Identification and Stratification of Diabetic Kidney Disease Using Serum Cystatin C and Serum Creatinine Based Estimating Equations in Type 2 Diabetes: A Comparative Analysis

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Abstract

**Background:** Cystatin C is a low molecular weight protein produced by all nucleated human cells, with a stable production rate. Its levels are not influenced by inflammation, infections, hepatic or renal diseases, or by dietary or constitutional factors. We compared serum cystatin C and serum creatinine based predicting equations to estimate glomerular filtration rate (GFR) in type 2 diabetes mellitus, using the staging of chronic kidney disease (CKD) defined by the National Kidney Foundation. We also explored the relationship of urine albumin, GFR, serum creatinine and cystatin C concentrations.

**Methods:** A cross-sectional study was performed at a tertiary care hospital in New Delhi. Consecutive patients with type 2 diabetes mellitus above the age of 35 years were enrolled. Fasting and 2-hour-postprandial blood glucose, fasting lipid profile, lipoprotein(a), haemoglobin, microalbuminuria, glycated haemoglobin (HbA1c), liver and renal function tests were assessed. Serum levels of Cystatin C were measured using immune-turbidometric method (Dade Behring analyzer BN2). Estimated GFR (eGFR) was calculated using Cockcroft-Gault formula, Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease-Epidemiology (CKD-EPI) Cys C formula. The three sets of eGFR were compared using repeated measure ANOVA. Linear regression analysis was performed to find the factor that affects the albumin excretion rate (AER) and e-GFR levels using all three equations.

**Results:** We assessed 172 patients with type 2 diabetes mellitus. Mean age of the patients was 61.4 ± 9.6 years with mean duration of diabetes of 11.40 ± 7.5 years. Approximately 70% of patients had hypertension. A family history of diabetes was present in 53.4% of subjects and a history of CAD in first degree relatives in 20.9%. The prevalence of coronary artery disease was 17.4%. Albumin excretion correlated with e-GFR estimated using each of the three equations. The best correlation was seen with the CKD-EPI equation derived e-GFR. The CKD-EPI equation also identified the maximum number of patients in the normo-albuminuria group as having CKD. Albuminuria correlated with blood urea levels (p = 0.014) and serum cystatin C levels (p < 0.005).

**Conclusion:** The new cystatin C based Chronic Kidney Disease Epidemiology equation identifies more patients in early CKD and also patients with normo-albuminuric CKD compared to the creatinine based Cockcroft-Gault equation or the Modification of Diet in Renal Disease formulae.

Editorial Viewpoint

- Cystatin C is a low molecular weight protein produced by all nucleated cells at a stable rate uninfluenced by various diseases and inflammatory conditions.
- This study finds that cystatin C based Chronic Kidney Disease Epidemiology equation identifies more patients in early CKD.

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Introduction

Diabetic nephropathy is the commonest cause of end-stage renal disease in the world. This is mainly due to the increasing prevalence of type 2 diabetes mellitus. It is characterized by microalbuminuria, subsequent macroalbuminuria, and declining GFR. However, there are patients with diabetes who have a combination of normoalbuminuria or microalbuminuria and impaired renal function, but not the traditional decline of GFR with the development of proteinuria.

Screening for diabetic nephropathy is currently done by measuring microalbuminuria, serum creatinine, and creatinine clearance (Ccr). Serum creatinine is the most widely used marker of glomerular filtration rate in clinical practice, although it has low sensitivity in early renal disease. The serum creatinine level depends on muscle mass and meat intake, and its estimation may have positive interference from glucose, protein and fructose. Isotopic and non-isotopic methods for the determination of GFR, though accurate, are expensive and complex making them impractical for routine use. The other common method used is the creatinine clearance, a test that compares serum creatinine level with creatinine concentrations in a 24-hour urine collection. This procedure is useful, but not as precise. The National Kidney Foundation (NKF) recommends that GFR should be estimated from prediction equations taking into account the serum creatinine concentration and some of the following variables: age, gender, and body size, calculated by formulae such as the Cockcroft-Gault equation or the modification of diet in renal disease (MDRD) equation.

Cystatin C is a low molecular weight (13kd) protein produced by all nucleated human cells, with a stable production rate. It is freely filtered by the glomerulus and catabolised primarily by proximal tubular cells. Its levels are not influenced by renal factors, such as inflammatory, infectious, and liver diseases, or by dietary or constitutional factors that could influence the production rate.

This study was designed to estimate (1) the prevalence of diabetic kidney disease in a group of patients with type 2 diabetes mellitus, (2) e-GFR using serum creatinine and serum cystatin C based predictive equations and (3) association of diabetic kidney disease covariates like hypertension, retinopathy, lipid levels and coronary artery disease (CAD).

Material and Methods

Consecutive patients with type 2 diabetes mellitus, above the age of 35 years, presenting to the Department of Medicine at Post Graduate Institute of Medical Education and Research and Dr RML Hospital, New Delhi, India were enrolled. Diabetes mellitus was defined in accordance with WHO criteria as fasting venous plasma glucose concentration ≥126 mg/dL or 2-hour-post-prandial plasma glucose ≥200 mg/dL on two or more occasions or a known case of diabetes mellitus on diet control/insulin/oral hypoglycemic agents.

Patients were excluded if they were on corticosteroids, had refractory hypertension, rapidly increasing proteinuria, nephrotic syndrome, active urinary sediments, urinary tract infection, untreated hypothyroidism, HIV seropositivity, >30% reduction in GFR within 2-3 months after initiation of Angiotensin converting enzyme inhibitor or angiotensin receptor blockers or a malignancy.

A detailed history was obtained regarding the onset, duration and treatment taken for diabetes mellitus. A history of smoking, alcohol consumption, and hypertension was also recorded. Hypertension was defined in accordance with JNC 7 criteria. The presence of CAD was assessed from a past history of CAD, history of coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA), a history of angina (Modified Rose questionnaire) and ECG changes (Minnesota codes). A family history of diabetes mellitus, hypertension, CAD and cerebrovascular accident was also recorded. Detailed physical examination was done with emphasis on measuring weight (kg), height (meter), blood pressure, waist and hip circumference and waist-hip ratio.

Laboratory investigations included fasting and 2-hour-post-prandial blood glucose, fasting lipid profile (total cholesterol, LDL, VLDL, HDL and triglycerides), haemoglobin, urinary albumin, HbA1c, lipoprotein(a), liver and renal function tests. A comprehensive ocular examination including direct ophthalmoscopy, indirect ophthalmoscopy with +20 D lens examination and slit-lamp examination was performed on all patients and diabetic retinopathy was classified according to Early Treatment Diabetic Retinopathy Study Research Group (ETDRS) criteria.

Serum creatinine was determined using the Autopak reagent kit utilizing the picrate method. Serum cystatin C was measured using immune-turbidimetric method using Dade Behring analyzer BN2 in specialized testing environment with a BN System™ or in the core lab with the Dimension Vista® System.

Gross albuminuria was tested using the Ames multiple reagent strips (Bayer Diagnostics India Ltd.). Patients whose urine specimen was negative for gross albuminuria were evaluated further for microalbuminuria by immunoturbidimetric assay and
those whose urine specimen was positive for gross albuminuria were evaluated for 24 hr urine albumin estimation.

Estimated GFR (eGFR) was calculated using the following estimating equations:

1. **Cockcroft-Gault formula:**
   
   \[ \text{eGFR} = [(140 - \text{age in years}) \times \text{Body weight in Kg}] / [72 \times \text{serum creatinine (mg/dl)}] \]
   
   *0.85 if female.

   This formula was standardized to body surface area (BSA). BSA was calculated using the formula of Dubois et al: BSA \( (\text{m}^2) = 0.007184 \times (\text{body weight in Kg})^{0.425} \times (\text{body height in cm})^{0.725} \), standard BSA = BSA/1.73.

2. **Modification of diet in Renal Disease:**
   
   \[ \text{eGFR} = 186 \times \text{(serum creatinine mg/dl)} - 1.154 \times \text{age in years} - 0.203 \times \text{BSA} + 0.742 \text{if female and multiplied by 1.210 if African American.} \]

3. **CKD-EPI Cys C formula:**
   
   \[ \text{eGFR} = 127.7 \times \text{(Cystatin C in mg/L)} - 1.17 \times \text{age in years} - 0.13 \times \text{BSA} + 0.91 \text{if female.} \]

According to the Kidney Disease Outcome Quality Initiative (KDOQI) guidelines of the NKF, CKD was be stratified into the following stages:

1. Stage 1: GFR ≥ 90 and Albumin excretion rate (AER) >30 mg per 24 hr.
2. Stage 2: GFR 60-89 and AER >30 mg per 24 hr.
5. Stage 5: GFR <15.

(All values of GFR are in ml/min/1.73m² BSA)

Those with GFR <60 ml per minute per 1.73 m² and AER <30 mg per 24 hour were considered to be at increased risk due to diabetes.

Patients were also stratified based on urinary albumin excretion into:

1. Normoalbuminuric (<30 mg/day or spot urine albumin/creatinine ratio of <30 µg/mg creatinine)
2. Microalbuminuria (30-300 mg/day or spot urine albumin/creatinine ratio 30-299 µg/mg creatinine)
3. Macroalbuminuria (>300 mg/day or albumin creatinine ratio > 300 µg/mg creatinine)

The study protocol was approved by the institutional review board of PGIMER and Dr. RML hospital, New Delhi. Informed, written consent was obtained from all patients.

**Statistical Analysis**

All analysis were performed using SPSS version 19.0 (IBM). Continuous variables were summarized as mean ± SD. Differences between groups were analyzed by repeat measure ANOVA, followed by the Bonferroni’s test for normally distributed values and by the Kruskal-Wallis test, as well as the Dunn’s test for non-parametric values. For non-parametric continuous variables, Mann-Whitney U test was used. Pearson’s correlation coefficient was employed to test the correlations between continuous variables. After correlation analysis, stepwise multiple linear regression analysis was performed to evaluate factors affecting the AER and e-GFR levels using all three equations. Multivariate logistic regression was performed to assess factors predicting renal impairment in normo-albuminuric patients. All results were considered significant if p <0.05.

**Results**

A total of 172 patients (97 men and 75 women) with type 2 diabetes were included in the study. The age of the patients ranged from 39 to 85 years with a mean age of 61.4 years. The duration of diabetes ranged from 9 months to 34 years with a mean of 11.40 years. Over two-thirds of patients (69.7%) were hypertensive and 17.4% had a history of CAD.

106 (61.6%) patients were hypertensive and were on medication. The mean systolic blood pressure was 133 mm Hg and the mean diastolic blood pressure was 81 mm Hg. Systolic blood pressure (SBP) >140 mmHg was recorded in 109 (63.4%) of patients and diastolic blood pressure (DBP) >80 mmHg was seen in 67 (39.0%) of patients.

Most patients in the study population were either overweight or obese. BMI ranged from 18.06 – 39.8 kg/m² with mean BMI being 27.0 Kg/m². Mean BMI for women (28.2 kg/m²) was higher than that for men (26.1 Kg/m²). No patient was underweight. Only 24 (14%) of patients had normal BMI whereas 83 (48.3%) were morbidly obese. 33 (19.2%) were overweight and 32 (18.6%) patients were obese.

Sixty-eight (39.5%) of patients had their HbA1c levels <7%. Mean waist circumference was higher in men (101.2 cms) compared to women (98.5 cms). 74 (98.7%) had a waist circumference more 80 cms. Eighty-eight (90.7%) of male patients had waist circumference more than 90 cms. Waist/hip ratio ranged from 0.75-1.17. Men had a higher mean waist hip ratio (1.02) than women (0.94). Of the 75 female patients, 69 (92.0%) had a waist hip ratio of more than 0.85 and 88 (90.7%) of males had a waist-hip ratio more than 0.95. Diabetic retinopathy was present in 27 patients (15.7%).

Forty-one (42.2%) men and 33 (44.0%) women were anemic. Other important biochemical parameters of the study patients are summarized in Table 1.

Thirty-four (19.7%) patients had hypercholesterolemia (total cholesterol level >200 mg/dL). 90 (52.3%) patients had LDL levels more than 100 mg/dL and 71
Table 1: Biochemical parameters of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men (n=79)</th>
<th>Women (n=67)</th>
<th>Total (n=146)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>13.21 ± 1.2</td>
<td>11.92 ± 1.33</td>
<td>12.65 ± 1.44</td>
</tr>
<tr>
<td>ESR (mm in 1st hour)</td>
<td>22.44 ± 21.11</td>
<td>36.78 ± 19.31</td>
<td>12.65 ± 1.44</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>144.72 ± 49.54</td>
<td>135.41 ± 42.93</td>
<td>140.66 ± 46.87</td>
</tr>
<tr>
<td>Post-prandial blood glucose (mg/dL)</td>
<td>209.35 ± 79.87</td>
<td>195.85 ± 63.78</td>
<td>203.46 ± 73.39</td>
</tr>
<tr>
<td>Blood urea (mg/dL)</td>
<td>30.54 ± 11.69</td>
<td>31.36 ± 17.50</td>
<td>32.02 ± 11.82</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.99 ± 0.37</td>
<td>0.85 ± 0.40</td>
<td>0.97 ± 0.28</td>
</tr>
<tr>
<td>Serum uric acid (mg/dL)</td>
<td>5.4 ± 1.43</td>
<td>5.84 ± 1.6</td>
<td>5.57 ± 1.62</td>
</tr>
<tr>
<td>Serum cystatin C (mg/L)</td>
<td>1.10 ± 0.23</td>
<td>1.20 ± 0.30</td>
<td>1.14 ± 0.51</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dL)</td>
<td>164.50 ± 34.14</td>
<td>176.69 ± 32.75</td>
<td>169.92 ± 34.04</td>
</tr>
<tr>
<td>Serum LDL (mg/dL)</td>
<td>106.81 ± 26.17</td>
<td>97.51 ± 31.70</td>
<td>101.65 ± 29.70</td>
</tr>
<tr>
<td>Serum HDL (mg/dL)</td>
<td>44.35 ± 16.04</td>
<td>45.68 ± 13.85</td>
<td>44.93 ± 15.02</td>
</tr>
<tr>
<td>Serum VLDL (mg/dL)</td>
<td>23.93 ± 13.92</td>
<td>25.39 ± 13.02</td>
<td>24.57 ± 13.52</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dL)</td>
<td>143.49 ± 60.64</td>
<td>145.26 ± 52.62</td>
<td>144.26 ± 57.12</td>
</tr>
<tr>
<td>lipoprotein(a) (mg/dL)</td>
<td>33.62 ± 33.07</td>
<td>29.01 ± 24.39</td>
<td>31.59 ± 29.57</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.61 ± 2.89</td>
<td>7.70 ± 1.49</td>
<td>7.65 ± 1.6</td>
</tr>
<tr>
<td>24 hour urinary albumin excretion rate</td>
<td>87.44 ± 327.2</td>
<td>44.35 ± 311.1</td>
<td>68.65 ± 257.41</td>
</tr>
</tbody>
</table>

Note: All values, except Hb, ESR and HbA1C, are in mg/dl, expressed as Mean ± SD

(41.2%) had triglycerides levels more than 150 mg/dL. 56 (74.6%) women and 41 (42.2%) men had low HDL levels.

One hundred thirty-three (77.3%) patients had normoalbuminuria, 31 (18%) had microalbuminuria and 8 (4.7%) had macroalbuminuria. Patients were stratified based on albumin excretion rate.

The MDRD equation identified 104 patients as having CKD and the CG equation identified 110 patients. However, using the CKD-EPI Cys C equation, 138 patients were identified as having CKD.

The CKD-EPI equation identified more women (86.7%) as having CKD compared to the other 2 equations. The distribution of CKD using the three equations is shown in Figure 1.

Based on NKF-KDOQI guidelines, using eGFR, patients with CKD were assigned to 5 stages of CKD. None of the patients had stage 5 CKD. Most patients had stage 2 or stage 3 CKD by all equations. The CKD-EPI Cys C equation classified the least number of patients in stage 0 or stage 1, but it stratified the highest number of patients (13 i.e. 7.6%) into stage 4. The CG equation stratified the least number of patients into stage 4. The MDRD equation placed higher number of patients in stage 0 compared to those placed in stage 0 by the CG and the CKD-EPI Cys C equations. These results are summarized in Figure 2.

Patients were stratified into 3 groups based on the albumin excretion rates (AER). Normoalbuminuric (AER <30 mg/day), microalbuminuric (AER 30-300 mg/day) and macroalbuminuric (AER >300 mg/day). Various clinical characteristics and biochemical parameters were compared in the three groups (Tables 2 and 3). Retinopathy was more prevalent in patients with micro-albuminuria. Blood urea, serum creatinine, serum cystatin C, fasting blood sugar, post-prandial blood sugar progressively increased with increasing albumin excretion.

Mean e-GFR was lowest in patients with the highest albumin excretion and serum cystatin C levels were highest in patients who had macro albuminuria as depicted in Figure 3.

The prevalence of non-albuminuric CKD was evaluated in the study population using the three equations. The CKD-EPI equation identified the maximum number of patients as having CKD (n=138) compared to the CG (n=110) or the MDRD (n=104) equation.

Repeat measure ANOVA test was used to compare mean e-GFR among the creatinine based CG and MDRD equations and the cystatin C based CKD-EPI equations. No significant difference was demonstrated between the creatinine based Cockcroft-Gault equation and Modification of Diet in Renal Disease equation but a significant difference was found between e-GFR measured by creatinine based equation and Cystatin C based CKD-EPI formula. The e-GFR estimated in a given patient using the CKD-EPI
Fig. 2: Comparison of performance of CG, MDRD and CKD-EPI\textsuperscript{Cys C} equations. CG: Cockcroft–gault, MDRD: Modification of diet in renal disease, CKD-EPI: Chronic kidney disease epidemiology

equation was significantly lower than that estimated using the CG and the MDRD equations.

In order to assess independent predictors of albumin excretion rate, predictor variables which on univariate analysis had a p-value $<0.10$ were entered into a multivariate linear regression model. Postprandial blood glucose and serum creatinine were found to be independent predictors of albumin excretion rate.

**Discussion**

We compared the estimated GFR in 172 patients with type 2 diabetes mellitus using three different predicting equations.

Using e-GFR calculated by the Cockcroft-Gault equation, 64\% of patients had CKD. Using the MDRD equation, this figure was 60.5\%. MDRD and the CKD-EPI equations classified more women as having CKD compared to men. This is consistent with the results of other studies as creatinine based equations underestimate e-GFR due to lower muscle mass in females.\textsuperscript{16}

The prevalence of CKD in patients with diabetes in our study is similar to that reported from other South-East Asian countries. A study by Lu et al from Shanghai China estimated the prevalence of CKD in type 2 diabetics to be 69\% using creatinine based CG equation.\textsuperscript{17} A study done in Italy by Mussap et al using Chromium 51 labeled EDTA estimated the prevalence of CKD in diabetics to be around 53\%.\textsuperscript{18} Approximately 1 in 6 people with type 2 diabetes develop overt nephropathy.\textsuperscript{19}

The cystatin C based CKD-EPI equation identified 80.20\% of our patients as having CKD. In diabetic patients, previous studies have shown that serum cystatin C is more sensitive than serum creatinine estimation of GFR in type 2 diabetic patients and Tan et al showed the same in type 1 diabetic patients.\textsuperscript{20} After adjustment for age, Christensson et al also demonstrated that serum cystatin C levels reflect mild diabetic nephropathy better.\textsuperscript{21} Shimizu et al showed a significant relationship between serum cystatin C levels and the prognostic stage in patients with type 2 diabetic nephropathy.\textsuperscript{22} Bicik et al showed that the sensitivity of serum cystatin C is higher in microalbunimuric patients with diabetes.\textsuperscript{23} On the other hand, Oddoze et al, demonstrated that serum creatinine and serum cystatin C are comparable in diagnostic accuracy in microalbuminuric and proteinuric diabetic patients.\textsuperscript{24} These studies differ from each other because of the use of different methods of GFR measurements, with different cut-off levels of GFR (80, 68 or 60 ml/min) and different methods of serum creatinine and serum cystatin C measurement.

**Table 2: Stratification of study population into normo, micro and macro- albuminuric group based on AER (albumin excretion rate) and comparison of anthropometric and demographic properties**

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria</th>
<th>Microalbuminuria</th>
<th>Macroalbuminuria</th>
<th>Test of significance</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>133 (77.3%)</td>
<td>31 (18%)</td>
<td>8 (4.7%)</td>
<td>Nil</td>
<td>nil</td>
</tr>
<tr>
<td>Male / Female</td>
<td>72 / 61</td>
<td>21 / 10</td>
<td>4 / 4</td>
<td>Chi-square</td>
<td>0.362</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>61.5 ± 9.5</td>
<td>60.8 ± 10.6</td>
<td>62.6 ± 9.3</td>
<td>Kruskal-wallis</td>
<td>0.871</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>26.9 ± 3.9</td>
<td>27.5 ± 3.4</td>
<td>26.4 ± 3.4</td>
<td>Kruskal-wallis</td>
<td>0.348</td>
</tr>
<tr>
<td>Duration of DM (in years)</td>
<td>11.0 ± 7.3</td>
<td>11.7 ± 7.7</td>
<td>16.9 ± 9.7</td>
<td>Kruskal-wallis</td>
<td>0.140</td>
</tr>
<tr>
<td>CAD</td>
<td>24 (18.01%)</td>
<td>4 (12.9%)</td>
<td>2 (25%)</td>
<td>Fisher’s exact Test</td>
<td>0.672</td>
</tr>
<tr>
<td>Duration of HTN (in years)</td>
<td>6.5 ± 8.8</td>
<td>8.3 ± 8.9</td>
<td>10.0 ± 8.0</td>
<td>Kruskal-wallis</td>
<td>0.921</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>15 (11.3%)</td>
<td>10 (32.3%)</td>
<td>2 (25%)</td>
<td>Fisher’s Exact Test</td>
<td>0.012</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>132.5 ± 14.1</td>
<td>136.3 ± 15.3</td>
<td>137.8 ± 20.9</td>
<td>Kruskal-wallis</td>
<td>0.435</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>80.6 ± 8.0</td>
<td>82.2 ± 7.6</td>
<td>82.0 ± 4.1</td>
<td>Kruskal-wallis</td>
<td>0.477</td>
</tr>
<tr>
<td>Waist circumference (in cms)</td>
<td>99.5 ± 9.3</td>
<td>102.5 ± 8.7</td>
<td>100.1 ± 11.0</td>
<td>Kruskal-wallis</td>
<td>0.154</td>
</tr>
<tr>
<td>WHR</td>
<td>1.0 ± 0.07</td>
<td>1.0 ± 0.08</td>
<td>1.0 ± 0.05</td>
<td>Kruskal-wallis</td>
<td>0.682</td>
</tr>
</tbody>
</table>

Note: All values except gender, CAD and retinopathy are in mean±SD. HTN= Hypertension, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, WHR= Waist to hip ratio.
Table 3: Stratification of study population into normo, micro and macroalbuminuric group based on AER (albumin excretion rate) and comparison of biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria</th>
<th>Microalbuminuria</th>
<th>Macroalbuminuria</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 133)</td>
<td>(n = 31)</td>
<td>(n = 8)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.7 ± 1.4</td>
<td>12.5 ± 1.6</td>
<td>12.7 ± 1.4</td>
<td>0.183</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>29.8 ± 13.5</td>
<td>30.9 ± 14.4</td>
<td>44.3 ± 19.2</td>
<td>0.014</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>1.4 ± 0.9</td>
<td>0.067</td>
</tr>
<tr>
<td>Sr. cystatin C (mg/L)</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.6</td>
<td>1.8 ± 0.7</td>
<td>0.005</td>
</tr>
<tr>
<td>eGFR by CG (ml/min)</td>
<td>87.8 ± 32.5</td>
<td>92.7 ± 34.7</td>
<td>57.9 ± 25.5</td>
<td>0.022</td>
</tr>
<tr>
<td>eGFR by MDRD (ml/min)</td>
<td>91.9 ± 30.8</td>
<td>87.0 ± 30.4</td>
<td>61.3 ± 37.0</td>
<td>0.041</td>
</tr>
<tr>
<td>eGFR by CKD-EPI (ml/min)</td>
<td>72.3 ± 37.4</td>
<td>95.3 ± 77.4</td>
<td>40.0 ± 22.4</td>
<td>0.002</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>135.1 ± 39.0</td>
<td>158.5 ± 65.1</td>
<td>164.4 ± 63.5</td>
<td>0.167</td>
</tr>
<tr>
<td>PPBS (mg/dL)</td>
<td>195.5 ± 62.7</td>
<td>226.1 ± 92.1</td>
<td>248.4 ± 124.0</td>
<td>0.207</td>
</tr>
<tr>
<td>HbA1C%</td>
<td>7.4 ± 1.3</td>
<td>8.4 ± 2.34</td>
<td>8.2 ± 1.6</td>
<td>0.055</td>
</tr>
<tr>
<td>AER (mg/day)</td>
<td>8.8 ± 7.2</td>
<td>98.1 ± 2.3</td>
<td>949.7 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>T. cholesterol (mg/dL)</td>
<td>168.6 ± 32.1</td>
<td>167.8 ± 39.1</td>
<td>199.6 ± 39.1</td>
<td>0.092</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>44.1 ± 12.6</td>
<td>48.7 ± 24.0</td>
<td>44.4 ± 6.3</td>
<td>0.665</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>101.3 ± 27.2</td>
<td>97.1 ± 34.0</td>
<td>124.0 ± 43.5</td>
<td>0.154</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>24.3 ± 12.6</td>
<td>26.3 ± 17.7</td>
<td>22.6 ± 8.7</td>
<td>0.861</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>143.5 ± 55.8</td>
<td>144.1 ± 67.9</td>
<td>157.3 ± 32.7</td>
<td>0.534</td>
</tr>
<tr>
<td>lipoprotein(a) (mg/dL)</td>
<td>28.0 ± 26.2</td>
<td>47.5 ± 39.1</td>
<td>28.2 ± 20.0</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Kruskal Wallis test was used for all comparisons. FBS= Fasting blood sugar, PPBS= Post-prandial blood sugar, AER= Albumin excretion rate. HDL=High density lipoprotein, LDL= Low density lipoprotein, VLDL= Very low density lipoprotein, TG= Triglyceride.

Figure 3: Cystatin C levels as compared to albumin excretion rate

Harmoinen et al25,26 and Mussap et al27 showed that serum cystatin C is more sensitive than serum creatinine for the estimation of e-GFR.

We stratified patients based on their albumin excretion rate into normo, micro and macroalbuminurics. 133 (77.3%) patients had normo-albuminuria, 31 (18%) had micro-albuminuria and only 8 (4.7%) had macro-albuminuria. Epidemiological and cross-sectional studies have reported variations in the prevalence of microalbuminuria in patients with type 2 diabetes. Vijay et al reported a prevalence of 15.7% in 600 type 2 diabetic patients from Chennai.27 Varghese et al reported a prevalence of 36.3% in 1425 type 2 diabetic patients in Chennai.28 The e-GFR calculated by the CG, the MDRD and the CKD-EPI formulas categorized 64%, 63.5% and 71.1% of normoalbuminuric patients as having CKD, respectively. These figures are comparable to those found by Yang et al who found a prevalence of CKD of 56% in normoalbuminuric diabetics.

The e-GFR was higher in patients with microalbuminuria than in those with normoalbuminuria. This is explained by the fact that most patients with microalbuminuria are in a state of hyperfiltration, leading to an increased GFR. CG and CKD-EPI based e-GFR follows the above rule. The MDRD equation underperforms in early CKD hence e-GFR calculated is mildly lower using the MDRD equation.

When predictors of AER were correlated with urinary albumin excretion, the presence of retinopathy correlated well with increasing albumin excretion (p = 0.012). Serum cystatin C levels also correlated well with increasing urinary albumin excretion (p = 0.005), as did blood urea levels (p = 0.041). eGFR calculated using all three equations correlated well with albumin excretion rate, with CKD-EPI equation having the highest correlation (p = 0.002) and MDRD the least (p = 0.041). The CG equation had a good correlation (p = 0.022). Among lipids, lipoprotein(a), had a good correlation p = 0.011.

In our study, patients were stratified based on e-GFR into 5 stages based on NKF-KDOQI guidelines. When the subjects were stratified using all three equations, no patient was found to be in stage 5 CKD. The CG equation classified the maximum number of patients into stage 2 (29.1%) followed by stage 3 (22.1%). Similarly the MDRD equation classified the maximum number of patients into stage 2(32%) followed by CKD stage 3 (15.1%). The CKD-EPI equation however placed the
maximum number of patients into stage 3 (34.9%) followed by Stage 2 CKD (32%).

In previous studies the MDRD equation performed well in stage 2 and 3 compared to very early CKD stages where the CG equation and cystatin C based formulas performed well in early stages of CKD. Rigalleau and colleagues estimated GFR using CG, MDRD and cystatin C based composite equations and found results similar to ours. The MDRD equation underestimated the number of CKD patients in stage 1 and 2 in their study and overestimated GFR in stage 3. The CG equation overestimated GFR in stage 1 and stage 2 and underperformed in stage 5. The cystatin C based equation provided the best estimates. In our study e-GFR based on the CG equation classified more patients into stage 1 compared to any other stage and put the least number of patients into Stage 5. e-GFR based on the MDRD equation placed most patients into stages 2 and 3 and placed the highest number of subjects in the no CKD group.

When e-GFR was predicted using the CKD-EPI equation, gender, BMI, duration of diabetes, duration of hypertension and HbA1c% correlated with e-GFR. When these factors were assessed using multivariate regression analysis HbA1c was the only independent predictor of e-GFR by CKD-EPI formula.

Conclusion

Albumin excretion, which is a major determinant of CKD progression, correlated with e-GFR estimated using each of the three equations. The best correlation was seen with the CKD-EPI equation derived e-GFR. The CKD-EPI equation also identified the maximum number of patients in the normo-albuminuria group as having CKD. Albuminuria correlated with blood urea levels (p = 0.014) and serum cystatin C levels (0.005). Serum creatinine levels did not correlate with proteinuria (p = 0.067).

In a multivariate regression analysis the only independent predictor of e-GFR by the CKD-EPI equation has HbA1c.

No significant difference was found between e-GFR calculated by the CG and MDRD equations. However, e-GFR estimated by serum cystatin C based CKD-EPI equation was significantly lower than that calculated by the CG and the MDRD equations.

The cystatin C based new Chronic Kidney Disease Epidemiology equation identifies more patients in early CKD and also patients with normoalbuminuric CKD compared to the creatinine based Cockcroft-Gault equation or the Modification of Diet in Renal Disease formulae.

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References

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