

Assessment of Cystatin C-Based GFR Estimating Equations as a Novel Reliable Biomarker for Renal Pathology Diagnosis in Patients with Mild to Severe Tubular Affection

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How to cite this paper: Ibrahim, M.A., Nagdi, N. and Kamel, C.R. (2022) Value of Cystatin C Based Equations for Assessment of Glomerular Filtration Rate in Relation to Renal Biopsy. *Open Journal of Nephrology*, 12, 426-440.
<https://doi.org/10.4236/ojneph.2022.124043>

Received: August 23, 2022

Accepted: December 5, 2022

Published: December 8, 2022

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Abstract

Background and Objective: Serum creatinine, a commonly used biomarker in determining glomerular filtration rate (GFR) and chronic kidney disease (CKD) stage, is highly variable biologically and does not rise until > 50% of renal function (RF) impairment occurs. Also, its production is not constant & is affected by many factors as muscle mass, age, inflammation. On the other hand, Cystatin C shows more stable production making it more suitable for assessment of kidney function. Also, It has been shown that the progression of CKD to renal failure, even in glomerular diseases, correlated better with the degree of tubular damage and interstitial fibrosis. So, our aim was to investigate the relation between kidney function assessed by different cystatin (Cys-C)-based estimated glomerular filtration rate (eGFR) in comparison to the gold standard Iohexol (Ioh) based measured (m)GFR in relation to the pathological degree of tubular damage in renal biopsy. To our knowledge, this is the first study that evaluates the relation of (Cys-C)-based eGFR to tubulointerstitial fibrosis in renal biopsy. **Methods:** This cross-sectional study was performed on 20 CKD cases who attended the Nephrology Department at Ain Shams University, where a renal biopsy was obtained, and individuals were allocated into two groups: group A (GA) with mild tubular affection (TA) and group B (GB) with moderate to severe TA. All participants were referred for measurement of GFR using Iohexol (Ioh) together with serum Cys-C level and eGFR was calculated using different Cys-C-based GFR estimating equations, which were further compared using Multivariate Linear Regression and Bland-Altman analyses. **Results:** Our results revealed a substantial statistical difference among the two studied groups regarding Hb, s

creatinine, urea. GB had significantly lower levels for both eGFR and mGFR (82, 93, 115, or 115) ml/min/1.73m², Vs. GA (200, 123, 162 or 124) ml/min/1.73m², according to GFR_{iohexol}, Stevens, Grubb, and CKD_EPI_CYST equations, respectively, $p < 0.05$. EGFR by CysC-based equations (Stevens, Grubb, and CKD_EPI_CYST) underestimated mGFR, when compared to Iohexol clearance with statistical significance in all patients (by $Z = -3.280\%$, -2.878% , -3.280% , respectively) and cases with mild tubular affection (by $Z = -3.11\%$, -2.657% , -2.972% , respectively) ($p < 0.05$), but with non-statistical significance in moderate to severe tubular affection category (B), $p > 0.05$. A significant correlation between CKD-EPI CYST and mGFR_{Iohexol} (Ioh) for GA was found ($R = 0.601$, $p = 0.030$), where there was a non-substantial relation between any of the used equations and the mGFR in category B ($p > 0.05$). There was no independent association between the eGFR results and Iohexol clearance. Stevens eGFR had the highest-level bias 33.9 compared with CKD_EPI_CYST (28) and Grubb eGFR (22.85). **Conclusion:** eGFR by CysC-based equations underestimate GFR in comparison to GFR-iohexol. There is significant correlation between eGFR by CysC-based equations and the gold standard GFR-iohexol only in mild degree of tubular affection and only with CKD-EPI-CYST equation. Stevens equation showed the highest bias while Grubb equation showed the least bias. Although cystatin-based equations have demonstrated a high level of correlation with measured GFR, they are still regarded as imprecise and cannot be established as equal to measured GFR or as a gold standard for GFR estimate.

Keywords

Cystatin C, Chronic Kidney Diseases, Glomerular Filtration Rate, Iohexol Clearance

1. Introduction

CKD is described as the existence of renal impairment or an estimated eGFR < 60 ml/min/1.73m² that lasts for 90 days or longer regardless of etiology and is graded into 6 phases depending on GFR (G1 to G5 with G3 split into 3a and 3b). It is a gradual decrease of kidney function that eventually necessitates the use of kidney dialysis or transplantation [1].

In glomerular diseases, although the disease course is usually prolonged, and in many cases there is a risk of chronic renal failure (CRF) development, its behaviour is difficult to predict. On the other hand, even advanced glomerular lesions seen in a biopsy do not necessarily have to be associated with a major impairment of renal function. Therefore, it is necessary to search for morphological and functional parameters that might facilitate the prediction for a further development of the disease [2].

In 1968, Risdon, Sloper and Wardener studied the associations between morphological parameters and renal function in patients with persistent glomerulo-

nephritis and found a strong relation between the level of renal function, the degree of tubular loss and interstitial fibrosis and risk of renal failure progression [3]. In subsequent years, Bohle *et al.* published a series of reports where they emphasised the importance of tubulointerstitial lesions [4].

GFR and chronic renal disease grading are generally determined by monitoring the concentrations of endogenous blood indicators like serum creatinine. Creatinine (Cr), on the other hand, is prone to substantial biological variation, and Cr concentration doesn't really increase till almost 50% of renal function is lost, resulting in erroneous CKD grading and false negatives [5]. In addition, in elderly people, serum Cr is not a useful indication of GFR. Moreover, to the significant influence of age on kidney structure and function, the same GFR level in various age groups may have varying pathophysiologic or non-pathophysiologic effects on renal function. Furthermore, the majority of the included studies demonstrated a gender difference in CKD prevalence. Females were more likely than males to have CKD. Females have less muscle mass than males, and muscle mass is a significant driver of blood creatinine levels [6].

To tackle these hurdles, Cystatin C has been demonstrated to be less susceptible to biological interference and more sensitive to early losses in renal function [5]. Cystatin C is a 13-kDa protein that is generated by all nucleated cells and belongs to the cysteine proteinase inhibitor class. Its production rate remains constant from 1 to 50 years of age. Cystatin C has gained widespread acceptance as an endogenous biomarker of GFR and is now routinely used in the assessment of CKD [7].

Reagents and clinical assays have varied significantly over time, resulting in a plethora of cystatin C-based estimated GFR equations (eGFR) with varying coefficients to account for the variation in concentrations measured [8].

The current work sought to evaluate the performance of Cystatin C-based eGFR equations evaluated by immunoturbidimetry in relation to the most constant renal pathological changes related to chronic kidney disease (CKD) which is tubular damage and tubulointerstitial fibrosis, in comparison to the gold standard mGFR by Iohexol clearance.

2. Patients and Methods

This cross-sectional study was performed on 20 cases with CKD who attended the Nephrology Department at Ain Shams University hospital in Cairo, where a renal biopsy was obtained, and individuals were allocated into two categories: patients with mild tubular affection [group A, (score 1, 2)] and those with moderate to severe tubular affection [group B, (score 3, 4)].

Prior to the start of the study, the proposed procedures were announced to all individuals who agreed to participate and satisfied the inclusion criteria. A detailed history was taken, which included demographic information (age, weight, and body mass index kg/m²). The full general examination included pulse, blood pressure, respiratory, cardiovascular, and abdominal.

2.1. Exclusion Criteria

The following were the exclusion criteria: diabetes, advanced liver and cardiovascular disease, severe muscle wasting, severe malnutrition, and history of dye sensitivity.

2.2. Methodology

After exclusion of patients with the above-mentioned exclusion criteria, informed signed consent of all study participants was taken. Ten (10 cc) of venous blood were withdrawn from every patient in each group under full aseptic condition after fasting overnight. Blood was transferred to an Eppendorf tube at 37°C for 30 minutes to clot and centrifuged at 4000 rpm for a further ten min. The obtained serum was put in aliquots kept at -70°C until the analysis time to determine marker serum level.

2.2.1. Measurement of Cystatin C

The CysC level in frozen-thawed serum was determined using a particle-enhanced turbidimetric immunoassay (PETIA) as reported early by [9]. EGFR calculated via the following 3 CysC-based equations:

$$\text{Stevens: } eGFR = 76.7 \times cys^{-1.19} \quad [10] \quad (1)$$

$$\text{Grubb: } eGFR = 87.62 \times cys^{-1.693} \times (0.94 \text{ if female}) \quad [11] \quad (2)$$

$$\text{CKD-EPI CYST: [12] Equation (3)}$$

- If serum cystatin is ≤ 0.8 : $\rightarrow 133 \times \min (s.cys/0.8)^{-0.499} \times 0.996^{\text{age}} \times 0.932$ if female
- If serum cystatin is > 0.8 : $\rightarrow 133 \times \max (s.cys/0.8)^{-1.328} \times 0.996^{\text{age}} \times 0.932$ if female

2.2.2. Routine Investigations

All participants were referred for routine laboratory investigation tests, including complete blood picture (CBC), coagulation profile, renal function examination (serum urea, Cr, Na, and K), hepatic function test (ALT, AST, serum albumin, uric acid), complete urine analysis and protein/creatinine ratio.

2.2.3. Measurement of GFR

The gold standard for measuring GER was serum IOHEXOL clearance. A 5 mL IV bolus of Ioh (Omnipaque 300) was administered. Blood samples were collected every 2, 3, 4, 5, and 24 hrs. The specimens had been centrifuged, and the values were obtained using High performance liquid chromatography (HPLC) and plotted into a curve to determine the area under the curve (AUC). Clearance was calculated according to the formula of one compartment model

$$Cl = \frac{\text{Dose}}{\text{AUC}} \quad (4)$$

where Dose is the full quantity of I_2 supplied during the bolus. The AUC is the

area under the curve correlating to the body's time spent in contact with Ioh. Plasma clearances (Cl_p) were then computed using the formula of Brochner-Mortensen *et al.*,

$$Cl_p = [0.990778 \times Cl_1] - [0.001218 \times Cl_2], [13] \quad (5)$$

Although the blood specimen number was onerous, the 24-hour sample, when incorporated in the Cl_p calculation, the GFR measurement became more reliable. Earlier blood specimens (T2 - T4 and T2 - T6) overestimated GFR, whereas for $GFR < 60$ mL per min per 1.73 m^2 a late timespan (24 hr) is necessary to decrease bias testing, that causes a 10% overstatement of GFR [14].

2.2.4. Renal Biopsy Examination

Renal biopsy was studied under a light and electron microscope, with a focus on tubular pathology. Tubular atrophy (TA), interstitial fibrosis (IF), interstitial edema (IE), interstitial inflammation, and acute tubular damage (ATD) all were evaluated semiquantitatively on a scale from 0 to 3 dependent on the proportion of cortex affected region (1, 1 to 25, 26 to 50, and more than 50%). Arteriosclerosis and arteriolosclerosis were graded from 0 to 3 (absent, mild, moderate, and severe) based on the degree of luminal constriction and artery wall thickening, respectively [15].

2.3. Ethical Consideration.

Approval of the study design was obtained from the Institutional Review Board (IRB) unit and the Research Ethical Committee in the faculty of Medicine; Ain Shams University.

2.4. Patient Consent

The proposed study methods were presented to all subjects, an oral and informed written permission consent document was signed by those who agreed to participate before sample collection.

2.5. Statistical Analysis

On an IBM personal computer, data was evaluated utilizing the SPSS (Statistical Package for Special Science) software, V_r 25. The Spearman's rank correlation coefficient analysis is utilized to ascertain the statistical dependency of two variables. The Mann-Whitney-U test is utilized to evaluate two sets of data whose distribution is unknown. Bias-Precision: the average difference between predicted and observed renal function was defined as bias, and the SD of this discrepancy was represented as precision. The Bland and Altman (BA) technique was utilized to show the discrepancies among calculated and measured GFR levels. Multivariate Linear Regression Analysis was utilized to look for an independent relationship between any of the estimated GFR outcomes and Iohexol clearance. The Wilcoxon test was used to compare Iohexol clearance to other eGFR techniques.

3. Results

Demographic characteristics of 20 CKD cases (40% were females), including 13 cases with mild tubular affection, and 7 cases with moderate to severe tubular affection, are presented in **Table 1**. The average age of all individuals involved in our current study was 35.9 ± 8.4 and 34.9 ± 16.2 , respectively. **Table 1** demonstrated that there is no statistically significant difference regarding age ($p = 0.847$), gender ($p = 0.052$), and BMI ($p = 0.863$) among the 2 groups of the current research. Additionally, there was a non-significant difference with respect to the degree of tubular affection and virology among all studied categories (A and B), $p > 0.05$.

The routine laboratory tests were presented in **Table 2**; the mean (hemoglobin) Hb value was 12.7 ± 2.9 and 8.6 ± 1.2 g/dl, for group A and B, respectively, with the same International Normalized Ratio (INR) $\sim 1.0 \pm 0.1$ in both groups. Our results revealed that there was a substantial statistical difference among the two studied groups regarding Hb, kidney function test (s. creatinine, Urea and serum uric acid), and ALT, $p < 0.05$, **Table 2**.

CysC-based eGFR was calculated using different equations (Stevens, Grubb, and CKD_EPI_CYST) in comparison to GFR_iohexol. As represented in **Table 3**, cases with moderate to severe tubular affection had significantly lower levels for both estimated and measured GFR (82, 93, 115, or 115) ml/min/1.73m², Vs. cases with mild tubular affection (200, 123, 162 or 124) ml/min/1.73m², according to GFR_iohexol, Stevens, Grubb, and CKD_EPI_CYST, respectively, $p < 0.05$.

CysC-based eGFR using Stevens, Grubb, and CKD_EPI_CYST formulas and mGFR_Iohexol were calculated for multiple correlations. Our results demonstrated a significant correlation between CKD-EPI-CYST and mGFR_Iohexol at the mild degree of tubular affection ($R = 0.601$, $p = 0.030$), whereas there was a

Table 1. Baseline characteristics of CKD patients among studied groups.

| | Group A (N = 13) | Group B (N = 7) | X ² | p Value |
|--------------------------------------------------|---------------------|--------------------|----------------|---------|
| Age (Years) | 35.9 ± 8.4 | 34.9 ± 16.2 | 0.196 | 0.847 |
| Gender | | | | |
| Male | 10 | 2 | 4.43 | 0.052 |
| Female | 3 | 5 | | |
| BMI (kg/m ²) | 26 ± 3.1 | 25.7 ± 3.4 | 0.175 | 0.863 |
| HTN | 5 (38.5%) | 2 (28.6%) | 0.196 | 0.526 |
| % of patients with active urinary sediment (AUS) | 3 (30%) | 4 (60%) | 2.32 | 0.151 |
| Virology (HCV) | 5 (38.5%) | 0 | 3.59 | 0.083 |

X² = Chi Square, HTN = hypertension.

Table 2. Comparison of laboratory profile among studied groups.

| | Group A (N = 13) | Group B (N = 7) | Z | p Value |
|--------------------------|---------------------|--------------------|-------|---------|
| Hg (g/dl) | 12.7 ± 2.9 | 8.6 ± 1.2 | 2.854 | 0.002* |
| INR | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.468 | 0.157 |
| s. creatinine (mg/dl) | 1.4 ± 1.4 | 5.0 ± 2.2 | 3.058 | 0.001* |
| BUN (mg/dl) | 22.6 ± 13.1 | 67.6 ± 32.4 | 3.052 | 0.001* |
| Na (mmol/L) | 134.1 ± 3.9 | 135.1 ± 6.8 | 1.114 | 0.275 |
| K (mmol/L) | 4.0 ± 0.7 | 4.2 ± 0.500 | 0.873 | 0.393 |
| UA (mg/dl) | 6.0 ± 0.7 | 7.9 ± 1.5 | 2.501 | 0.011* |
| Albumin (mg/dl) | 2.2 ± 0.9 | 2.7 ± 0.9 | 1.112 | 0.275 |
| TP(mg/dl) | 5.4 ± 1.1 | 5.6 ± 1.1 | 0.638 | 0.536 |
| ALT (U/L) | 16.1 ± 6.8 | 11.9 ± 4.5 | 2.080 | 0.037* |
| Protein/creatinine ratio | 2.8 ± 1.4 | 5.4 ± 6 | 0.833 | 0.438 |

Hg = Hemoglobin; INR = International Normalized Ratio; BUN = Blood Urea Nitrogen; TP = Total Protein.

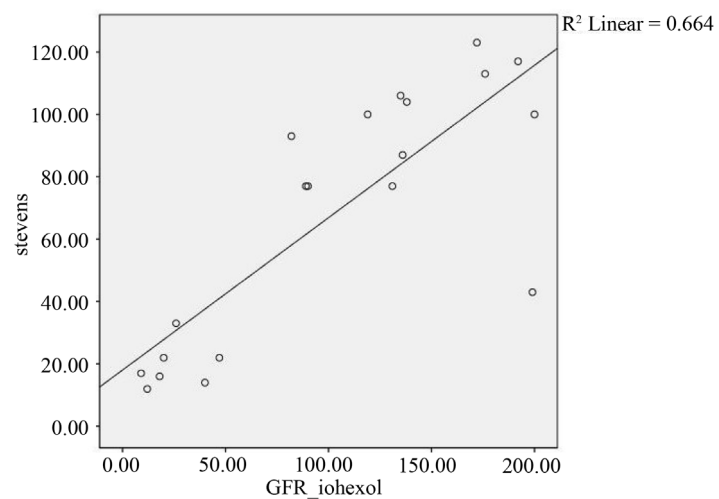
Table 3. Comparison between GA and GB as regards CysC-based eGFR using various equations.

| | Group A (N = 13) | | | Group B (N = 7) | | | Z | p Value |
|--------------|------------------|--------|-----|-----------------|--------|-----|--------|---------|
| | Min | Median | Max | Min | Median | Max | | |
| GFR_iohexol | 9 | 136 | 200 | 12 | 26 | 82 | -3.051 | 0.001* |
| Stevens | 17 | 100 | 123 | 12 | 22 | 93 | -2.899 | 0.002* |
| Grubb | 10 | 127 | 162 | 7 | 13 | 115 | -2.895 | 0.002* |
| CKD_EPI_CYST | 15 | 110 | 124 | 11 | 16 | 115 | -2.736 | 0.005* |

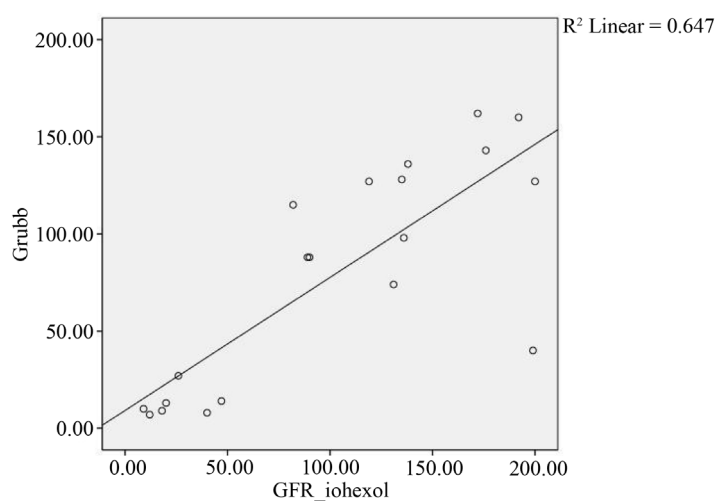
Z: Mann Whitney Test.

non-substantial relation among all the used equations and measured GFR at moderate to severe tubular affection ($p > 0.05$). For all patients, a strong significant statistical correlation between all equations and measured mGFR, with comparable correlation coefficients ($R = 0.799$, $p = 0.0001$) was found, as illustrated in **Table 4** and **Figure 1**.

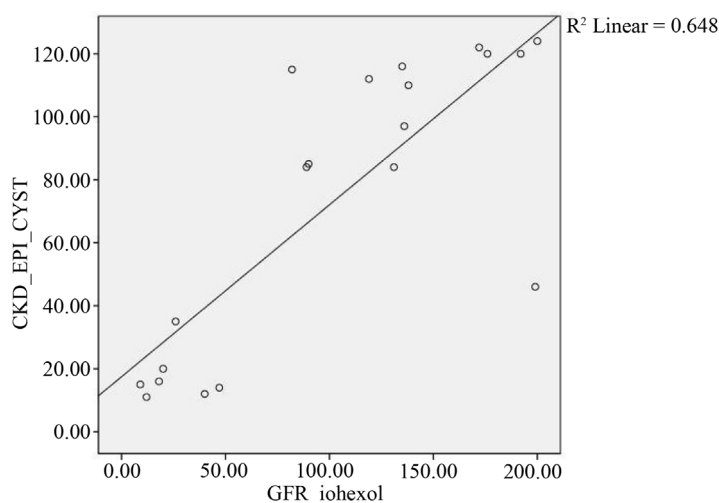
Table 5 presented the comparison between Iohexol clearance and different methods of eGFR in all patients and after patient's division according to the degree of tubular affection by renal biopsy. Our results revealed that eGFR by cystatin-based equations (Stevens, Grubb, and CKD_EPI_CYST) underestimate mGFR, when compared to Iohexol clearance with statistical significance in all patients (by $Z = -3.280\%$, -2.878% , -3.280% , respectively) and cases with mild tubular affection (by $Z = -3.11\%$, -2.657% , -2.972% , respectively) ($p < 0.05$), but with non-statistical significance in moderate to severe tubular affection category (B), $p > 0.05$.



(a)



(b)



(c)

Figure 1. Correlation between eGFR estimated by (a) Steven's equation (b) Grubb's equation or (c) CKD-EPICYST equation and iodhexol clearance (mGFR) as a Gold standard measure in all patients.

Table 4. Correlations among various eGFR estimate techniques and iohexol clearance as mGFR a gold standard measure: (mild tubular affection, moderate to severe, and all patients).

| GFR_iohexol | Group A | | | Group B | | | All patients | | |
|-------------|---------|-------|--------|---------|-------|-------|--------------|---------|---------|
| | S | G | ESK | S | G | ESK | S | G | ESK |
| R | 0.490 | 0.485 | 0.601* | 0.667 | 0.714 | 0.464 | 0.799** | 0.799** | 0.799** |
| P-Value | 0.089 | 0.093 | 0.030 | 0.102 | 0.071 | 0.294 | 0.0001 | 0.0001 | 0.0001 |
| Number | 13 | 13 | 13 | 7 | 7 | 7 | 20 | 20 | 20 |

S = Stevens, G = Grubb, CEC = CKD_EPI_CYST, R = Spearmanns correlation coefficient.

Table 5. Comparison of Iohexol clearance mGFR and various techniques of eGFR in all patients and after patient division based on degree of tubular affection (mild tubular affection, moderate to severe) by renal biopsy.

| | Group A | | | | | Group B | | | | | All patients | | | | |
|--------------|---------|-----|-----|--------|-------|---------|-----|-----|--------|-------|--------------|-----|-----|--------|-------|
| | Mdn | Min | Max | Z | p | Mdn | Min | Max | Z | p | Mdn | Min | Max | Z | p |
| GFR_iohexol | 136 | 9 | 200 | | | 26 | 12 | 82 | | | 105 | 9 | 200 | | |
| Stevens | 100 | 17 | 123 | -3.111 | 0.002 | 22 | 12 | 93 | -0.420 | 0.674 | 77 | 12 | 123 | -3.280 | 0.001 |
| Grubb | 127 | 10 | 162 | -2.657 | 0.008 | 13 | 7 | 115 | -1.101 | 0.271 | 88 | 7 | 162 | -2.878 | 0.004 |
| CKD_EPI_CYST | 110 | 15 | 124 | -2.971 | 0.003 | 16 | 11 | 115 | -0.420 | 0.674 | 85 | 11 | 124 | -3.280 | 0.004 |

Z: Wilcoxon Test; Mdn = Median.

Our results showed no independent association between any of the estimated GFR results and Iohexol clearance. Stevens eGFR had the highest-level bias 33.9 compared with CKD_EPI_CYST eGFR (28) and Grubb eGFR (22.85), **Table 6**, and **Figure 2**.

4. Discussion

GFR is commonly used to assess kidney function. It is most often calculated in clinical practice utilizing endogenous surrogate indicators. The most often utilized endogenous marker is serum creatinine. Serum cyst-C is a relatively recent endogenous indicator that has the benefit of being produced continuously via all nucleated body cells and being catabolized almost entirely at the proximal tubule. Serum cyst-C had been found in clinical investigations to be an accurate diagnostic of GFR, [16].

The CKD Epidemiology (CKD-EPI) formula, introduced in 2009, appears to be better accurate in calculating GFR than prior ones. Because creatinine procedures were not standard throughout the intervening institutions, resulting in discrepancies in creatinine readings, all of these formulas lack appropriate validation at the GFR at that they were used. Lastly, Cr-depend GFR estimates have numerous disadvantages and are dependent on numerous variables, and the precision of these formulas is hotly debated [17].

Cyst-C has been suggested as a new endogenous GFR biomarker. Although newer research has questioned these findings, serum cyst-C level appears to be

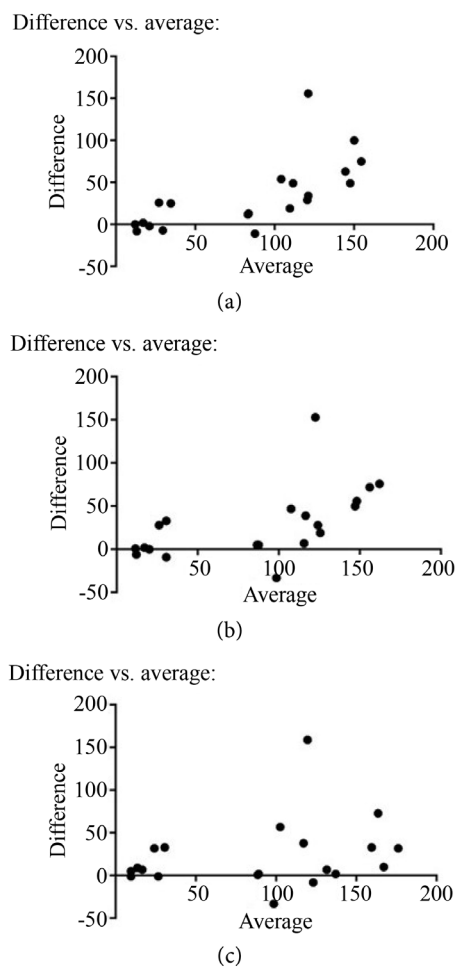


Figure 2. Bland-Altman plot comparing (a) Stevens' equation, (b) Grubb's equation and (c) CKD-EPI CYST equation with Iohexol clearance (mGFR).

Table 6. Multivariate linear regression and bland altman analysis.

| Multivariate Linear Regression Analysis (MLRA) | | | | | |
|------------------------------------------------|------------|---------|--------------|----------------|---------|
| | (Constant) | Stevens | Grubb | CKD EPI CYST | |
| B | -8.045 | 3.222 | -0.760 | -0.179 | |
| T | -0.211 | 0.900 | -0.410 | -0.097 | |
| p Value | 0.836 | 0.384 | 0.688 | 0.924 | |
| Bland Altman Analysis: | | | | | |
| | Stevens | Grubb | CKD EPI CYST | Kroskal wallis | p value |
| Bias | 33.9 | 22.85 | 28.65 | 0.642 | 0.725 |
| Precision | 41.6 | 40.1 | 40.8 | | |

unaffected by muscle mass, gender, aging, or dietary condition. Inflammation, fever, or other factors may not affect serum cystatin C levels. Furthermore, it appears to be a more accurate GFR indicator in diseases such as liver cirrhosis, dia-

betes mellitus, and the geriatric. Because of these qualities, several people have recommended cyst-C as a better exact measure of GFR than Cr, especially in persons of minor GFR impairment; however, these investigations are not only scarce but also conflicting and cover a small number of individuals [18].

Notwithstanding the theoretical benefits of cyst-C and the more refined formulae, the dispute persists, and no formula has been securely developed to measure GFR at any phase. As a result, the need for updated formulas is mostly owing to the lack of accuracy in estimating GFR, especially when the gold standard techniques of GFR assessment differ from one research to another [19]. Several formulae have been established based on creatinine and cystatin C. In this context, recent research wherein renal function was assessed using Iohexol clearance as the gold standard of GFR and Cr or cyst-C formulas is noteworthy [20].

In terms of demographic data, our analysis found no statistically significant difference among the 2 studied categories (A and B). eGFR estimated by Cystatin C-based equations had a strong correlation with mGFR estimated by Iohexol with comparable correlation coefficients (R), which is consistent with several studies, including one by Godwill *et al.*, who found that cyst-C levels were substantially linked with assessed GFR [21]. Also, our findings matched those of Abdallah *et al.*, who discovered a substantial association between the Cystatin C-based formula in the examined CKD patients and the measured GFR in the same patients [22].

Stevens *et al.* conducted a pooled analysis in which they estimated GFR utilizing serum Cyst-C alone and in conjunction with serum Cr, correlated significantly with GFR measured by Iothalamate but also to produce more reliable estimations, a formula combining serum cyst with serum Cr, age, gender, and race was proposed [10].

In a separate investigation, Inker *et al.* evaluated the efficacy of the Cyst_CKD_EPI formula alone and in contrast to the combined Cr-cyst-C formula, finding that the combined formula provided a highly precise and accurate assessment of GFR [12]

In accordance with our findings, Hojs *et al.* found that cystatin-based equations underestimated measured GFR and lacked accuracy [20]; nevertheless, these findings contrast other research by Gupta *et al.*, who reported cyst-based equations overestimated measured GFR [23]. Our findings also revealed a substantial degree of bias between cystatin C-based equations and Iohexol clearance, with a non-statistically significant tendency toward larger bias with Steven's equation and the least bias with Grubb's equation.

Steven's equation was compared to other several equations in a study by Harman *et al.* found ten research that looked at 14 different cyst-C based estimating formulae: Grubb *et al.*, Arnal-Dade, Macisaac *et al.* Stevens formula demonstrated the least bias and the maximum accuracy versus observed GFR utilizing kidney or Cl_p of contrast media, radioactive elements, or inulin (2013) [23].

Another research by Chudleigh *et al.* evaluated the performance of multiple

cystatin-based equations and discovered that all models underestimated GFR, with the Stevens equation showing less bias than the Rule and Perkins equations but higher bias than the Tan and MacIsaac equations [24].

Sharma *et al.* discovered that the diagnostic accuracy of several cystatin C equations varied with GFR in their investigation. This problem must be addressed when using these equations in clinical practice and in future research on eGFR equations [25].

According to Rule *et al.*, the various methodologies (urinary inulin clearance, plasma ^{99m}Tc -DTPA clearance, and plasma Iohexol clearance) employed as a GFR assessments gold standard reference could potentially contribute to part of the among-investigation variations, where variations in GFR assessment procedures are likely to be a substantial origin of diversity, [26].

Furthermore, Delanaye *et al.* believe that a significant cause of variance is the lack of established calibration for cyst-C testing. On comparing various cyst-C procedures, where considerable discrepancies have been recorded, and therefore when employing cystatin C-based equations, it is vital to understand that cystatin C estimations vary depending on whether the test is performed using a turbidimetric or nephelometric approach [27]. Other results of the present investigation include a strong relationship between the degree of tubular affection and Hb level, which was shown to be lower in group B patients compared to those in group A.

Patients with varied etiologies were studied, and it was shown that the prevalence of anemia was closely linked to a decline in GFR [28]. The present investigation also demonstrated that patients in category B (moderate to severe tubular affection) had higher levels of serum uric acid, which is consistent with a study by Zhou *et al.* that found hyperuricemia to be a marker for tubulointerstitial lesions [29].

5. Conclusion

According to our findings, GFR calculated using cystatin-based equations underestimates GFR when compared to GFR evaluated using iohexol. Only in moderate tubular affection and with the CKD EPI CYST equation is there a substantial association between GFR evaluated by cystatin-based equations and gold standard GFR iohexol. The Stevens equation had the greatest bias, whereas the Grubb equation had the least bias. Although cystatin-based equations have demonstrated a high level of correlation with measured GFR, they are still regarded as imprecise and cannot be established as equal to calculated GFR or as a gold standard for GFR estimate.

Acknowledgements

Approval of the study design was obtained from the Institutional Review Board (IRB) unit and the Research Ethical Committee in the faculty of Medicine; Ain Shams University. The proposed study methods were presented to all subjects,

an oral and informed written permission consent document was signed by those who agreed to participate before sample collection. Ethically compliant with the Helsinki Ethical Declaration.

Conflicts of Interest

The authors reported no possible conflicts of interest.

Funding

Funded by the authors.

Ethically compliant with the Helsinki Ethical Declaration and the committee of ethics of Ain.

Shams University Hospitals.

Data Available on reasonable request from Dr Cherry Reda via her email.

Contributions

Research idea and study design: MAI; data acquisition: NN; data analysis/interpretation: MAI, CRK; supervision or mentorship: MAI, CRK. CRK takes responsibility that this study has been reported honestly, accurately and transparently, and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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