine equation for comparison. We also compared previously published cystatin C-based equations with the original coefficients. Of the eight cystatin C-based equations previously published, four had cystatin levels estimated by turbidimetric method [4-

TABLE I BASELINE CHARACTERISTICS OF THE INDEX AND VALIDATION DATASET

Characteristics	*Index (n=100)*	*Validation (n=42)*
Boys *n* (%)	83 (83)	34 (81)
Age (y)	8 (4.1 ,12)	9 (5, 12)
Height cm	119 (100, 136)	123 (102, 148)
Height SD score	-0.69 (-1.9 ,0.29)	-0.72 (-2.7, 0.71)
Weight kg	22 (15, 30)	20 (15 ,41)
Weight SD score	-1.2 (-1.8, 0.03)	-1.1 (-2.1, 0.43)
Body surface area, m^2	0.86 (0.62, 1.1)	0.84 (0.65, 1.3)
Body mass index, kg/m^2	15.2 (13.6 ,16.8)	14.7 (13.8, 16.3)
Serum creatinine,mg/dL	0.8 (0.6, 0.9)	0.7 (0.6, 0.9)
Serum cystatin C (mg/L)	0.8 (0.65, 0.95)	0.7 (0.45, 0.85)
#GFR, mL/min/1.73 m^2	74 (33, 97.6)	79 (72, 84)

SD:, standard deviation; GFR: glomerular filtration rate; Values are median (interquartile range); # by ^{99m}Tc- DTPA.

6,15] and the remaining used nephelometry [7,16-18]. Equations by Bokenkamp, *et al.*[4], Filler, *et al.*[5] and Grubb, *et al.* [6] with original coefficients yielded very high bias, poor precision and accuracy when tested in the validation dataset.

Cystatin C-based equation overestimated while the combined cystatin C-and creatinine-based equation underestimated the measured GFR. Cystatin C-based equation [GFR=96.9 -30.4 ×x cystatin C] had significantly less median bias (1.9 *vs* -12.4 mL/min/1.73 m^2) (Signrank test, *P*=0.05), higher precision (13.1 *vs* 25.6 mL/min/1.73 m^2) and accuracy (92.1% *vs.* 75.7%) as compared to creatinine-based equation. The combined cystatin C-and creatinine-based equation [GFR $11.45 \times (\text{height/creatinine})^{0.356} \times (1/\text{ cystatin C})^{0.188}$] had similar bias (-1.4 mL/min/1.73 m^2), precision (15.2 ml/min/1.73 m^2) and accuracy (91.2%) as cystatin C-based equation (***Table* II**). ***Fig.* 1** shows Bland and Altman analyses of comparison of GFR values derived from prediction equations with the measured GFR.

DISCUSSION

We observed that cystatin C-based equation was significantly better than creatinine-based equation in terms of bias, precision and accuracy in children with CKD with GFR between 60-90 mL/min/1.73 m^2. Adding creatinine to cystatin C-based equation did not improve the bias precision and accuracy of the cystatin C equation. Previously published cystatin C-based equations with the original coefficients performed poorly when tested on the validation dataset, thus justifying generation of new cystatin C-based equations. This is likely due to differences in the methods of GFR and creatinine estimations, and differences in the levels of GFR of the study subjects [15].

The cystatin C-based equations derived in this study have several limitations. The equations were derived in children who had renal dysfunction and may not perform well in non-CKD population. The precision of both cystatin C and the combined cystatin C- and creatinine-based equation was poor. The serum creatinine measurement in our study was performed by kinetic Jaffe method which was not traceable to the reference method that measures creatinine by isotope dilution gas chromatography/mass spectrometry, thus limiting its use in laboratories performing enzymatic creatinine estimations.

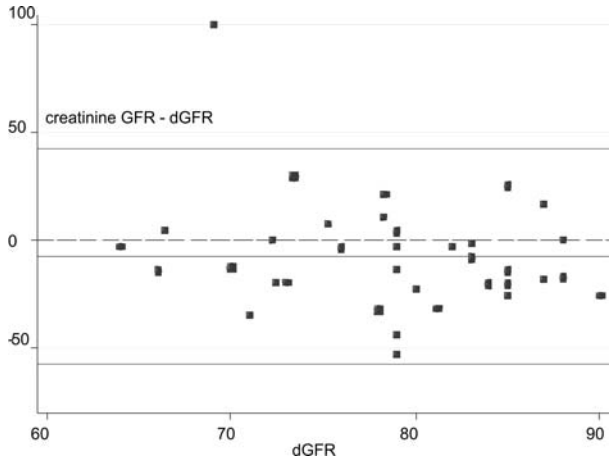
The accuracy of creatinine-based equation for predicting reference GFR within 30% has ranged from 25-79% [5,7,15,16] as compared to 78-87% [5,16] of cystatin C-based equations. The combined cystatin C-and creatinine-based equation had higher accuracy (82-97%) [7,15,16,18]. In almost all studies, including ours, cystatin C-based equation had better accuracy than creatinine-based equations. When using cystatin C-based equation, it is important to recognize that the estimates of cystatin C vary depending on whether turbidimetric or nephelometric method is used for the assay [19].

Although equations combining cystatin C and creatinine are generally superior to those based upon

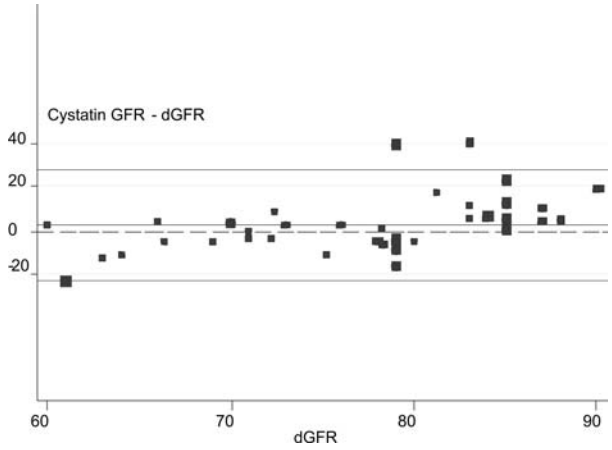
TABLE II COMPARISON OF THE PERFORMANCE OF PREDICTION EQUATIONS ON THE VALIDATION DATASET

eGFR equation	*Median bias (95% CI)#*	*Precision*	*% of eGFR within 10% of dGFR*	*% of eGFR within 30% of dGFR*
0.42 × height/creatinine	-12.4 (-19.9, 1.0)	25.6	29.7	75.7
96.9 -30.4 × cystatin C	1.92 (-5.1 , 6.9)*	13.1	60.5	92.1
$11.45 \times (\text{height/creatinine})^{0.356} \times (1/\text{ cystatin C})^{0.188}$	-3.93 (-10.4, 7.1)	15.21	44.1	91.2

#median of (eGFR- dGFR) values; eGFR: estimated glomerular filtration rate; dGFR: ^{99m}Tc- DTPA GFR; eGFR, bias, in mL/min/1.73 m^2, cystatin C in mg/L, creatinine in mg/dL, height in cm.



(a)



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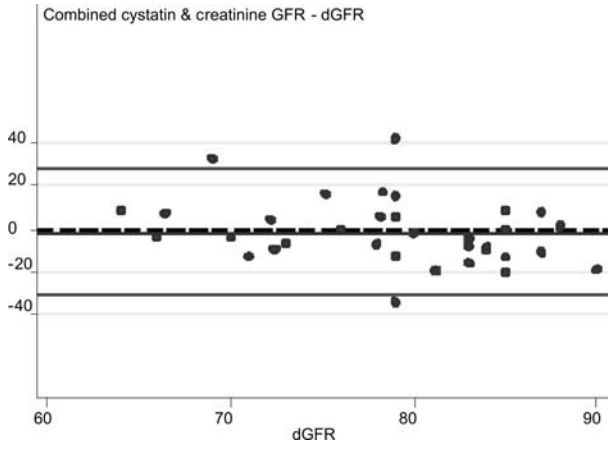

(c)

FIG. 1 *Bland and Altman analysis showing comparison of ^{99m}Tc-DTPA GFR (dGFR) with creatinine GFR (a), cystatin C GFR (b) and combined cystatin C and creatinine GFR (c) in the validation dataset.*

either creatinine or cystatin C alone, this may not be the case in specific clinical context. It has been suggested that in situations where creatinine or cystatin C are known to be invalidated as markers of GFR, only cystatin C or creatinine-based GFR estimate should be used [20]. We also found that combined cystatin C-and creatinine-based equation was not better than cystatin C-based equation in children with early decline in GFR. More studies examining the performance of cystatin C-based equation in healthy children and its predictive value in detecting early CKD are needed. We suggest that in early CKD clinicians should understand the limitations of creatinine-based GFR equation, and preferably use cystatin C-based GFR equations.

We conclude that cystatin C-based equation has better performance than creatinine-based equation in estimating GFR in children with early CKD.

Contributors: PH: conceptualized and designed the study, analyzed and interpreted the data; drafted the article; LR and RG: performed and interpreted the biochemical analyses, and revised the manuscript for important intellectual content; RK: conducted the laboratory test and interpreted them, and revised the manuscript for important intellectual content; AB: designed the study and revised the manuscript for important intellectual content. He will also act as guarantor of the study. The final manuscript was approved by all authors.

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WHAT IS ALREADY KNOWN?

- Cystatin-based GFR equation is superior to creatinine-based equation for estimating GFR in children with advanced chronic kidney disease (CKD).

WHAT THIS STUDY ADDS?

- Cystatin C-based equation is significantly better than creatinine-based equation in terms of bias, precision and accuracy in estimating GFR in children with early CKD.
- Addition of creatinine to the cystatin C-based equation does not improve its performance in early CKD.

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