Cystatin C as a marker of GFR in comparison with serum creatinine and formulas depending on serum creatinine in adult Egyptian patients with chronic kidney disease.

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Abstract: Background and aim of the study: There is no literature available on the performance of cystatin C in adult Egyptian patients with Chronic Kidney Disease (CKD). Our study was aimed to compare the diagnostic performance of serum Cystatin C, serum creatinine, Cystatin C-based formula and creatinine-based formulas with measured glomerular filtration rate (GFR) in adult Egyptian patients with CKD. Methods: The study was conducted on 80 patients were known as CKD[42 of them where males (52.3%) and 38 females (46.7%)] with mean age 56.58 ± 13.06 years, attending the Nephrology Department, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. Serum cystatin C was measured with Human Cystatin C ELISA – Biovendor. TheeGFR was calculated using Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and simple cystatin C formulas. GFR was measured using 99mTC - diethylenetriaminepenta acetic acid (DTPA) renal scan method. Results: There was significant correlation between serum Cystatin C and measured GFR (r=-0.8792; p<0.0001) than between serum creatinine and measured GFR (r=-0.5861). There was significant correlation between Cystatin C –based formula in the studied CKD patients and the measured GFR in the same patients(r= 0.899; p< 0.0001) better than the correlation between measured GFR in the studied CKD patients and GFR calculated from the MDRD formula (r = 0.788; P< 0.0001) and C&G formula (r = 0.683; P< 0.0001) in the same patients. The receiver operating characteristic curve (ROC) analysis showed that serum cystatin C had bigger AUC and higher sensitivity(AUC:0.902; sensitivity:97.6%) than serum creatinine (AUC: 0.711; sensitivity: 72.6%). Also the cystatin C-based formula and MDRD, had bigger AUC (0.875; 0.930 respectively) and higher sensitivity (97.5%; 90.5% respectively) than the C&G formula (0.872; 81.0%), but no statistically significant differences between the formulas was found. Conclusion: The present study suggest that serum Cystatin C is a good alternative marker to serum creatinine in CKD patients and that Cystatin C-based formula, which requires just one variable (serum cystatin C), achieved a diagnostic performance that was at least comparable if not better than the creatinine-based formulas using more variables.

Keywords: GFR, eGFR; CKD; Cystatin C; 99mTC-DTPA

1- Introduction

Chronic kidney disease (CKD) is an important public health problem classified into stages according to the level of GFR. Therefore, estimation of the GFR is essential for the evaluation of patients with CKD and is useful tool to screen for CKD also in high-risk groups as persons with diabetes mellitus. GFR estimation allows us to detect early impairment of kidney function, prevent further deterioration and complications, correct the dosage of drugs cleared by the kidney so as to avoid potential drug toxicity, and manage CKD patients. Recently, the National Kidney Disease Education Program (NKDEP) recommended reporting GFR values above 60 mL/min/1.73 m² not as an exact number but simply as >60 mL/min/1.73 m², and contrary for the values of 60 mL/min/1.73 m² and below the exact numerical estimate should be reported[1]. For clinicians the GFR below 60 mL/min/1.73 m² is very important. The values indicate the presence of CKD and represent an increased risk of impaired kidney function, progression to kidney failure, and premature death caused by cardiovascular events of patients with CKD[2,3].

Serum creatinine level, the most commonly used surrogate measure for glomerular filtration rate (GFR), does not increase until renal function decreases to 50% of it’s normal value. It’s excretion rate varies with age, sex, physical exercise and lean body mass. The population variance of serum creatinine level is large making it a poor measure for comparison with reference range[4]. Creatinine clearance is often inaccurate, but it is widely used in clinical practice. The gold standard tests such as 51cr - labeled EDTA, 99mTc labeled DPTA or Iohexol are too cumbersome to use in clinical setting[5].

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Over the last decades several different markers for estimation of GFR have been proposed. Despite all known disadvantages, serum creatinine and predictive equations, such as the Cockcroft-Gault (C&G) formula and abbreviated modification of diet in renal disease (MDRD) formula, have become the most commonly used marker to estimate GFR in clinical practice as in most studies [6-8]. Furthermore, estimation of GFR derived from MDRD formula is recommended in annual evaluation of all patients with type 2 diabetes mellitus (DM2) [9]. Unfortunately, both these formulas are also limited by lack of validation in the full range of GFR to which they are applied [10].

Recently, serum cystatin C low-molecular-weight protease inhibitor, that is freely filtered across the glomerular membrane and then reabsorbed and metabolized in the proximal tubule, was proposed as a new endogenous marker of GFR [11,12]. The previous reports have suggested that serum cystatin C is a better indicator of GFR than serum creatinine in patients with spine injury, liver cirrhosis, diabetes, mild to moderate impaired kidney function, and in elderly patients [13-17].

**Aim of the study**

To compare the diagnostic performance of serum CystatinC, serum creatinine, cystatin C-based formula and creatinine-based formulas with measured glomerular filtration rate (GFR) in adult Egyptian patients with CKD.

**2- Subjects and methods**

This study was conducted on 80 patients were known as CKD[42 of them where males (52.3%) and 38 females (46.7%)] with mean age 56.58 ± 13.06 years, attending the Nephrology Department, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. The patients with cardiac failure, malignancy and liver cirrhosis were excluded from the study. Written consent was obtained from all the study participants.

All patients subjected to full history taking and clinical examination. Blood sample (10 cc) was drawn from each patient. Venous blood is collected and divided in tubes as follows: tubes in which blood samples were centrifuged and serum aliquoted and stored at -70°C until cystatin C measured, tubes in which blood samples were centrifuged and serum aliquoted were routine investigations where done, tubes containing EDTA for blood picture and tubes containing citrate for prothrombin time and concentration and INR.

Routine investigations as complete blood picture, kidney function tests:[Serum urea, creatinine, sodium and potassium and uric acid], liver function tests: [Alanine transaminase (ALT), Aspartate transaminase (AST), Prothrombin time (PT) and concentration (PC) and international normalized ratio (INR) and serum albumin, ESR, random blood sugar and urine analysis were done.

GFR was measured using 99mTc - diethyleneetriaminepenta acetic acid (DTPA) renal scan method.

**Serum Cystatin C (Human Cystatin C ELISA – Biovendor) Assay procedure:**

Reagents prepared, Standards, controls and samples are diluted as follows: Each concentration is diluted 400x in two steps (10x and 40x). 100µl were pipetted of each standard, control and sample into appropriate wells. The plate is incubated at room temperature for 30 minutes; shaken at about 300 rpm on the orbital microplateshaker. The wells are washed 3 times with wash solutions, remaining washing solution was removed. 100µl of conjugate solution was added in each well. Plate incubated at room temperature for 30 minutes; shaken at about 300 rpm on the orbital microplateshaker. The wells are washed 3 times with wash solutions, remaining washing solution was removed. 100µl of substrate solution was added and protected from light by covering plate with aluminum foil. Incubation at room temperature for 20 minutes. Adding 100µl of stop solution stopped the color development. Optical density was determined in the plate by reading absorbances at 450nm. (Yang X, 2006[18]).

**GFR was calculated** according to C&G, MDRD, and cystatin C based formulas:

**C & G formula:**

\[
GFR \text{ (ml/min/1.73m}^2) = \left(\frac{140-\text{age}}{72} \times \text{weight (kg)}\right) \times \text{s.Cr (mg/dl)}.
\]

**MDRD formula.**

\[
GFR=\frac{1.2 \times \text{s.Cr (mg/dl)} \times 0.85 \times \text{weight (kg)}}{1.73 \times \text{age} \times 0.203}
\]

**Simple cystatin C formula:**

\[
100 \times \text{CystatinC(mg/L)}
\]

**IV-Statistical Analysis**

The results were expressed as means ± standard deviation of the means (SD) or percentage. Pearson’s correlation coefficient was used for defining the correlation between measured GFR and serum creatinine, serum cystatin C, the GFR calculated from the serum creatinine-based formulas, and the GFR calculated from the cystatin C formula. In order to determine the diagnostic accuracy of the serum cystatin and Cysatin C-based formula in comparison with the other markers of GFR, receiver-operating characteristic (ROC) plots were constructed and
analysed. The area under the curve (AUC) describes the test’s overall performance and is used to compare different tests. Sensitivity and specificity were calculated. The measured GFR was used as the gold standard and the cut-off value was set at 60 mL/min/1.73 m² for CKD as defined by the National Kidney Foundation [22]. The analysis was performed using Statistical Analysis System, version 6.03, on an IBM at personal computer and MedCalc for windows (version 12.7.5). P value <0.05 was considered significant.

3- Results.

The Demographic features of the studied CKD patients are shown in Table 1

The mean age of the studied 80 CKD patients was 56.58 ± 13.06 (range: 24-68. years). The mean value of BMI was 26.82 ± 7.47.

The laboratory profile of the studied CKD patients is shown in Table 2:

The mean value of s.creatinine, within the studied CKD patients was 1.97 ± 0.32 (mg/dl), while for BUN, the mean value within the studied CKD patients was 38.4 ± 15.33 (mg/dl).

The mean value of serum albumin within the studied CKD patients was 3.41 ± 0.42 (g/dl).

The mean value of serum cystatin C within the studied CKD patients was 1.64 ± 0.27 (mg/l).

The mean value of measured GFR in the studied CKD patients was 35.46 ± 8.57 (mL/min/1.73 m²).

The mean value of the C&G, MDRD and Cystatin C-based formulas in the studied CKD patients are shown in Table 3.

The mean value of CG in the studied CKD patients was 43.16 ± 15.83 (mL/min/1.73 m²).

The mean value of MDRD in the studied CKD patients was 33.14 ± 11.72 (mL/min/1.73 m²).

The mean value of cystatin C-based formula in the studied CKD patients was 36.54 ± 7.50 (mL/min/1.73 m²).

Correlation between serum Cystatin C and serum creatinine with measured GFR and estimated GFR

There was significant correlation between serum Cystatin C and measured GFR (r=0.8792; p< 0.0001) than between serum creatinine and measured GFR (r=0.5861). Serum creatinine correlates with eGFR_{CG&MDRD} (r=0.8647; p<0.0001 and r=0.9213; p<0.0001) better than the correlation of cystatin C with eGFR_{CG&MDRD} (r=0.6120 and r=0.5467). The ROC analysis showed that serum cystatin C (AUC: 90.2%; sensitivity: 97.6%; specificity: 78.9%) had bigger AUC and higher sensitivity than serum creatinine (AUC: 0.711; sensitivity: 72.6%; specificity: 65.8%). (Fig.1;Table 4)

For the correlation between serum Cystatin C and creatinine with age and BMI, there was no significant correlation with serum cystatin C (r=0.0260, and r=0.0843, respectively; p>0.05) and serum creatinine showed significant correlation with age (r=−0.6225; p<0.01) and BMI (r=−0.5137; p=0.0223).

Correlation between the Cystatin C –based formula and measured GFR.

There was significant correlation between Cystatin C – based formula in the studied CKD patients and the measured GFR in the same patients (r= 0.899; p< 0.0001).

Correlation between the creatinine –based formulas and measured GFR.

There was Statistically significant correlation between measured GFR in the studied CKD patients and GFR calculated from the MDRD formula (r= 0.788; P< 0.0001) and C&G formula (r= 0.683; P< 0.0001) in the same patients. The Correlation between the Cystatin C –based formula and the C&G, and the MDRD in the studied CKD patients. There was a significant correlation between Cystatin C –based formula and CG (r= 0.671; p< 0.01). There was a significant correlation between Cystatin C –based formula and MDRD (r= 0.613; p< 0.01).

The ROC and AUC analysis of creatinine-based formulas (C&G and MDRD) and cystatin C-based formula in the studied CKD patients.

The ROC curve analysis (cut-off for GFR 60 mL/min/1.73 m²) showed that the cystatin C-based formula and MDRD, had bigger AUC(0.875;0.930 respectively) and higher sensitivity(97.5%; 90.5% respectively) than the C&G formula(0.872;81.0%), but no statistically significant differences between the formulas was found ( Figure 2).

Diagnostic accuracy (AUC, sensitivity, and specificity) at the cut-off value for GFR 60 mL/min/1.73 m² of the different creatinine-based formulas, and the cystatin C-based formula are presented in table 5.

The Cystatin C –based formula showed the nearest mean value (36.54 ± 7.50 mL/min/1.73 m²) to the measured GFR mean value in the studied patients (35.46 ± 8.57 mL/min/1.73 m²) with a difference of only 1.08 mL/min/1.73 m², compared with 2.32 mL/min/1.73 m² with MDRD and with 7.7 mL/min/1.73 m² with CG.

The MDRD formula underestimated the GFR with -2.32 mL/min/1.73 m². While the CG showed maximum lack of precision with an overestimation of GFR with 7.7 mL/min/1.73 m², and the most accurate formula for our patients was the cystatin C-based formula with only an overestimation of 1.08 mL/min/1.73 m².
Table 1: Demographic features of the studied CKD patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD patients (n= 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yrs</td>
<td>24-68</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>56.58 ± 13.06</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 42 (52.3%)</td>
</tr>
<tr>
<td></td>
<td>Female 38 (47.7%)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>71.48 ± 17.26</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.67 ± 0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.82 ± 7.47</td>
</tr>
</tbody>
</table>

Values are the mean ± SD or (n) = number tested or (%) = percent.

Table 2: Laboratory profile of the studied CKD patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD patients (n= 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin(g/dl)</td>
<td>3.41 ± 0.42</td>
</tr>
<tr>
<td>BUN(mg/dl)</td>
<td>38.4 ± 15.33</td>
</tr>
<tr>
<td>S. creatinine(mg/dl)</td>
<td>1.97 ± 0.32</td>
</tr>
<tr>
<td>Measured GFR(mL/min/1.73 m²)</td>
<td>35.46 ± 8.57</td>
</tr>
<tr>
<td>S.Cystatin C(mg/l)</td>
<td>1.64 ± 0.27</td>
</tr>
</tbody>
</table>

Values are the mean ± SD or (n) = number tested.

Table 3: The mean values for the Creatinine-based formulas of the studied CKD patients compared with Cystatin C–based formula in the same patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD patients (n= 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&amp;G( mL/min/1.73 m²)</td>
<td>36.54 ± 7.50</td>
</tr>
<tr>
<td>MDRD( mL/min/1.73 m²)</td>
<td>34.14 ± 11.72</td>
</tr>
</tbody>
</table>

Values are the mean ± SD or (n) = number tested.

Table 4: Diagnostic accuracy (AUC, sensitivity, specificity) and comparison of ROC curves of serum cystatin C and creatinine as markers of GFR.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>P value</th>
<th>P* value</th>
<th>P** value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cystatin C</td>
<td>0.902</td>
<td>88.1%</td>
<td>78.9%</td>
<td>0.0008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>0.711</td>
<td>72.6%</td>
<td>65.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P* value calculated according to cystatin C–based formula.
P** value calculated according to MDRD formula.

AUC: Area under the curve.

4- Discussion

The Kidney Disease Outcomes Quality Initiative (K/DOQI) CKD guidelines have established a five-stage classification of patients with CKD that is based solely on kidney function. The guidelines state that the stage of kidney disease should be determined for each CKD patient and that a clinical action plan should be developed on the basis of the stage of disease[23]. Thus inaccurate estimation of kidney function may be responsible for misclassification of some patients and lead to inappropriate evaluation or treatment of these patients[24].

The formulas that are most widely used to estimate kidney function and that are recommended in adults by K/DOQI guidelines are the Cockcroft-Gault (CG) formula[19] and simplified Modification of Diet in Renal Disease (MDRD) formula[20]. However,
the formulas have some well-known limitations. Therefore, new alternatives like creatinine-based CKD-EPI equation, cystatin C-based formulas, and equation that use both serum creatinine and serum cystatin C were developed.

It has been demonstrated that the (CG) formula can overestimates GFR at low renal function levels and underestimate high GFR values. Other GFR overestimation biases were demonstrated for overweight patients, young and females subjects.

The MDRD clearly underestimates GFR in subjects with normal renal function. Levey et al, emphasized the need for caution in applying the MDRD formula to individuals with a Scr. within the normal range because it has not been validated in people without renal disease. That the MDRD underestimated GFR was also recently demonstrated in renal failure in females and in overweight patients, while the opposite is true of lean subjects.

Many studies comparing the MDRD and CG equations using GFR measured by reference to iohexol confirmed these findings: CG was relatively more accurate in subjects with mild or no renal insufficiency while the MDRD performed better in kidney transplanted patients with renal failure.

A substantial body of evidence has developed over the past several years which supports the use of Cystatin C as an alternative and more sensitive endogenous marker for the estimation of GFR than serum creatinine and serum creatinine based GFR estimations.

In our study, we evaluated the performances of the serum Cystatin C, serum creatinine, Cystatin C-based formula and creatinine-based formulas (CG and MDRD) as markers of GFR in a cohort of 80 Egyptian CKD patients. An important characteristic of our study is that, included patients whose s.creatinine ranged from 0.6 to 3.0 mg/dl, thus the performances of CG and MDRD formulas and Cystatin C-based formula could be assessed over a wide range of kidney function.

Furthermore, because all patients included in the study are Egyptians, the performance of the MDRD and CG could be assessed in a group of patients whose anthropometric characteristics are different from Europeans and Americans.

Our present study showed that serum cystatin C is the most useful endogenous marker of GFR. We also have shown that the simple cystatin C-based formula achieved at least a comparable if not better a diagnostic performance than the creatinine-based formulas.

The present study showed that, the correlation of serum cystatin C with measured GFR was better than the correlation of serum creatinine with measured GFR. These results suggest that cystatin C is a good marker of renal function in patients with renal impairment, as has been reported in non-diabetic patients, patients with renal transplant and in healthy patients.

Cystatin C is proposed to reflect GFR independent of age and BMI. Also in the present study, similar findings have been observed. Our present study showed that, there was no significant correlation of serum cystatin C with age and BMI ($r=0.0260$ and $r=-0.0843$, respectively; $p>0.05$) and serum creatinine showed significant correlation with age ($r=0.6225; p<0.01$) and BMI ($r=-0.5137; p=0.0223$). Serum creatinine correlated well with eGFR (C&G and MDRD) than serum cystatin C, which may be due to the eGFR being calculated using serum creatinine levels.

There is also evidence that confirms the influence of creatinine with BMI. In the study by O’Riodan et al. among 53 geriatric outpatients aged >70 years, cystatin C was considerably more accurate than creatinine in estimating GFR, with values greater than reference range having a 97% sensitivity in detecting GFR <60 ml/min/1.73m$^2$ compared with a sensitivity of only 37% for creatinine.

Similarly, the present study also revealed that cystatin C was found to be more accurate than creatinine in estimating GFR with 97.5% sensitivity, compared with a sensitivity of 72% for creatinine in all age groups of patients. An increased serum cystatin C concentration may provide a clinically important indication of a decreased GFR, even if serum creatinine concentration remains unchanged. This fact confirms that cystatin C is less dependent on age groups.

Some authors even concluded that the cystatin C formula is complementary to the serum creatinine-based equations or can be used in place of the serum creatinine-based equations.

Similarly in our present study, the correlation between the “gold standard” measured GFR and the cystatin C-based formula was better than the correlation between the measured GFR and GFR calculated with the MDRD and C&G formulas. According to our results, the cystatin C-based formula and MDRD formula had bigger AUC and higher sensitivity than C&G formula, but no statistically significant difference in diagnostic accuracy between the cystatin C-based formula and creatinine-based formulas was found.

In our study, the MDRD formula underestimated the GFR with -2.32 mL/min/1.73 m$^2$. While the CG showed maximum lack of precision with an overestimation of GFR with 7.7 mL/min/1.73 m$^2$, and the most accurate formula for our patients was the cystatin C-based formula with only an overestimation of 1.08 mL/min/1.73 m$^2$. 
As in other studies, the two most important biases of the CG and the MDRD formulas go in the opposite direction, i.e. they respectively overestimate and underestimate the GFR in healthy and overweight subjects, particularly among females. A certainly normal Scr (0.85mg/dl) gives rise to a GFR estimation of 87ml/min/1.73m² by the MDRD formula (which is likely to prompt an erroneous diagnosis of 'mild renal failure'), while the CG formula would suggest 'hyperfiltration'\cite{36,39,44,65}.

Conclusion
The results of the present study suggest that Cystatin C is a good alternative marker to creatinine in CKD patients and that Cystatin C-based formula, which requires just one variable (serum cystatin C), achieved a diagnostic performance that was at least comparable if not better than the creatinine-based formulas using more variables.

References


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