A Practical Approach to Glomerular Filtration Rate Measurements: Creatinine Clearance Estimation Using Cimetidine

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Abstract. Determination of creatinine clearance (Ccr) is not a reliable indicator of glomerular filtration rate (GFR), owing to tubular secretion of creatinine. It has been reported that Ccr measurements can approximate true GFR after cimetidine (Ci) administration. In this study, GFR was estimated by Cockcroft and Gault’s equation (C \(_{CG}\)) based on measurement of plasma creatinine, and Ccr was determined by the standard clearance equation using 4- and 24-hr urine samples (Ccr\(_4\) and Ccr\(_{24}\), respectively) in 17 patients and 10 healthy controls. After cimetidine administration (800 mg, 3 times daily), GFR values were recalculated at the same time periods (C \(_{CG-Ci}\), Ccr\(_{Ci4}\) and Ccr\(_{Ci24}\), respectively). The results were all compared to those obtained by the \(^{99m}\)Tc-DTPA protein-free double-sample method (C\(_{DTPA}\)), which is a reference method for GFR determination. The coefficient of variation (CV%) for Ccr\(_{24}\)/C\(_{DTPA}\) was high before cimetidine administration; Ccr\(_{24}\) and Ccr\(_{Ci24}\) values were significantly different from C\(_{DTPA}\) (CV 23.1%, Ccr\(_{24}/C_{DTPA} = 1.17\), \(p = 0.005\); and CV 14.1%, Ccr\(_{Ci24}/C_{DTPA} = 0.92\), \(p = 0.006\), respectively). Ccr\(_4\) values obtained before cimetidine ingestion showed large variation and were significantly different from C\(_{DTPA}\) (CV 15.5%, Ccr\(_4)/C_{DTPA} = 1.11\), \(p = 0.001\)). Ccr\(_{Ci4}\) values after cimetidine were similar to C\(_{DTPA}\) (CV 6.9%, Ccr\(_{Ci4}/C_{DTPA} = 1.01\), \(p = 0.28\)). C \(_{CG}\) estimates were higher before cimetidine intake (CV 12.4%, C \(_{CG}/C_{DTPA} = 1.21\), \(p < 0.001\)), whereas C \(_{CG-Ci}\) values were not significantly different from C\(_{DTPA}\) values (CV 7.0%, C \(_{CG-Ci}/C_{DTPA} = 1.01\), \(p = 0.67\)). This study shows that GFR estimations by C \(_{CG}\), Ccr\(_4\), Ccr\(_{24}\), or Ccr\(_{Ci24}\) are insufficiently reliable. On the other hand, C \(_{CG-Ci}\) and Ccr\(_{Ci4}\) results are acceptable for true GFR estimations. (received 18 January 2001; accepted 20 March 2001)

Key words: glomerular filtration rate, creatinine clearance, cimetidine, Cockcroft-Gault equation, renal function

Introduction

The determination of glomerular filtration rate (GFR) is a valuable indicator of renal pathophysiology and functional renal mass [1,2]. Plasma creatinine concentration, the most common index for evaluation of renal function, is influenced by many variables, such as body muscle mass, age, physical activity, diet, and analytical method. Moreover, plasma creatinine values in patients with renal disease may be normal until renal function has decreased to approximately one-half of normal levels. Classical estimations of creatinine clearance (Ccr\(_{24}\)) are subject to error, since creatinine is not only filtered through the glomeruli, but is also secreted by the tubules, and since accurate collection of 24-hr urine specimens is difficult, particularly in children and the elderly [3,4]. Cimetidine (Ci), an H\(_2\)-receptor antagonist, inhibits the tubular secretion of creatinine. It has been reported that creatinine clearance approximates true GFR after cimetidine administration [3]. Estimation of inulin clearance, which is accepted as the “gold standard” for GFR measurements, cannot be applied in every diagnostic center, since it is invasive and is often considered impractical [5]. GFR studies that use radionuclides such as \(^{125}\)I-iothalamate, \(^{51}\)Cr-EDTA (ethylenediaminetetra-acetic acid), and \(^{99m}\)Tc DTPA (diethylenetriaminepenta-acetic acid) are costly and complicated, but they provide good analytical precision and recovery [6,7].

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This study compares GRF values estimated by Cockcroft and Gault’s equation \( (C_{\text{C-G}}) \) based on analysis of plasma creatinine, and by the standard creatinine clearance equation, based on urine collections for 4 hr \( (C_{\text{cr4}}) \) and 24 hr \( (C_{\text{cr24}}) \). The effects of cimetidine on determinations of \( C_{\text{C-G}}, C_{\text{cr4}} \) and \( C_{\text{cr24}} \) were investigated. Finally, the reliability of these methods was established by comparisons with \(^{99m}\text{Tc-DTPA}\) clearance results obtained by the protein-free double-sample method \( (C_{\text{DTPA}}) \), which is equivalent to the inulin clearance assay \([6,7]\).

**Materials and Methods**

**Study population.** Seventeen patients (12 male, 5 female, age 20-48 yr) with histopathologically-proven renal disease and 10 healthy controls (5 male, 5 female, age 22-40 yr) were enrolled in the study. The renal histopathogy findings included membranoproliferative glomerulonephritis (6 patients), focal glomerulosclerosis (4 patients), polycystic kidney (2 patients), and membranous glomerulonephritis (2 patients). Patients with diabetes mellitus, pregnancy, cimetidine allergy, body mass index \( (\text{BMI}) \) >30 kg/m\(^2\), or those who were receiving drugs such as trimethoprim and salicylates (which inhibit tubular secretion of creatinine) and cephalosporin, guanidine, pyruvate, and ascorbic acid (which interfere with creatinine analysis) were excluded from the study \([8-10]\). The protocol was approved by the Medical Ethics Committee of the Gulhane School of Medicine. The study was fully explained to the subjects and their informed consent was obtained.

**Study Design.** Base-line blood samples (with EDTA anticoagulant) and urine collections (4-hr and 24-hr) were obtained from all subjects. Creatinine was assayed in plasma and urine. \( C_{\text{cr4}} \) and \( C_{\text{cr24}} \) were calculated by the standard clearance equation \((#1)\); \( C_{\text{C-G}} \) was calculated by the Cockcroft-Gault equation \((#2)\) \([11]\).

\[
C_{\text{cr}} = \frac{(U_{\text{cr}} \times V)}{P_{\text{cr}}} \times 1.73/A \quad (#1)
\]

\[
C_{\text{C-G}} = \left[\frac{(140 - \text{age}) \times \text{BW}}{P_{\text{cr}}}\right] \times (1.23/P_{\text{cr}}) \quad (#2)
\]

\( C_{\text{C-G}} \): Cockcroft-Gault estimate of glomerular filtration rate \((\text{ml/min/1.73 m}^2)\)

\( \text{BW} \): body weight \((\text{kg})\)

\( P_{\text{cr}} \): plasma creatinine \((\mu\text{mol/L})\)

\( A \): body surface area \((\text{m}^2)\)

**Clearance measurements after cimetidine.** The dosage schedule and study protocol are outlined in Fig. 1. On the first day, the subjects were hydrated orally (at least 200 ml/hr) for 4 hr to ensure sufficient urine flow and to suppress the reabsorption of cimetidine. The volumes of the urine specimens were measured, and, after centrifugation, an aliquot of each urine specimen was stored at \(-40^\circ\text{C}\) until assay. Blood samples were collected into tubes with EDTA anticoagulant, and, after centrifugation, the plasma samples were stored at \(-40^\circ\text{C}\) until assay. Body surface areas were estimated from body height and weight measurements using the equation of DuBois and DuBois \([12]\). Plasma and urine creatinine concentrations were measured in duplicate by a kinetic Jaffé method using a Technicon Dax-48 analyzer (Bayer Diagnostica, Germany).

On the morning of the second day, \(^{99m}\text{Tc-DTPA}\) was administered to the subjects and blood samples were drawn for GFR measurements by the \(^{99m}\text{Tc-DTPA}\) protein-free double-sample method (see below). All subjects were instructed to rest during the test period. In 10 controls, assays by the \(^{99m}\text{Tc-DTPA}\) method were performed before and after the administration of cimetidine in order to evaluate the effect of cimetidine on GFR and \( C_{\text{DTPA}} \).

**Determination of true GFR.** DTPA was reconstituted in 2-4 ml (total volume) of sterilized saline with 1850-3700 MBq \((50-100 \text{ mCi})\) \(^{99m}\text{Tc}\) pertechnetate. Radiochemical quality control included estimation of the free and unchelated hydrolyzed-reduced \(^{99m}\text{Tc}\). After reconstitution, 2 aliquots containing equal activity \((110 \text{ MBq})\) and volume of \(^{99m}\text{Tc-DTPA}\) were prepared; one was used as the standard and the other as the patient dose. The patients were hydrated with 10-15 ml/kg of oral fluids prior to scintigraphic analysis. Patient dosages of \(^{99m}\text{Tc-DTPA}\) were measured with a dose calibrator (Veenstra Instruments, VDC-202, Austria). Blood samples were withdrawn from the contralateral arm into EDTA-anticoagulated tubes at 60 and 180 min after the injection. The blood
Fig. 1. Two-day protocol for administration of cimetidine and measurement of creatinine and \(^{99m}\text{Tc-DTPA}\) clearances.

Table 1. Observations and renal clearances in controls and patients before and after cimetidine administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 10) (mean ± SD; CV%)</th>
<th>Patients (n = 17) (mean ± SD; CV%)</th>
<th>All subjects (n = 27) (mean ± SD; CV%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>29.8 ± 10.2</td>
<td>34.9 ± 7.7</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.2 ± 3.1</td>
<td>25.9 ± 1.9</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Plasma creatinine*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pre-cimetidine)</td>
<td>74.0 ± 5.4</td>
<td>112.6 ± 43.9</td>
<td>98.3 ± 39.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(post-cimetidine)</td>
<td>86.7 ± 8.3</td>
<td>140.4 ± 67.0</td>
<td>120.5 ± 59.0</td>
<td></td>
</tr>
<tr>
<td>(\text{Ccr}_4) **</td>
<td>114.8 ± 18.9</td>
<td>78.7 ± 28.3</td>
<td>94.2 ± 27.5</td>
<td></td>
</tr>
<tr>
<td>(\text{Ccr}_{ci4})</td>
<td>105.6 ± 12.9</td>
<td>71.2 ± 28.4</td>
<td>83.8 ± 29.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Ccr}_{24}) **</td>
<td>115.2 ± 18.4</td>
<td>84.6 ± 36.6</td>
<td>95.9 ± 34.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Ccr}_{ci24})</td>
<td>97.4 ± 15.2</td>
<td>67.9 ± 33.3</td>
<td>78.9 ± 31.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{CCr}-\text{G}) **</td>
<td>123.7 ± 14.8</td>
<td>85.9 ± 31.0</td>
<td>99.9 ± 31.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{CCrG}-\text{G})</td>
<td>105.7 ± 12.8</td>
<td>72.0 ± 28.2</td>
<td>84.5 ± 28.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{CDPTA}) **</td>
<td>106.9 ± 13.3</td>
<td>71.9 ± 30.1</td>
<td>84.9 ± 30.3</td>
<td></td>
</tr>
<tr>
<td>(\text{CCr}_4/\text{CDPTA})</td>
<td>1.07 ± 0.09 (8.4)</td>
<td>1.14 ± 0.21 (17.9)</td>
<td>1.11 ± 0.17 (15.5)</td>
<td></td>
</tr>
<tr>
<td>(\text{CCr}_{ci4}/\text{CDPTA})</td>
<td>0.99 ± 0.05 (5.1)</td>
<td>1.02 ± 0.08 (7.8)</td>
<td>1.01 ± 0.07 (6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{CCr}_{24}/\text{CDPTA})</td>
<td>1.08 ± 0.14 (13.0)</td>
<td>1.23 ± 0.32 (26.0)</td>
<td>1.17 ± 0.27 (23.1)</td>
<td></td>
</tr>
<tr>
<td>(\text{CCr}_{ci24}/\text{CDPTA})</td>
<td>0.91 ± 0.10 (11.0)</td>
<td>0.93 ± 0.16 (17.2)</td>
<td>0.92 ± 0.13 (14.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{CCr}-\text{G}/\text{CDPTA})</td>
<td>1.16 ± 0.05 (4.3)</td>
<td>1.24 ± 0.18 (14.5)</td>
<td>1.21 ± 0.15 (12.4)</td>
<td></td>
</tr>
<tr>
<td>(\text{CCrG}-\text{G}/\text{CDPTA})</td>
<td>0.99 ± 0.03 (3.0)</td>
<td>1.02 ± 0.09 (8.8)</td>
<td>1.00 ± 0.07 (7.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(\mu\text{M/L}\) ** ml/min/1.73 m\(^2\)
268 samples were centrifuged at 2000 rpm for 10 min. The plasma samples were centrifuged in ultrafiltration tubes (Amicon, USA) for 45 min at 7500 rpm to prepare 99% protein-free ultrafiltrates. Aliquots of plasma, ultrafiltrate, and diluted standard were counted in a gamma counter (Wallac 1260 Multigamma II, LKB, Finland). GFR was computed by the following equation [6,13,14]:

\[
GFR = \frac{D \ln(P_1/P_2)}{(T_2 - T_1)} \times e^{(T \ln P_2 - T_2 \ln P_1)/(T_2 - T_1)}
\]

- D = injected dose (cpm)
- T₁ = time of first plasma sample (60 min)
- T₂ = time of second plasma sample (180 min)
- P₁ = first sample activity (cpm)
- P₂ = second sample activity (cpm)

**Statistical Analysis.** Statistical analyses were performed by using SPSS and EXCEL software. The data were expressed as means ± SD. Comparisons of results obtained with and without cimetidine ingestion were based on paired-sample t-test. Correlation coefficients between GFR estimates and C\textsubscript{DTPA} results were computed by linear regression analysis. The ratios of the various clearances to C\textsubscript{DTPA} were calculated before and after the administration of cimetidine. These ratios showed how accurately the clearance values approached the true GFR value. This method is preferred to correlation coefficients as it analyzes the agreement between two methods (as CV%), not the relationship between them [15]. In a perfect correlation, the ratio points lie along the line of equality. A p value ≤0.05 was considered statistically significant.
Results

Following cimetidine administration, plasma creatinine levels increased both in the patients (mean 24%, range 12.5-46) and the controls (mean 18%, range 11.3-22) (Table 1, Fig. 2a). The absolute and percentage increases of plasma creatinine after cimetidine administration showed significant correlation with the basal serum creatinine levels (Fig. 2b,c, respectively).

To study the effect of cimetidine on CDTPA, assays were performed by the 99mTc-DTPA protein-free double-sample method before and after cimetidine administration in 10 controls; no significant difference was found (r 0.997, slope 0.974, intercept 1.52).

The results for Ccr were lower than Ccr, but were significantly different from CDTPA. The Ccr values showed close agreement with the CDTPA values; after cimetidine intake, the Ccr and CDTPA values were not significantly different (Table 1, Fig. 3).

The Cc-G values estimated by Cockcroft and Gault’s equation were significantly higher than the...
However, following cimetidine intake, the CCiC-G results did not differ significantly from the CDTPA values (Table 1, Fig. 5).

**Discussion**

Inulin, the “gold standard” compound for measurements of GFR, is completely filtered by the glomeruli and not metabolized by the kidney. However, inulin clearance is seldom measured routinely, even in research laboratories, because of technical difficulties [5]. New test compounds, such as cystatin C, sinistrin, and iohexol, have been extensively studied in searching for a practical technique for GFR measurements [16-21]. Evaluating GFR by immunonephelometric measurement of cystatin C has been reported to give reliable results, including children and renal transplant patients [17,20].

Cimetidine can be used to inhibit the tubular secretion of creatinine (3,22-27). Ixkes et al [3] reported that daily intake of 2400 mg of cimetidine inhibits the tubular secretion of creatinine and that the effect remains constant for 6 hr. In the present study, tubular secretion of creatinine was also inhibited completely in patients and controls after the administration of 2400 mg of cimetidine per day in divided doses. There

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**Fig. 4.** Regression analyses for estimation of creatinine clearance by the Cockcroft and Gault equation, based on measurements of plasma creatinine, versus 99mTc-DTPA clearance (CDTPA). In panels a and b, the regression lines are solid and the lines of equality are dotted. Panel a: before cimetidine (CrC-G versus CDTPA); panel b: after cimetidine (CrC-G versus CDTPA). In panels c and d are plotted, respectively, the differences of (C_{C-G} - C_{DTPA}) and (C_{C-G} - C_{DTPA}), as a function of each subject’s CDTPA value.
were no side effects except transient fatigue, which was noted in one patient. Hilbrands et al [24] reported that cimetidine administration may cause rare side effects, including tinnitus, fatigue, dyspepsia, diarrhea, and sleeplessness.

Other investigators have also observed that, after cimetidine administration, the clearance values for creatinine closely approximate those obtained for inulin, $^{125}$I-iotophalamate or $^{51}$Cr-EDTA [23,25]. As shown in Fig. 2, a prominent increase was observed in plasma creatinine levels after cimetidine ingestion. The alteration of creatinine levels after cimetidine suggests that tubular secretion of creatinine shows significant correlation with the basal levels of plasma creatinine. Similar results have been reported by others [3,25]. The mechanism for tubular secretion of creatinine has not been completely explained. It has been suggested that creatinine is secreted by both cationic and anionic transport systems, due to its amphoteric properties, though the cationic route is probably dominant [28]. Cimetidine, an $H_2$-receptor antagonist, evidently inhibits the cationic transport of creatinine competitively, but has no effect on GFR [28-30].

In the present study, cimetidine had no significant effect on glomerular filtration of $^{99m}$Tc-DTPA and the resultant $C_{\text{DTPA}}$ values in 10 controls. Olsen et
al [31] likewise found that cimetidine did not influence the clearances of inulin or $^{125}$I-iothalamate. In the present study, Ccr$_4$, Ccr$_{24}$ and C$_{C-G}$ values were diminished after cimetidine administration and their respective correlations with C$_{DTPA}$ were closer than were observed without cimetidine.

Van Acker et al [25] and Ixkes et al [3] reported that estimates of creatinine clearance after cimetidine were not significantly different from inulin clearance values. Coresh et al [32] found that creatinine clearance values were higher than $^{125}$I-iothalamate GFR values. In the present study, neither Ccr$_{24}$ nor Ccr$_{C_{i24}}$ values were in agreement with C$_{DTPA}$. Partial inhibition of creatinine secretion and miscollections of urine during the 24-hr period are likely reasons for the discordance among these studies (3,23-25,32).

In the present study, the GFR estimates obtained by Cockcroft and Gault’s equation were 21% higher than C$_{DTPA}$ values when the C$_{C-G}$ values were based on creatinine concentrations in plasma collected before cimetidine administration. Coresh et al [32] likewise found that such C$_{C-G}$ values were higher than true GFR values. However, the present study shows that, after cimetidine, C$_{G_{iC-G}}$ values showed close agreement with C$_{DTPA}$ values. The Ccr$_{C_{i4}}$ values were also closely comparable to C$_{DTPA}$ values.

In summary, following cimetidine administration, the clearance estimates obtained by Cockcroft and Gault’s equation and the creatinine clearance values obtained with 4-hr urine collections are more practical and reliable methods for estimating GFR and evaluating renal function, when compared to others. Further studies are needed to validate this conclusion in pediatric and geriatric populations.

References