

COMPARISON OF RENAL FUNCTION ASSESSMENT BY CYSTATIN C AND CREATININE BASED EQUATIONS FOR e-GFR IN TYPE 2 DIABETICS IN DIFFERENT STAGES OF ALBUMINURIA

Ayesha Qamar, Tariq Mahmood Ahmad, Asma Hayat, Mohammad Alamgir Khan, Mohammad Najam Ul Hasnat*, Saqibah Rehman

Army Medical College/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *King Edward Medical University Lahore Pakistan

ABSTRACT

Objective: To compare e-GFR estimated by creatinine or cystatin C based and combined creatinine and cystatin C based equations in type 2 diabetics in different stages of albuminuria.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology, Army Medical College Rawalpindi in collaboration with endocrinology outpatient department Military Hospital Rawalpindi, from Nov 2015 to Nov 2016.

Material and Methods: A total of 119 type 2 diabetic subjects of either gender, aged 30- 60 years were enrolled in the study with duration of diabetes less than 15 years and were divided into further sub groups on the basis of degree of albuminuria determined by spot urine albumin to creatinine ratio (uACR). Fifty age matched disease free controls with no history of any systemic disease were also included in the study. Known patients of type 1 diabetes, chronic inflammatory disorders, uncontrolled hypertension, thyroid disease, chronic kidney disease, on lipid lowering drugs, steroids, ACE inhibitors and pregnant ladies were excluded from the study. Serum creatinine serum cystatin C were assessed on fully automated chemistry analyzer selectra. E-GFR was calculated by online GFR calculator by National Kidney Foundation. Comparison of means of e-GFR calculated by various equations was carried out by one way ANOVA and post-hoc Tukey tests. Degree of agreement between various equations for the estimation of GFR was assessed by kappa statistics. A *p*-value less than 0.05 were considered statistically significant.

Results: Mean e-GFR (ml/min/1.73m²) was lowest in cystatin C based CKD-EPI equation (89.56 ± 39.84) followed by combined cystatin C and creatinine based CKD-EPI (92.34 ± 37.88). Values of e-GFR by creatinine based CKD-EPI equation (95.84 ± 27.24), and by creatinine based MDRD equation (105.37 ± 64.98) were both higher. In creatinine based MDRD, equation normo albuminuria and micro albuminuria groups did not show statistically significant difference as compared to each other and control group. The mean value of e-GFR was found to be lowest in the normo albuminuric diabetics when estimated by cystatin C based CKD-EPI equation (88.82 ± 46.98) followed by combined creatinine and cystatin C based CKD-EPI equation (95.73 ± 42.96).

Conclusion: Cystatin C based CKD-EPI equation for e-GFR identifies more patients with glomerular dysfunction in normo-albuminuric stage of DKD as compared to cystatin C and creatinine based CKD-EPI and creatinine based MDRD equations. Therefore, e-GFR estimated by serum cystatin C based CKD-EPI formula is a better option for assessing the renal status in patients of early DKD.

Keywords: Cystatin C, Diabetic kidney disease, e-GFR.

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INTRODUCTION

Diabetic kidney disease (DKD) is a clinical syndrome characterized by the following:

- Persistent albuminuria (>300 mg/d or >200 µg/min) that is confirmed on at least twice 3-6 months apart
- Progressive decrease in the glomerular filtration rate (GFR)
- Elevated arterial blood pressure¹.

GFR is defined as the clearance of a

Correspondence: Dr Ayesha Qamar, Department of Pathology Rawal Institute of Health Sciences Islamabad Pakistan

Email: ayesha.qamar7001@gmail.com

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substance in the plasma which is exclusively metabolized by the kidneys and freely filtered by the glomeruli². In a prevalence study the Asian and Hispanic patients were having the highest prevalence of micro albuminuria (43.2% and 43.8%) and macro albuminuria (12.3% and 10.3%).

Serum creatinine has been widely used as a marker of GFR but it is not sensitive enough to detect decreased renal function at an early stage of disease without albuminuria³. Various novel biomarkers have been studied for earlier detection of diabetic nephropathy including glomerular dysfunction, tubular dysfunction and oxidative stress markers⁴. Cystatin C, a low molecular weight protein is produced by virtually all nucleated cells of the body. Its concentration is almost totally dependent on GFR. Other studies have demonstrated that serum Cystatin C is an early renal dysfunction marker in diabetic patients⁵. In contrast to creatinine it is not much affected by age, gender, muscle mass and protein diet like creatinine and therefore preferable as compared to creatinine in renal dysfunction assessment in the elderly, children, in persons with low muscle mass⁶ and in normo albuminuria stage of diabetic kidney disease⁷⁻⁹.

The aim of our study was to compare the various equations for the estimation of GFR in different stages of diabetic kidney disease according to degree of albuminuria and to find the equation that identifies maximum number of patients with renal dysfunction in the early stage of normoalbuminuria.

MATERIAL AND METHODS

The study was conducted in the department of chemical pathology Army Medical College in collaboration with endocrinology OPD Military Hospital Rawalpindi, from Nov 2015 to Nov 2016 after seeking approval from the ethical review committee of the college. Sample size was calculated using the WHO sample size calculator. Population proportion=9.1%. A total of 119 type 2 diabetic subjects aged 30-65 years were enrolled

in study by non probability purposive sampling, diagnosed as per latest ADA criteria (HbA1C levels $\geq 6.5\%$ and or fasting blood glucose levels $\geq 7\text{mmol/l}$). Population was further divided into three sub groups depending on the albumin creatinine ratio (ACR). The people having normal range ACR ($<30\text{ mg/g}$) were placed in the normo-albuminuria group, those having ACR in the range of $30-300\text{ mg/g}$ in micro albuminuria group and those having ACR more than 300 mg/g were placed in the macroalbuminuria group.

Age and sex matched disease free controls were also included. All the study participants gave informed consent. Subjects having type 1 diabetes, uncontrolled hypertension, chronic inflammatory disorders, thyroid disease and those on ACE inhibitors steroids and lipid lowering drugs were excluded from the study. A total of 5ml venous blood sample and spot urine sample were obtained from the participants after an overnight fast. Serum was separated by centrifuging blood at 4000rpm for 5 minutes and this serum was analyzed for creatinine and Cystatin C levels. Serum creatinine was measured by Jaffe's kinetic method on fully automated chemistry analyzer selectra. Serum Cystatin C was measured by quantitative turbidimetric test on Selectra. Latex particles coated with polyclonal anti-Cystatin C antibodies were agglutinated when mixed with samples containing Cystatin C. The agglutination caused an absorbance change. Cystatin C concentration was then determined by the interpolation from a calibration curve prepared from calibrators of known concentrations. Cystatin C based GFR and creatinine based e-GFR were calculated by the specific equations by online e-GFR calculator by National Kidney Foundation:

MDRD (Serum Creatinine Based Equation 2009)

- $E\text{-GFR} = 186 \times (\text{serum creatinine (mg/l)})^{-1.154} \times \text{age}^{-0.203}$. (A correction factor of 0.742 was used for women.)

CKD-EPI-Serum Cystatin C Based Equation (2012)

- E-GFR = $127.7 \times (\text{Cystatin C in mg/l})^{-1.17} \times (\text{age in years})^{-0.13} \times (\times 0.91 \text{ if female})$.

CKD-EPI-Creatinine Equation (2009)

- E-GFR = $141 \times \min(\text{SCr}/\kappa, 1)^\alpha \times \max(\text{SCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 [\text{if female}] \times 1.159 [\text{if Black}]$.

CKD-EPI-Creatinine-Cystatin C Equation (2012)

- E-GFR = $135 \times \min(\text{SCr}/\kappa, 1)^\alpha \times \max(\text{SCr}/\kappa, 1)^{-0.601} \times \min(\text{Scys}/0.8, 1)^{-0.375} \times \max(\text{Scys}/0.8, 1)^{-0.711} \times 0.995^{\text{Age}} \times 0.969 [\text{if female}] \times 1.08 [\text{if black}]$.

Urine microalbumin was estimated by quantitative turbidimetric test on Selectra and

The mean values of e-GFR estimated by different equations were compared in various study groups according to degree of albuminuria by the ANOVA and post-hoc Tukey tests. Kappa statistics assessed the degree of agreement between different equations. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

One hundred and nineteen type 2 diabetics fulfilling the inclusion criteria were included in the study from the endocrinology outpatient department of the Military Hospital Rawalpindi. Fifty age and sex matched disease free controls were also selected.

The biochemical and demographic data of the participants i.e. cases and controls is shown in

Table-I: Baseline characteristics of study participants.

Parameters	Controls Mean ± SD	Cases Mean ± SD	Normal reference ranges	<i>p</i> -value (t-test)
e-GFR by MDRD formula by creatinine (e-GFR1) (ml/ min/ 1.73m ²)	155.80 ± 42.25	105.3721 ± 64.98715	More than or equal to 60 ml/min/1.73m ²	0.083
e-GFR by CKD-EPI creatinine (e-GFR3) (ml/ min/ 1.73m ²)	124.10 ± 14.36	95.8450 ± 27.24439		0.03
e-GFR by CKD-EPI cystatin C Creatinine (e-GFR4) (ml/min/1.73m ²)	131.70 ± 18.97	92.3488 ± 37.88008		0.02
e-GFR by CKD-EPI cystatin C based (e-GFR2) (ml/min/1.73m ²)	126.40 ± 19.47	84.5659 ± 39.84930		<0.001
Age in years	49.1872 ± 10.98	50.1783 ± 10.77066		
Serum creatinine (µmol/L)	41.60 ± 13.37	73.2036 ± 25.19218	0.5-1.1mg/dL	<0.001
Serum cystatin C (mg/L)	0.63 ± 0.14	1.0206 ± 0.35143	0.51-0.98mg/dL	<0.05

urinary creatinine by jeffe's kinetic method on selectra. Urinary albumin / creatinine ratio (u ACR) was calculated to overcome the day to day variation in urine volume and variation due to improper collection.

Data were analyzed on Statistical package for social sciences version 21. Mean and SDs were calculated for serum creatinine cystatin C AND e-GFR estimated by various equations. Independent sample t-test was applied for comparison of mean value between two groups.

table-I. Out of the 119 cases 57 (49%) were females and 62 (51%) were male patients. Among the 50 controls 24 (48%) were males and 26 (52%) were females.

Mean age was 49.1872 ± 10.98 years in controls and 50.1783 ± 10.77066 years in cases. Descriptive statistics (mean ± SD) were calculated for numerical variables like age, serum cystatin C, serum Creatinine and estimated GFR assessed by various equations. These results revealed that mean values of serum cystatin C were higher in

cases as compared to controls (p -value <0.001). Mean values of various parameters (Cystatin C creatinine and e-GFR) in all study groups were compared in the study groups using one way ANOVA which revealed p -value was less than 0.001.

Serum Cystatin C mean value was significantly different in the normo-albuminuric diabetic group as compared to control group (p -value=0.009) due to raised levels of serum Cystatin C in a number of normo-albuminuric diabetic subjects. The mean value of serum cystatin C did not show statistically significant difference between micro albuminuria and macro albuminuria groups. (p -value=0.9).

Estimated GFR mean values were also lower

The diabetic cases were subdivided into three groups based on the degree of albuminuria by spot urine ACR and difference in mean values of e-GFR assessed by various equations among these groups was carried out separately. The comparison of each two subgroups was carried out by the post-hoc Tukey test. The difference in means were significant statistically between controls and normo albuminuria groups among e-GFR calculated by all cystatin C based equations (p -value <0.05) and not significantly different for the creatinine based MDRD equation (p -value=0.08) (table-II).

The e-GFR values by all equations showed progressive decrease in levels with increasing degree of albuminuria. The value of mean e-GFR

Table-II: Post-hoc Tukey test for comparing mean values of serum cystatin C among the study groups. (Difference of means is significant at 0.05 level).

(I) patient categories according to albuminuria	(J) patient categories according to albuminuria	Sig p -value
Control	Normo albuminuria	.009
	Microalbuminuria	<.001
	Macroalbuminuria	<.001
Normo albuminuria	Control	.009
	Microalbuminuria	.228
	Macroalbuminuria	<.001
Micro albuminuria	Control	<.001
	Normo albuminuria	.228
	Macroalbuminuria	.002
Macro albuminuria	Control	<.001
	Normo albuminuria	<.001
	Microalbuminuria	.002

in cases as compared to controls. Mean value of serum creatinine was 73.2036 ± 25.1921 $\mu\text{mol/L}$ in cases and 41.60 ± 13.37 $\mu\text{mol/L}$ in controls and was significant statistically. The mean value of serum cystatin C was 1.11 ± 0.06 mg/L among controls and 1.53 ± 0.34 mg/L among cases and was found to be statistically significant. Difference in means of e-GFR estimated by CKD-EPI equations using creatinine only, Cystatin C only, creatinine and Cystatin C both and MDRD both controls and cases were statistically significant (table-I).

(ml/min/1.73m²) was the lowest in the cystatin C based CKD-EPI equation (89.56 ± 39.84) followed by combined cystatin C creatinine based CKD-EPI (92.34 ± 37.88), followed by creatinine based CKD-EPI (95.84 ± 27.24), followed by creatinine based MDRD equation (105.37 ± 64.98). Statistically significant difference was seen in the mean values of e-GFR by various equations in the controls and cases and between various groups devised according to degree of albuminuria except the creatinine based MDRD equation in which normo albuminuria and micro

albuminuria groups did not show statistically significant difference as compared to each other and control group. The mean value of e-GFR was found to be lowest in the normo albuminuric diabetics when estimated by cystatin C based CKD-EPI equation (88.82 ± 46.98) followed by the combined creatinine and cystatin C based CKD-EPI equation (95.73 ± 42.96). There was

equations in cases and controls (tables-III, IV & V).

DISCUSSION

Diabetic patients with DKD are at a much higher risk of progressing to end stage kidney disease (ESRD) along with increased mortality and morbidity associated with cardiovascular

Table-III: Agreement between e-GFR by MDRD (Creatinine) and by CKD-EPI (Cystatin C) Equations.

		Value	Asymp Std Error ^a	Approx T ^b	Approx Sig.
Measure of Agreement	Kapp^a	.062	.022	7.545	<.001
N of Valid Cases (e-GFR by MDRD equation by Creatinine. *e-GFR by CKD-EPI Cystatin C based)		129			

(a): Not assuming the null hypothesis. (b): Using the asymptotic standard error assuming the null hypothesis.

Table-IV: Measure of agreement between CKD-EPI (Creatinine) and CKD-EPI (Cystatin C) Equations.

		Value	Asymp. Std. Error ^a	Approx. T ^b	Approx Sig
Measure of Agreement	Kapp^a	.022	.015	2.602	.009
N of Valid Cases (e-GFR by CKD-EPI creatinine *e-GFR by CKD-EPI cystatin C based)		129			

(a): Not assuming the null hypothesis. (b): Using the asymptotic standard error assuming the null hypothesis.

Table-V: Measure of agreement between CKD-EPI (Creatinine) and CKD-EPI (Cystatin C) Equations.

		Value	Asymp. Std. Error ^a	Approx. T ^b	Approx Sig.
Measure of Agreement	Kappa	.030	.017	3.583	<.001
N of Valid Cases (e-GFR by CKD EPI cystatin C* e-GFR by CKD-EPI combined cystatin C and creatinine based)		129			

(a): Not assuming the null hypothesis. (b): Using the asymptotic standard error assuming the null hypothesis.

statistically significant agreement between the cystatin C based and creatinine cystatin C combined equations for estimation of GFR (p -value<0.05).

Kappa statistics showed a good agreement between the MDRD and CKD-EPI (Cystatin C based) and a also a very good concordance between CKD-EPI cystatin C based and combined cystatin C and creatinine based

disease. Although albuminuria assessment remains an essential tool for risk stratification and progression analysis of DKD but recent evidence has called into question its sensitivity and specificity. It has been found that only 30% of micro-albuminuric subjects progress to overt nephropathy after 10 years of followup¹⁰.

GFR is the best marker of renal function. The ideal methods for determining GFR in

research setting are inulin and ^{51}Cr -EDTA plasma clearance. But they are extremely time consuming and expensive plus and require expertise so not routinely available in clinical practice. Serum creatinine is the analyte used for estimation of GFR in common practice. The sensitivity of serum creatinine is poor in the early stages of kidney function impairment as by time an increase in serum level is seen a considerable decrease in GFR has already taken place³.

Formulae using serum creatinine for the estimation of GFR such as MDRD are not reliable at GFR more than 60 ml/min/1.73m². Cystatin C based assays like CKD-EPI equation for estimation of GFR appear to be better options than the other time consuming, expensive alternatives which need to be investigated and explored further.

The primary determinant of the Cystatin C levels in the blood is the rate of its filtration at the glomerulus and is an excellent marker of GFR¹¹. Value of Serum Cystatin C was raised in the diabetic groups (mean \pm SD=1.022 \pm 0.33) as compared to the control group (mean \pm SD=0.63 \pm 0.14) and in individual group comparison control group showed significant difference from normo-albuminuria (mean \pm SD=0.98 \pm 0.33), micro albuminuria (1.09 \pm 0.31) and macro albuminuria (1.18 \pm 0.33) groups. The Post- hoc test implied that serum Cystatin C levels were raised significantly in a considerable number of diabetic participants with normo-albuminuria (*p*-value less than 0.05). Therefore it can be used in early diagnosis/screening of DKD even before the onset of albuminuria. Similar findings of raised Cystatin C levels in normo-albuminuric diabetic subjects as compared to controls were recorded recently by researchers in their studies¹²⁻¹⁵. The mean value of e-GFR estimated by all cystatin C based and creatinine based formulae did not show a statistically significant difference when comparison of normo-albuminuria and microalbuminuria groups was carried out by post-hoc Tukey test (*p*-value>0.05 each). This was because of higher than expected value of e-GFR in a number of patients with micro

albuminuria than in patients of normo-albuminuria. This is explained by the fact that many patients with micro albuminuria are in a state of hyperfiltration, leading to an increased GFR. There was however a statistically significant difference between mean values of serum cystatin C in normo-albuminuria and microalbuminuria groups as compared to macroalbuminuria subgroup (*p*-value 0.00 and 0.02 respectively). Similar findings were observed recently in a cross sectional study carried out at a tertiary care hospital at New Delhi on 172 patients with type 2 diabetes⁹.

Estimated GFR calculated using the CKD EPI Cystatin C equation was the lowest among the equations followed by the CKD EPI combined equation. In general inclusion of cystatin C resulted in lower e-GFR. Estimated GFR calculated using the CKD EPI Cystatin C equation was the lowest among the equations followed by the CKD EPI combined equation. In general inclusion of cystatin C resulted in lower e-GFR. Similar results were obtained in other studies^{13,16}. In the present study cystatin C based CKD-EPI equation yielded the lowest GFR in normoalbuminuric subjects followed by the cystatin C Creatinine based combined e-GFR. While the creatinine based MDRD equation yielded a higher GFR. Therefore cystatin C is a better and earlier tool for the e-GFR estimation in DKD. In another study the highest percentage of e-GFR values within \pm 30% of measured GFR by gold standard were estimated using the combined equation incorporating both cystatin C and creatinine¹⁷. Our study has the limitation of smaller sample size and cross sectional study design but the findings also concur with other studies so these should be confirmed in larger longitudinal studies in comparison with gold standard.

CONCLUSION

Cystatin C based CKD-EPI equation for e-GFR identifies more patients with glomerular dysfunction in normo-albuminuric stage of DKD as compared to combined cystatin C and

creatinine based CKD-EPI and creatinine based MDRD equations. Therefore, e-GFR estimation by serum cystatin C based CKD-EPI equation is a better option for assessing the renal functions status in patients of early DKD.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any authors.

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