

Evaluation of the New Race-Free 2021 CKD-EPI Equations Incorporating Creatinine and Cystatin C as a Marker of Glomerular Filtration Rate in Renal Transplant Recipients

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ABSTRACT

Objective: To evaluate bias and accuracy of race-free 2021 CKD-EPI equations in our adult population of renal transplant recipients.

Study Design: Cross-sectional study.

Place and Duration of Study: Chemical pathology and Endocrinology Departments of Armed Forces Institute of Pathology in collaboration with Armed Forces Institute of Urology, Rawalpindi, Pakistan, from Dec 2021 to May 2022.

Methodology: Fifty post-transplant adult patients with stable renal functions were included. Estimated GFR (eGFR) was calculated by the new CKD-EPI equations (2021), incorporating creatinine alone and creatinine combined with cystatin C, classical (2009) creatinine equation, cystatin C (2012) and creatinine combined with cystatin C (2012) equations with help of eGFR calculator app by National Kidney Foundation. GFR was measured by creatinine clearance. Performance of new CKD-EPI equations was evaluated in comparison with old CKD-EPI equations taking measured GFR (mGFR) by creatinine clearance as gold standard.

Results: The 2021 race free creatinine equation showed the largest mean bias (17.6 ml/min/1.73m²) followed by 2009 creatinine equation (11.06 17.6 ml/min/1.73m²). CKD-EPI 2012 equation incorporating Cystatin C alone performed the best with the least mean bias of 4.3ml/min/1.73m². Similarly, 2012 equation incorporating both creatinine and cystatin C showed better results (mean bias = 7.3 ml/min/1.73m²) than new equation incorporating both cystatin C and creatinine (mean bias = 9.78 ml/min/1.73m²).

Conclusion: The new race free 2021CKD-EPI creatinine equation showed highest bias, least precision and weak positive correlation with mGFR as compared to previously used eGFR equations in our cohort of renal transplant recipients.

Keywords: CKD-EPI 2021equations, Creatinine, Cystatin C, eGFR, Race-Free.

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INTRODUCTION

Chronic kidney disease (CKD) affects more than 10% of general population worldwide, with a 21.2% prevalence in Pakistan.^{1,2} The recommended treatment for stage-5 kidney disease is renal transplantation, as it has reduced morbidity and mortality as compared to patients undergoing dialysis.²

Precise measurement of glomerular filtration rate (GFR) is crucial for care of renal transplant recipients, which is either done by direct measurement of GFR or estimation by different equations.³ Measured glomerular filtration rate (mGFR) is the gold standard, but due to its invasiveness, cost and lengthy procedure, it cannot be done routinely.⁴ About 70 different equations have been evolved so far and some

are specifically developed for renal transplant recipients.⁵ Among these equations Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations are routinely used because of their high accuracy.⁶

Serum creatinine concentration depends on several factors like age, race, muscle mass, gender and catabolic state. Therefore 2009 CKD-EPI equation included race as one of the variables.⁷ Different studies have demonstrated that equations incorporating serum cystatin C calculate GFR more accurately and precisely in kidney transplant recipients than conventional creatinine-based equations.⁸ It has also been suggested that CKD-EPI equation using cystatin C combined with creatinine to be utilised more for assessing GFR.⁹

This study was directed to assess the bias and accuracy of race free 2021 CKD-EPI equations in

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comparison with old CKD-EPI 2009 and 2012 equations in our adult renal transplant recipients.

METHODOLOGY

This cross-sectional study was conducted at the Chemical Pathology and Endocrinology Departments of Armed Forces Institute of Pathology, in collaboration with Armed Forces Institute of Urology Rawalpindi, Pakistan, from December 2021 to May 2022. Permission was sought from the Institutional Ethical Review Board (IRB no: cons-CHP-5/READ-IRB/21/1029) prior to commencement.

Inclusion Criteria: Post-transplant adult patients of either gender, who had stable renal functions, at least one or more years had passed since the renal transplant was done and were not currently suffering from any infection or urological disorder were included.

Exclusion Criteria: Patients who had change in serum creatinine concentration of more than 10% within the preceding 4 weeks, had hypoproteinemia or proteinuria, were on diuretics, who had history of cardiovascular diseases, chronic liver diseases, thyroid disorders, myopathies or any malignancy, patients on steroids and women who were pregnant or breast feeding and patients who had inadequate 24 hours urine collection were excluded.

We included all patients who met our inclusion criteria, who presented to our departments during the study period.

Five ml of blood was taken in clot activator gel tube for analysis of serum cystatin C and creatinine, and 24 hours urinary sample was taken for measuring creatinine clearance. Procedure for collection of 24 hours urine was explained to the patients and written instructions were provided to avoid errors in 24 hours urine collection. Serum cystatin C was analyzed on Advia 1800 by latex enhanced immunoturbidimetric assay. Analytical sensitivity of this assay was 0.25 mg/L with <10%CV. Serum creatinine was also analyzed on Advia 1800 by kinetic photometric assay. eGFR was calculated using the new CKD-EPI equations (2021) incorporating creatinine alone and creatinine combined with cystatin C and old equations incorporating creatinine (2009), cystatin C, 10 (2012) and creatinine combined with cystatin C (2012) with help of eGFR calculator app by National Kidney Foundation.

Data analysis was carried out with Statistical Package for Social Sciences (SPSS) version 25 and

Microsoft Excel 2010. Shapiro Wilk test was used to assess the distribution of data for continuous variables. Variables showing parametric distribution were expressed as Mean $\pm$ SD, whereas those showing non parametric distribution were expressed as median (IQR). Qualitative variables were expressed as frequencies and percentages. Spearman coefficient was used to assess the correlation between measured GFR and estimated GFR with different equations. Wilcoxin Signed Rank test was used to see the difference between pairs of measured and estimated GFR values. Bland Altman plot was used to assess the accuracy and precision of eGFR equations. A p -value ≤ 0.05 was taken as significant.

RESULTS

A total of 50 post-transplant patients with median age 44.5(28.7) years were inducted in the study, of whom 35(70%) were males and 15(30%) were females. Nineteen patients (38%) were using cyclosporine and 31(62%) were using tacrolimus as immunosuppressant. Shapiro Wilk test showed parametric distribution for height, BMI, serum creatinine and creatinine clearance (p -value>0.05) whereas non-parametric distribution for age, weight, serum cystatin C, urea and eGFR values by the five equations (p -value <0.05).

Table-I: Baseline Characteristics of Study Participants (n=50)

Variables	Values
Age (years), Median (IQR)	44.5(28.7)
Weight (kgs), Median (IQR)	69.0(12.7)
Height (cm), Mean $\pm$ SD	168.9 $\pm$ 6.3
BMI (kg/cm ²), Mean $\pm$ SD	24.4 $\pm$ 3.6
Time post- transplant (years), Median (IQR)	3.0(2.2)
Serum Cystatin C, Median (IQR)	1.3(0.6)
Serum Creatinine (μ mol/L), Mean $\pm$ SD	113.6 $\pm$ 25.7
Serum Urea (mmol/L), Median (IQR)	5.70(2.85)
Creatinine Clearance (ml/mim/1.73m ²), Mean $\pm$ SD	50.9 $\pm$ 16.5
eGFR-creatinine-2021 (ml/mim/1.73m ²), Median (IQR)	66.50(35.5)
eGFR-cystatin C 2012 (ml/mim/1.73m ²), Median (IQR)	53.5(39.7)
eGFR-creatinine- cystatin C -2021 (ml/mim/1.73m ²), Median (IQR)	60.5(32.0)
eGFR-creatinine-2009 (ml/mim/1.73m ²), Median (IQR)	58.0(32.5)
eGFR-creatinine- cystatin C -2012 (ml/mim/1.73m ²), Median (IQR)	57.0(30.2)
Cause of CKD (Renal Transplantation)	n(%)
Diabetes Mellitus	19(38.0%)
Hypertension	14(28.0%)
Membranous glomerulonephritis	4(8.0%)
Congenital atrophy	6(12.0%)
Polycystic kidney disease	3(6.0%)
Focal segmental glomerulosclerosis	4(8.0%)

*CKD: Chronic Kidney Disease

eGFR equations were compared taking mGFR by creatinine clearance as gold standard. Spearman correlation analysis showed strong positive correlation between mGFR and 2012 cystatin C equation ($r=0.891$, $p<0.001$), 2021 combined creatinine and cystatin C equation ($r=0.854$, $p<0.001$) and 2012 combined cystatin C and creatinine equation ($r=0.741$, $p<0.001$) whereas weak positive correlation between mGFR and 2021 creatinine ($r=0.554$, $p<0.001$) and 2009 creatinine equation ($r=0.596$, $p<0.001$), which can be seen in Figure-1.

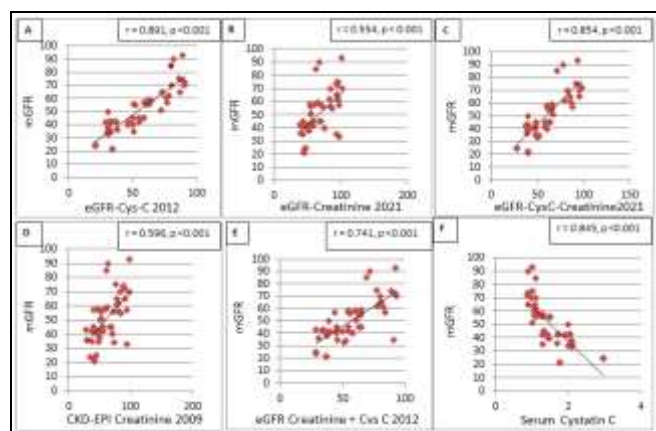


Figure-1: Correlation of CKD-EPI equations (eGFR) with creatinine clearance (mGFR). (A) Correlation between mGFR and eGFR Cystatin C 2012 (B) Correlation between mGFR and eGFR creatinine 2021 (C) Correlation between mGFR and eGFR cystatin C and creatinine 2021 (D) Correlation between mGFR and eGFR creatinine 2009 (E) Correlation between mGFR and eGFR cystatin C and creatinine 2012 (F) Correlation between mGFR and serum cystatin C. Solid line is the trend line. (n=50)

A statistically significant difference was found between the five pairs of GFR values i.e. mGFR and eGFR values by the five equations. CKD-EPI 2012 cystatin C equation showed the least difference ($Z = -2.83$, $p=0.005$), whereas CKD-EPI 2021 creatinine equation showed the highest difference ($Z = -5.39$, $p<0.001$) from the measured GFR values as shown in Table-II.

Table-II: Difference Between Measured and Estimated Glomerular Filtration Rates using New and Old Equations (n=50)

Equations	Measured GFR (ml/min/1.73m ²) Median (IQR) (n=50)	Estimated GFR (ml/min/1.73m ²) Median (IQR) (n=50)	p-value
CKD EPI cys- 2012	47.5(19.5)	53.5 (39.7)	0.005
CKD EPI cys-cr-2012	47.5(19.5)	57.0 (30.2)	<0.001
CKD EPI cr- 2009	47.5(19.5)	58.0 (32.5)	<0.001
CKD EPI cys-cr-2021	47.5(19.5)	60.5 (32.0)	<0.001
CKD EPI cr- 2021	47.5(19.5)	66.5 (35.5)	<0.001

*Cys: Cystatin C, Cr: Creatinine

The bias between measured and estimated GFR is shown by Bland Altman plot (Figure-2). Generally,

cystatin C based eGFR values showed less bias with smaller limits of agreement and less scattering of Bland Altman plot (high precision). On the other hand creatinine based eGFR values had more bias, larger limits of agreement and more scattering of Bland Altman plot (low precision) as shown in Figure-2.

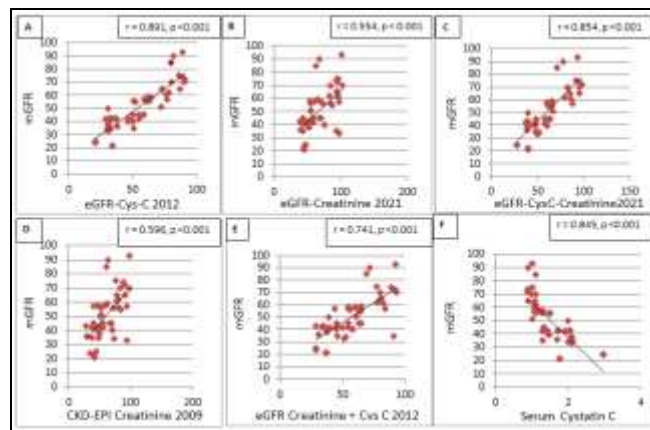


Figure-1: Correlation of CKD-EPI equations (eGFR) with creatinine clearance (mGFR). (A) Correlation between mGFR and eGFR Cystatin C 2012 (B) Correlation between mGFR and eGFR creatinine 2021 (C) Correlation between mGFR and eGFR cystatin C and creatinine 2021 (D) Correlation between mGFR and eGFR creatinine 2009 (E) Correlation between mGFR and eGFR cystatin C and creatinine 2012 (F) Correlation between mGFR and serum cystatin C. Solid line is the trend line. (n=50)

The new race free 2021 creatinine based CKD-EPI equation showed the largest bias (17.66 ml/min/1.73m²), least precision (SD=17.61) and accuracy (P30=52%) whereas cystatin C based 2012 CKD-EPI equation had least bias (4.3 ml/min/1.73m²), highest precision (SD=10.0) and highest accuracy (P30=88%). Comparison of all the equations is shown in Table-III.

Table-III: Comparison of New and Old CKD-EPI Equations (n=50)

Equation	Correlation coefficient (r)	Mean±SD GFR (ml/min/1.73m ²)	Limits of agreement		Mean difference (bias) (ml/min/1.73m ²)	SD of mean difference (precision)	P30 (Accuracy within 30%)
			Upper	Lower			
CKD-EPI cys- 2012	0.891	55.28±20.5	23.9	-15.3	4.3	10.0	88
CKD-EPI cys-cr-2012	0.741	58.28±18.9	33.0	-18.4	7.3	13.1	74
CKD-EPI cys-cr-2021	0.854	60.76±19.4	30.7	-11.1	9.7	10.6	66
CKD-EPI cr- 2009	0.596	61.99±19.4	44.7	-22.6	11.0	17.1	60
CKD-EPI cr- 2021	0.554	68.64±20.0	52.1	-16.8	17.6	17.6	52

*P30 = % of estimated GFR values within ± 30% of measured GFR. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, Cys: Cystatin C, Cr: Creatinine

DISCUSSION

Accurate measurement of GFR is critical in kidney transplant recipients for their better management. In our study, we evaluated both the old,

race-dependent and new, race-free CKD-EPI equations in renal transplant recipients. CKD-EPI 2012 equation incorporating cystatin C alone performed the best with the least mean bias of 4.3ml/min/1.73m² and excellent precision (SD=103ml/min/1.73m²). It also showed the strong positive correlation ($r=0.891$, $p<0.001$) with measured GFR and least difference ($z=2.83$, $p=0.005$) between pairs of measured and estimated GFR. Similarly, the 2012 equation incorporating both creatinine and cystatin C showed better results (mean bias=7.3 ml/min/1.73m²) than the new 2021 equation incorporating both cystatin C and creatinine (mean bias=9.78 ml/min/1.73m²) followed by 2009 creatinine equation (mean bias =11.06 ml/min/1.73m²). The 2021 race free creatinine equation showed the largest mean bias (17.6 ml/min/1.73m²), least precision, weakest positive correlation ($r = 0.554$, $p<0.001$) and largest difference ($z= -5.39$, $p=<0.001$) between the pairs of measured and estimated GFR.

New race free creatinine equation also showed much higher bias (5.5 ml/min/1.73m² vs 2.4 ml/min/1.73m²), less precision (16.6 ml/min/1.73m² vs 16.1 ml/min/1.73m²) and accuracy (68.3 vs 74.2%) than the old creatinine equation in a study.¹¹

Sukhanishi *et al.*¹² also reported that the new CKD-EPI equation demonstrated more positive bias in their study in Indian cohort of renal transplant recipients. Hsu *et al.*¹³ have shown that eliminating the race factor in the 2009 CKD-EPI equation does not enhance the accuracy in renal function estimation. However, Hundemer *et al.*¹⁴ has reported lower bias for the newer creatinine equation (-0.2 mL/min/1.73 m²) with similar precision and accuracy. These differences can be attributed to racial differences. Fu *et al.*¹⁵ concluded in their study that applying the 2021 race-free equation increases eGFR by a significant amount and relocate a considerable number of CKD patients to an upper eGFR class in their study in European population.

Estimated GFR equation based on serum cystatin C is a thoroughly evaluated equation with least bias.¹⁶ Hundemer *et al.*¹¹ reported that the cystatin C (2012) equation showed median bias of 3.6 mL/min/1.73 m², precision of 13.3 mL/min/1.73 m², and accuracy, i.e. p30 of 80%, this is comparable to our study results. In our study 2012 Cystatin C equation had mean bias of 4.3 mL/min/1.73 m² precision of 10 mL/min/1.73 m² and p30 of 88%. Various studies have demonstrated that CKD-EPI equation incorporating both creatinine

and cystatin C has the largest reliability,¹⁷ and formulas incorporating cystatin C may be more beneficial for the exact estimation of GFR.^{17,18} Serum creatinine concentration depends largely on muscle mass while this is not the case with serum cystatin C concentration. Cystatin C does not depend on race and is superior to creatinine in cases of atypical muscle mass, but it has other limitations.¹⁹ Cystatin C-based 2012 equation outperformed all eGFR equations based on creatinine in our local cohort of renal transplant recipients. Latest research suggests that both biomarkers are complementary to each other and accuracy of eGFR is increased to about 10% when used together.²⁰

LIMITATIONS OF STUDY

A small sample size and single centered study are the limitations of our study. We recommend further studies with large sample size and involving different centers for better understanding the performance of these equations in different geographical and ethnic groups.

CONCLUSION

The new, race-free 2021CKD-EPI creatinine equation showed highest bias, least precision and weak positive correlation with mGFR as compared to previously used eGFR equations in our cohort of renal transplant recipients.

Conflict of Interest: None.

Discolure:

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

AB & MA: Conception, study design, drafting the manuscript, approval of the final version to be published.

SB & KMR: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

SU & UN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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