ASSOCIATION OF DIFFERENT EGFR METHODS, CALCIUM METABOLISM AND ANEMIA IN DIABETIC CHRONIC KIDNEY DISEASE: AN INDIAN PERSPECTIVE (EXPERIENCE)

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Key words: modification of diet in renal disease, Cockcroft-Gault, parathyroid hormone, Cr\textsuperscript{51}-EDTA, anemia

SUMMARY

The aim was to evaluate performance of Cockcroft Gault (CG), modification of diet in renal disease (MDRD) formula in the prediction of glomerular filtration rate (GFR) as compared to Cr\textsuperscript{51}-EDTA GFR (isotope GFR) in diabetic chronic kidney disease (dCKD) and their relation with calcium and iron metabolism parameters. One hundred ambulatory patients with diabetes without nephrotic syndrome, renal replacement, renal transplantation or any severe coexisting illness underwent clinical assessment, blood collection after overnight fast and isotope GFR estimation using Cr\textsuperscript{51}-EDTA. Advanced CKD (GFR < 30 mL/min/1.73m\textsuperscript{2}) was found in 42.5\% of patients. Compared to isotope GFR, CG formula and MDRD equation overestimated and underestimated GFR, respectively, with CG having a lower diagnostic accuracy. Correlation between parathormone and isotope GFR was maximal in CKD stage 4. The odds ratio for anemia for GFR < 30mL/min/1.73m\textsuperscript{2} was 2.21. In conclusion, MDRD is a better predictor of renal function as compared to CG in Indian dCKD patients.

INTRODUCTION

Cockcroft-Gault (CG) formula or modification of diet in renal disease (MDRD) equation have been commonly used for estimation of glomerular filtration rate (GFR) in patients with chronic kidney disease (CKD) (1-4). Both these equations were developed among nondiabetic CKD patients and their validity among patients with diabetes (the most common cause of CKD worldwide) has not been studied extensively. A few but not all studies have suggested that MDRD is more accurate and robust among diabetics with CKD (5) and is not influenced by body mass index (BMI) (6,7). CG tends to overestimate GFR among patients with obesity (6). Obesity is common among patients...
with diabetes. Although considered superior to CG, the use of MDRD among patients with diabetes has not been validated in India.

Secondary hyperparathyroidism (SHPT), a grossly underdiagnosed and an important complication of end-stage renal disease (ESRD) is believed to begin during the earlier stages of CKD (8). As many as 56% of patients with GFR <60 mL/min are believed to have SHPT (9). The presence of SHPT results in faster progression of CKD along with increased risks for the need of dialysis, health care costs and death (10). The pathogenesis of this SHPT is not well known. Hyperphosphatemia, hypocalcemia, vitamin D deficiency, decreased expression of calcium and vitamin D receptors and parathormone (PTH) resistance are believed to have some role in the development of SHPT (11). The relationship between GFR and PTH has not been well studied. The stage of CKD at which PTH begins to rise, and the risk factors associated with increased PTH have not been well characterized.

Anemia is also a common problem in patients with CKD. GFR <60 mL/min/1.73m² was strongly associated with a higher prevalence of anemia in patients with CKD (the third national health and nutrition examination survey) (12). It has been suggested that a decline in serum hemoglobin (Hb) may be observed even before overt changes in renal function (13), and is believed to be the result of both erythropoietin resistance as well as a decreased erythropoietin production secondary to tubule-interstitial damage (14). However, the relation between the stage of renal dysfunction and severity of anemia in patients with CKD is not well studied.

Hence, the aim of our study was to compare the performance of CG formula and MDRD equation against isotopic GFR (using $^{51}$Cr-EDTA) measurement (15) for estimation of GFR among patients with diabetes. We aimed to study the relationship measures of calcium metabolism (iPTH, calcium, phosphorus, alkaline phosphatase) with isotopic GFR, CG-GFR and MDRD-GFR. We also aimed to find the point of decrease in GFR when PTH beings to increase in serum and the model of GFR estimation that correlates best with PTH increase. We plan to evaluate the occurrence of anemia in patients with diabetic CKD in our region and study whether the degree of anemia can predict the severity of CKD.

**MATERIAL AND METHODS**

Patients with diabetes attending the diabetic clinic of Department of Endocrinology and Metabolism, IPGMER and SSKM Hospital, Kolkata, were considered for the study. The study was conducted from November 2008 until November 2011. The study protocol was approved by the institutional ethics committee. Patients between 18 to 65 years of age were considered eligible. Excluded were patients who had nephrotic range proteinuria (>3 g in 24 hours), clinical edema, undergone dialysis, renal transplantation, severe coexisting illness like primary hyperparathyroidism, uncontrolled congestive cardiac failure, septicemia, or were non-ambulatory. All patients underwent detailed clinical examination. Records were taken on the duration of diabetes, number of drugs used for treatment, dose and use of insulin. Data on age, sex and smoking status (never, past, or current) were collected. Height (to ±0.1 cm) was measured in all patients using a wall-mounted stadiometer, and body weight (to ±100 g) was measured using an electronic calibrated scale. Blood samples were collected from patients after an overnight (12 hour) fast for analysis of renal function, hepatic function, lipid profile, HBA1c, fasting and postprandial blood glucose, hemoglobin, iron indices [ferritin, iron, total iron binding capacity (TIBC)], calcium, phosphorus, 25-hydroxy vitamin-D [25(OH)D] and intact parathyroid hormone (iPTH). Lipid profile, fasting and postprandial blood glucose and creatinine were measured using clinical chemistry analyzer (Daytona, serial number 58260536, Furuno Electric, Nishnomeya, Japan). Serum ferritin, iron and TIBC were estimated using enzyme linked immunosorbent assay (Diagnostic Automation, California, USA). iPTH was estimated using solid phase, enzyme labeled chemiluminescent immunometric assay (Immule 1000, Siemens, Gwynedd, UK). Serum 25(OH)D was estimated using chemiluminescence microparticle immunoassay (Architect 25-OH vitamin-D assay, Abbott, USA).
Patients with 25(OH)D >30 ng/mL were included in the study. The entire study protocol was explained to the patients and only those who gave their written informed consent and met all the inclusion and exclusion criteria were included in the study.

For estimation of isotope GFR, clearance of the radionuclide marker was measured after intravenous injection of $^{51}$Cr-EDTA. After a single bolus of 100 μCi (3.7 MBq) of $^{51}$Cr-EDTA, four venous blood samples were drawn at 75, 105, 135, and 165 min and urinary samples were collected at 90, 120, 150, and 180 min, as previously described (15). The $^{51}$Cr-EDTA radioactivity was measured on a γ counter.

Cockcroft-Gault (CG) GFR was calculated using the formula:

$$eCCr = \frac{[(140-\text{age}) \times \text{weight} \ (\text{in kilograms}) \times (0.85 \ \text{if female})]}{[\text{creatinine} \ (\text{in mg/dL}) \times 72]}$$

(16).

Using serum creatinine in mg/dL, MDRD GFR was calculated using the formula:

$$eGFR = 186 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times [1.212 \ \text{if black}] \times [0.742 \ \text{if female}]$$

(17).

Patients

Two hundred and thirty four patients were initially screened. Eighty-six patients were excluded because of 25(OH)D <30 ng/mL, 16 patients had edema, 6 patients had nephrotic range proteinuria, 12 patients refused to give consent for the study, 7 patients had received renal replacement therapy in the past, 4 patients had diabetic foot disease, 2 patients had uncontrolled hypertension, and one had coexistent tuberculosis. One hundred patients with diabetes (58 men and 42 women) who met all the inclusion and exclusion criteria were included in the study. Out of the 100 patients, 22 patients had type 1 diabetes (T1DM) and 78 patients had type 2 diabetes (T2DM).

Statistical analysis

Continuous data were expressed as mean ± standard deviation, and analyzed by t-test and ANOVA, as appropriate. Results of the CG formula and MDRD equation were compared with isotopic GFR by correlation. The sensitivity and specificity of both formulas were assessed from nonparametric ROC curves generated by plotting sensitivity versus 1 – specificity, giving the ideal test a sensitivity = 1 and specificity = 1. Correlations among methods of GFR estimation, PTH and other parameters were done using Spearman rank based coefficient. Categorical variables were compared using χ²-test. P<0.05 was considered statistically significant.

RESULTS

The mean age of the study population was 46.60±13.7 years. The mean body mass index (BMI) was 23.78±4.6 kg/m², mean serum creatinine 2.38 ± 0.3 mg/dL, mean HbA1c 8.41±1.2% and mean albuminuria 429±260 mg in 24-hour urine collection.

The mean isotopic GFR was 37.18±17.22 mL/min/1.73m². Of the 100 patients selected, 10 patients were in stage 2 CKD, 45 patients were in stage 3 CKD, 29 patients in stage 4 CKD and 16 patients were in stage 5 CKD (using isotopic GFR for stratification).

Comparison of clinical, anthropometric (BMI), GFR, ferrokinetic and calcium metabolism parameters of patients with diabetic CKD between males and females is shown in Table 1. In our study, BMI was significantly higher and hypertension was significantly more common among male diabetic CKD patients as compared to females (Table 1). Serum creatinine was significantly higher among female diabetic CKD patients. However, isotope GFR was not significantly different in females as compared to males (Table 1). Serum iron was significantly lower, with a lower total iron binding capacity (TIBC) among males as compared to females (Table 1).

The mean CG formula overestimated GFR (43.88 ±17.90 mL/min/1.73m²; P<0.05) and the mean MDRD equation underestimated GFR (32.28±18.06 mL/min/1.73 m²; P<0.001) when compared to isotopic GFR. There was a statistically significant positive correlation of both CG (r=0.74, P<0.0001) and MDRD (r=0.81, P<0.0001) estimations of GFR with isotopic GFR, with a slight advantage for the MDRD equation.
Table 1. Comparison of clinical, anthropometric (BMI), GFR, ferrokinetic and calcium metabolism parameters in males as compared to female patients with diabetic CKD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=58)</th>
<th>Female (n=42)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45.3±18.2</td>
<td>48.4±13.1</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of DM</td>
<td>188.87±74.89</td>
<td>168.00±99.02</td>
<td>NS</td>
</tr>
<tr>
<td>Family h/o DM</td>
<td>57.1</td>
<td>70.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>58</td>
<td>35</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.97±5.75</td>
<td>20.78±2.74</td>
<td>0.04</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>2.16±1.17</td>
<td>2.70±1.0</td>
<td>0.04</td>
</tr>
<tr>
<td>HbA1c</td>
<td>8.49±9.2</td>
<td>8.33±1.90</td>
<td>NS</td>
</tr>
<tr>
<td>Isotope GFR</td>
<td>3.5.90±17.25</td>
<td>39.01±17.31</td>
<td>NS</td>
</tr>
<tr>
<td>MDRD GFR</td>
<td>30.16±13.97</td>
<td>35.2±22.53</td>
<td>NS</td>
</tr>
<tr>
<td>CGF GFR</td>
<td>42.33±11.48</td>
<td>46.02±22.58</td>
<td>0.03</td>
</tr>
<tr>
<td>Hemoglobin (gm/dL)</td>
<td>8.93±3.05</td>
<td>9.67±2.15</td>
<td>0.09</td>
</tr>
<tr>
<td>Ferritin</td>
<td>213.19±320.35</td>
<td>331.28±539.10</td>
<td>NS</td>
</tr>
<tr>
<td>Iron</td>
<td>44.43±9.03</td>
<td>54.19±26.72</td>
<td>0.05</td>
</tr>
<tr>
<td>TIBC</td>
<td>259.60±45.80</td>
<td>322.55±126.48</td>
<td>0.01</td>
</tr>
<tr>
<td>Corrected calcium</td>
<td>8.86±1.23</td>
<td>8.78±1.63</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate</td>
<td>3.75±0.49</td>
<td>3.49±0.69</td>
<td>0.08</td>
</tr>
<tr>
<td>iPTH</td>
<td>122.03±86.53</td>
<td>144.16±108.86</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2. Correlation of isotope GFR with estimated GFR models, ferrokinetic parameters and measures of calcium metabolism

<table>
<thead>
<tr>
<th>Pearson correlation coefficient (r)</th>
<th>Isotope GFR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDRD</td>
<td>0.788</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CG</td>
<td>0.692</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb</td>
<td>0.586</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron</td>
<td>0.452</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corrected Ca</td>
<td>0.278</td>
<td>0.042</td>
</tr>
<tr>
<td>Phosphate</td>
<td>-0.405</td>
<td>0.043</td>
</tr>
<tr>
<td>iPTH</td>
<td>-0.577</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The Cockcroft-Gault formula overestimated high values of GFR according to the Bland-Altman procedure (mean +6.7 mL/min/1.73 m²); this was not the case for the MDRD equation (mean +4.9 mL/min/1.73 m²) (Fig. 1a, Fig. 1b).

The ROC curve analysis showed that the maximum diagnostic accuracy of the CG formula for the diagnosis of renal failure (GFR <60 mL·min⁻¹·1.73 m²) was lower than the MDRD equation (CG formula AU- 0.868, cutoff limit 56.5; MDRD equation AUC- 0.927, cutoff limit 54.7; P < 0.05). This was mainly due to better sensitivity of the MDRD equation estimation (CG formula sensitivity 77.9% and specificity 81.1%; MDRD equation sensitivity 91.9% and specificity 78.4%) (Fig. 2).
The mean serum phosphorus, corrected calcium and plasma iPTH in our population was 3.64±0.59 mg/dL, 8.83±1.40 mg/dL and 131.13 ±96.23 pg/mL, respectively. There was good correlation with 99mTc GFR with iPTH (r=-0.577) as well as with MDRD (r=-0.585). There was moderate correlation of phosphorus with isotope GFR (r=-0.202) and MDRD (r=-0.376), but poor correlation with corrected calcium (Table 2).

When defined by CKD stages, correlation was maximum in CKD stage 4 (r=-0.819) compared to stage 3 (r=-0.736) and stage 5 (r=-0.571) (Table 3).

In our study, 42.5% of patients had GFR <30 mL/min/1.73m2 (isotope GFR). Normal serum hemoglobin (>11 g/dL) was seen in only 27.4% of CKD patients in our study. The majority of patients (67.1%) had hemoglobin of 7-11 g/dL and 5.5% patients had hemoglobin <7 g/dL. When defined by CKD stages, correlation was maximum in CKD stage 5 (r=-0.571) as compared to stage 3 (r=-0.736) and stage 5 (r=-0.571) (Table 3).

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DISCUSSION

MDRD equations and CG formula have their imperfections. Overestimation at low GFR levels and the influence of weight reduce the sensitivity and accuracy of the CG formula (6,7). The MDRD equation is more difficult to calculate in clinical practice and underestimates GFR at high levels, but it has better accuracy in diagnosing and stratifying chronic renal failure in diabetic patients, which is an important advantage for a prediction formula. The MDRD equation is more accurate for the diagnosis and stratification of renal failure in diabetic patients (7). In our study, GFR calculated by both MDRD and CG formula correlated well with GFR measured by isotope scan. However, MDRD showed better correlation with isotope GFR than CG formula in diabetic patients. Thus, MDRD is a better method than CG in predicting CKD stages in Indian diabetic patients.

An elevated serum PTH level is considered to be one of the earliest markers of abnormal bone mineral metabolism in CKD (18,19). Early prediction as well as detection of increased PTH is important as it is an independent predictor of cardiovascular morbidity and mortality, endothelial dysfunction and systemic inflammation (18,19). However, there are limited data on iPTH elevation in patients with early CKD. There have been few reports suggesting that iPTH begins to rise in serum once the GFR falls below 60 mL/min (CKD stage 3) (20,21). In our study, there was a good negative correlation of iPTH with $^{99m}$Tc GFR as well as with MDRD GFR. Maximum correlation of iPTH with $^{99m}$Tc GFR was observed in stage 4 CKD as compared to stage 3 and 5. Hence, the rise in serum levels of iPTH can be more accurately predicted from GFR in patients with CKD stage 4.

Detection of anemia is important in patients with diabetic CKD in view of the commonly associated cardiovascular disease and hypoxia induced organ damage (22). It has been reported that anemia is more common in diabetic CKD patients with significantly lower serum hemoglobin as compared to nondiabetic CKD patients across all stages of CKD (23). In our study, the odds ratio for developing anemia in diabetic CKD patients was high (2.21), especially from stage 4 CKD onwards. Serum hemoglobin showed better correlation with isotope GFR than other indices of iron metabolism like serum iron, ferritin and TIBC. In patients with advanced stages of CKD, there was better correlation between isotope GFR and hemoglobin. Hence, frequent hemoglobin monitoring is more important as compared to other ferrokinetic parameters in patients with advanced diabetic CKD.

In diabetic patients, we need to be more vigilant for CKD if hemoglobin falls below 9.3 gm/dL, as it has 78% sensitivity for predicting CKD stage 3.

One of the major limitations of this study was the cross-sectional nature. Further prospective studies are warranted in a larger cohort of patients in view of these observations in our population.

To conclude, it may be said that MDRD GFR is a better predictor of renal function among Indians with advanced diabetic kidney disease, as it correlated better with isotope GFR as compared to CG formula. Rise in serum iPTH is more predictable after stage 3 CKD. Anemia is a significant problem among patients with diabetic kidney disease in our country, especially among patients with advanced CKD. Serum hemoglobin of less than 9.3% has a 78% sensitivity of predicting stage 3 CKD among patients with diabetes.
REFERENCES


