The need for robust validation for MDRD-based glomerular filtration rate estimation in various CKD populations

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Abstract

Background. Currently, estimated glomerular filtration rate (eGFR) equations have been validated only in Caucasians and African-Americans and is not applicable to people of other races/ethnicities as shown in studies conducted in two Asian populations: Chinese and Japanese. Because of this, it is important that eGFR equations are validated in its prospective population before applying it in the clinical setting and in epidemiologic studies. Therefore, we examined all eGFR equations available: reexpressed isoﬁte dilution mass spectroscopy (IDMS)-traceable Modiﬁcation of Diet in Renal Disease (MDRD) equation, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, Chinese equation and Japanese equation.

Methods. A total of 350 adult Thai CKD patients were studied. The Tc-DTPA plasma clearance was used as a reference for glomerular ﬁltration rate (GFR). The serum creatinine was determined by IDMS reference enzymatic methods (CrEnz) and Jaffe’s kinetic assay (CrJaffe) as indicated in each equation.

Results. The disagreement between the reference GFR and eGFR (reference GFR minus eGFR) was 9.6 mL/min/1.73 m² for the reexpressed IDMS-traceable MDRD equation, 8.0 mL/min/1.73 m² for CKD-EPI equation, 1.9 mL/min/1.73 m² for eGFR equation from the Chinese study and 20.9 mL/min/1.73 m² for the eGFR equation from the Japanese study. The Thai equation for the reexpressed MDRD was 1.129. The reexpressed MDRD equation for Thai is as follows: 175 × CrEnz⁻¹.154 × Age⁻⁰.203 × 0.742 (if female) × 1.129 (if Thai). When stepwise multiple regression analysis was used, the Thai eGFR formula is: 375.5 × CrEnz⁻⁰.848 × Age⁻⁰.364 × 0.712 (if female).

Conclusions. Differences in race/ethnicity can signiﬁcantly affect the results obtained from MDRD-based eGFR equation. It is highly recommended that each population should validate eGFR equations before applying the equation in epidemiologic studies or clinical use.

Keywords: CKD; eGFR equation; glomerular filtration rate

Introduction

Currently, there are many estimated glomerular filtration rate (eGFR) formulas available. However, its usage is limited because there are variations of eGFR equations due to a diverse anthropometry among different ethnic groups. The original Modiﬁcation of Diet in Renal Disease (MDRD) equation was modiﬁed as the ‘reexpressed MDRD formula’ after standardizing and readjusting for serum creatinine. The reexpressed MDRD formula used traceable high-level isotope dilution mass spectroscopy (IDMS) reference serum creatinine measured by enzymatic methods (CrEnz) to ensure accuracy. Recently, another formula known as the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was developed for the Caucasian and the African-American chronic kidney disease (CKD) population [1–4]. The reexpressed MDRD formula and CKD-EPI have not been validated in non-Caucasian and non-African-American patients. Recently, two studies conducted in Asian populations showed that the MDRD-based formula underestimated glomerular filtration rate (GFR) in Chinese patients by 23% and overestimated GFR in Japanese patients by 12% [5, 6]. By using the MDRD-based formula, the racial factors for Chinese and Japanese populations were 1.23 and 0.88, respectively [5–7]. This discrepancy creates a problem for other Asian populations in determining which value they should use for racial factor to accurately detect and monitor renal damage.

Furthermore, in a recent epidemiologic study conducted in the Thai population, it was shown that Thais had a higher prevalence of Stages III and IV CKD than in Caucasians from the USA and Australia [8] when the original MDRD formula was used. What is even more disturbing is the fact that the prevalence in Thailand for CKD Stages III and IV were higher than in Taiwan, which was known to have a high prevalence.
eGFR equation in CKD

of CKD [8]. For Thai nephrologists, this information is of utmost concern and whether the eGFR determined by the MDRD formula currently in use was accurate or not.

Therefore, there is an urgent need to validate all current eGFR formulas namely, the reexpressed MDRD [3], the CKD-EPI [4], the Chinese [5] and the Japanese equations [7] in the Thai population.

Materials and methods

Patients

The study was approved by the Ethical Committee for Research, Chulalongkorn University, Bangkok, Thailand. CKD patients with a stable condition and >18 years old were recruited into the study. CKD was diagnosed and classified according to the K/DOQI guideline [9]. The study was conducted in an ambulatory setting and began at 08:00–09:00 AM in order to avoid the diurnal variations in the renal function [10]. Patients having acute deterioration of the renal function, amputation, malnutrition, in a bedridden state, with infection, in an edematous state, gastrointestinal bleeding, heart failure or hospitalized were excluded. Women of childbearing age without a reliable contraceptive method, patients on renal replacement therapy, patients taking methyldopa, levodopa, ascorbic acid, cinetidine, trimethoprim, antibiotics, steroids or flucytosine were also excluded. All hypertensive patients were classified according to the JNC VII guidelines [11].

Reference GFR measurement

The reference GFR was determined by collecting plasma from 10 different time points using the $^{99m}$Tc-Diethylene Triamine Pentaacetic Acid ($^{99m}$Tc-DTPA) plasma clearance method, which was performed at the Department of Radiology, Chulalongkorn University. $^{99m}$Tc-DTPA was purchased from the Office of Atoms for Peace, Bangkok, Thailand, with a radiopurity of >95% and $^{99m}$Tc-DTPA bound to plasma protein of <5%. The same protocol was applied to all patients. In brief, heparin lock was inserted in the arm to obtain dated samples (>=10). Blood specimens were drawn to assess plasma radioactivity at 5, 10, 20, 30, 60, 90, 120, 180 and 240 min post $^{99m}$Tc-DTPA injection. Plasma radioisotope activities were then plotted as a function of time to calculate a time-activity curve for GFR (Figure 1). The GFR equation was determined by using bi-exponential fitting method [12].

$$GFR = \frac{D}{\text{area under time-activity curve} - \int_0^t c(t) \, dt}$$

D is the dosage of injected $^{99m}$Tc-DTPA. The result was normalized by the body surface area, which was calculated according to Du Bois D and Du Bois EF [13].

Calibration for the serum creatinine assay

Fasting serum creatinine was measured by using a Roche Diagnostics (Indianapolis, IN) CREA plus (11775642) enzymatic assay (CrJaffe) on a COBAS INTEGRA 400 plus analyzer. The measured CrJaffe values were adjusted by using traceable high-level IDMS reference serum creatinine as recommended by the National Kidney Disease Education Program [14]. The IDMS reference serum creatinine (SRM 967) was purchased from the National Institute of Standards and Technology. The certified concentrations of serum creatinine were 0.847 ± 0.018 mg/dL for level 1 and 3.877 ± 0.082 mg/dL for level 2.

The serum creatinine was also measured by Jaffe’s kinetic assay (CrEnz) by using Roche Diagnostics (Indianapolis, IN) CREA/RC (12217333) on a COBAS INTEGRA 400 plus analyzer without any adjustments from traceable high-level IDMS reference. Values obtained from CrJaffe and CrEnz were utilized in each eGFR formula accordingly. For example, CrJaffe values were used for the Chinese equation, whereas the values from CrEnz were used for the reexpressed MDRD, CKD-EPI and Japanese equation.

eGFR calculation

The eGFR values were calculated by using the reexpressed IDMS-traceable MDRD equation, CKD-EPI equation, Chinese equation and Japanese equation (Table 1).

Statistical analysis

Bland–Altman plots were used to assess the agreement between the eGFR and the reference GFR [15]. The regression of the average and the difference between the reference GFR and the eGFR (reference GFR minus eGFR) were analyzed. The Thai coefficient for the reexpressed MDRD equation was determined by using linear regression analysis.

A stepwise multiple regression was used to determine variables that could predict GFR. Of 350 patients, 250 samples (also known as the training sample) were randomly selected and entered into the stepwise regression models. A P value <0.001 was used as the criterion for entering a variable into the model.

eGFR values obtained from new equations derived from the regression analysis were compared to the currently, available formulas by using validated samples (N = 100). The correlation coefficient was used to assess the overall fit of the model. The 50th percentiles of the distribution of absolute difference between reference and eGFR indicated the typical size of errors in predicting the GFR value, and the 75th and 90th percentiles assessed the sizes of the larger errors that occurred for each equation. Bias was defined by the mean absolute difference between the reference and eGFR. Precision was defined as the standard deviation value of the mean absolute difference. Accuracy was defined as the proximity of the estimation compared with the reference and is a measure encompassing both precision and bias. The measure of accuracy was calculated using combined root mean square error and percentage of GFR within 10, 15 and 30% of reference GFR. The accuracy of the equation was compared by the $\chi^2$ test.

Statistical analysis was performed on a desktop computer, using MedCalc Software version 10 (MedCalc Software bvba, Mariakerke, Belgium).

Results

Characteristics of the patients

A total of 350 cases with various CKD stages were included in the study. The patients’ characteristics are listed in Table 2. The averages of the body mass index and the body surface area were 25.3 ± 4.8 kg/m² and 1.67 ± 0.18 m², respectively. The most common etiology of CKD was diabetic nephropathy. The mean $\text{CrEnz}$ was 2.02 ± 1.32...
Table 1. eGFR equations currently available

<table>
<thead>
<tr>
<th>eGFR methods</th>
<th>Gender</th>
<th>Serum Cr</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reexpressed MDRD equation [3]</td>
<td>Not applicable</td>
<td>Cr_{Tfenz}</td>
<td>$175 \times (Cr_{Tfenz})^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if female})$</td>
</tr>
<tr>
<td>CKD-EPI equation [4]</td>
<td>Female</td>
<td>$Cr_{Tfenz} \leq 0.7$ mg/dL</td>
<td>$144 \times (Cr_{Tfenz}0.7)^{-1.209} \times (0.993)^{0.869}$</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>$Cr_{Tfenz} &gt; 0.7$ mg/dL</td>
<td>$144 \times (Cr_{Tfenz}0.7)^{-1.209} \times (0.993)^{0.869}$</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>$Cr_{Tfenz} \leq 0.9$ mg/dL</td>
<td>$141 \times (Cr_{Tfenz}0.9)^{-1.37} \times (0.993)^{0.869}$</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>$Cr_{Tfenz} &gt; 0.9$ mg/dL</td>
<td>$141 \times (Cr_{Tfenz}0.9)^{-1.37} \times (0.993)^{0.869}$</td>
</tr>
<tr>
<td>Chinese equation [5]</td>
<td>Not applicable</td>
<td>$Cr_{Tfenz}$</td>
<td>$175 \times (Cr_{Tfenz})^{-1.234} \times (age)^{-0.179} \times (0.79 \text{ if female})$</td>
</tr>
<tr>
<td>Japanese equation [7]</td>
<td>Not applicable</td>
<td>$Cr_{Tfenz}$</td>
<td>$194 \times (Cr_{Tfenz})^{-1.094} \times (age)^{-0.287} \times (0.739 \text{ if female})$</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of patients enrolled in the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (N = 350)</th>
<th>Male (N = 193)</th>
<th>Female (N = 157)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: years (mean ± SD)</td>
<td>59.5 ± 13.6</td>
<td>60.2 ± 15.4</td>
<td>58.1 ± 14.9</td>
</tr>
<tr>
<td>Weight: kg (mean ± SD)</td>
<td>60.0 ± 15.1</td>
<td>65.6 ± 12.5</td>
<td>58.8 ± 14.6</td>
</tr>
<tr>
<td>Height: cm (mean ± SD)</td>
<td>1.61 ± 0.08</td>
<td>1.66 ± 0.06</td>
<td>1.54 ± 0.05</td>
</tr>
<tr>
<td>Body mass index: kg/m² (mean ± SD)</td>
<td>25.3 ± 4.8</td>
<td>24.9 ± 3.9</td>
<td>25.8 ± 5.4</td>
</tr>
<tr>
<td>Body surface area: m² (mean ± SD)</td>
<td>1.67 ± 0.18</td>
<td>1.75 ± 0.16</td>
<td>1.59 ± 0.18</td>
</tr>
<tr>
<td>Proteinuria: g/day (mean ± SD)</td>
<td>0.87 ± 0.60</td>
<td>0.86 ± 0.60</td>
<td>0.87 ± 0.59</td>
</tr>
<tr>
<td>Hematuria &gt; 3 cells/hpf % case</td>
<td>24.8%</td>
<td>24.6%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Serum creatinine (Cr_{Tfenz}): mg/dL (mean ± SD)</td>
<td>2.02 ± 1.32</td>
<td>2.26 ± 1.52</td>
<td>1.69 ± 1.15</td>
</tr>
<tr>
<td>Serum BUN: mg/dL (mean ± SD)</td>
<td>26.52 ± 17.12</td>
<td>28.22 ± 17.12</td>
<td>25.91 ± 16.6</td>
</tr>
<tr>
<td>Albumin: g/dL (mean ± SD)</td>
<td>4.1 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Reference GFR: mL/min/1.73 m² (mean ± SD)</td>
<td>55.86 ± 30.40</td>
<td>56.04 ± 28.48</td>
<td>59.63 ± 36.30</td>
</tr>
<tr>
<td>DM: (% case)</td>
<td>33.5%</td>
<td>31.5%</td>
<td>34.0%</td>
</tr>
<tr>
<td>Mean arterial pressure: mmHg (mean ± SD)</td>
<td>99.0 ± 14.0</td>
<td>98.6 ± 13.9</td>
<td>99.6 ± 13.2</td>
</tr>
<tr>
<td>Hypertension: % case</td>
<td>74.0%</td>
<td>74.0%</td>
<td>75.0%</td>
</tr>
<tr>
<td>Risk or cause of CKD: % case</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>30.8%</td>
<td>30.1%</td>
<td>31.8%</td>
</tr>
<tr>
<td>Non-diabetic glomerulopathy</td>
<td>11.2%</td>
<td>10.4%</td>
<td>12.1%</td>
</tr>
<tr>
<td>Single kidney</td>
<td>1.5%</td>
<td>2.1%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Obstructive uropathy</td>
<td>1.5%</td>
<td>1.6%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Cystic kidney disease</td>
<td>1.5%</td>
<td>2.1%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Ischemic nephropathy</td>
<td>1.0%</td>
<td>1.0%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Unknown</td>
<td>52.0%</td>
<td>52.3%</td>
<td>51.6%</td>
</tr>
</tbody>
</table>

mg/dL [95% confidential interval (CI) of 1.80–2.21]. The mean reference GFR was 55.86 ± 30.40 mL/min/1.73 m² (95% CI of 51.10–59.05 mL/min/1.73 m²).

Assessing agreement between eGFR values from different equations and the reference GFR

To assess the agreement between eGFR values and the reference GFR, Bland–Altman plots were produced (Figure 2). The agreement was compared by calculating the bias estimated on the mean of differences ± the limits of agreement of each eGFR equation that can be expressed as $9.6 \pm 23.1 \text{ mL/min/1.73 m²}$ for the reexpressed IDMS-traceable MDRD equation, $8.0 \pm 22.0 \text{ mL/min/1.73 m²}$ for the CKD-EPI equation, $1.9 \pm 30.4 \text{ mL/min/1.73 m²}$ for the Chinese equation and $20.9 \pm 27.1 \text{ mL/min/1.73 m²}$ for the Japanese equation. From the linear regression analysis of the eGFR and the reference GFR, the regression performance showed $r^2 > 0.8$ for all eGFR equations. The slopes of the regression lines varied by each equation: 0.95 in the reexpressed MDRD equation, 0.93 in CKD-EPI equation, 0.76 in the Chinese MDRD equation and 1.37 in the Japanese equation. The slope of reexpressed MDRD equation was closer to the identical line (slope = 1.0) compared with the slope of other equations.

Thai racial coefficient factor for MDRD equation and Thai eGFR equation

The study population was randomly divided into two cohorts: a training group of 250 cases and a validating group of 100 cases. The Thai racial coefficient factor was obtained from the regression model of the reexpressed MDRD equation and reference GFR from the training group: 1.129 (95% CI: 1.091–1.162), ($p = 0.938$). Thus, the reexpressed MDRD GFR is presented as follows: $175 \times (Cr_{Tfenz}^{-1.154}) \times \text{Age}^{-0.203} \times (0.742 \text{ if female}) \times 1.129$ (if Thai). The reason for selecting the racial factor from the reexpressed MDRD instead of CKD-EPI was because the slope of reexpressed MDRD and reference was closest to 1.00.

The Thai eGFR equation was obtained from the linear regression model by using the data from the training group consisting of demographic, clinical and laboratory variables. Variables that correlated significantly with the reference GFR ($P < 0.001$) were $Cr_{Tfenz}$, BUN, age, albumin and body surface area. A newly derived equation was obtained from a stepwise regression model, which included $Cr_{Tfenz}$ and age. The Thai eGFR formula was expressed as $37.55 \times (Cr_{Tfenz}^{-0.848}) \times \text{Age}^{-0.364} \times (0.712 \text{ if female})$ ($r^2 = 0.869$).
Diagnostic performance of the equations

The newly derived equations were compared to the existing equations to assess its diagnostic performance (n = 100). The accuracy of the newly derived equations was significantly higher compared to the existing equations by accuracy within 10, 15 and 30% (P < 0.05) (Table 3). The newly derived equations yielded a more accurate prediction of GFR than did the other equations in terms of r² statistics and error, especially for the mean absolute bias, precision, 50th, 75th and 90th percentiles for absolute error.

Sensitivity and specificity of eGFR equations and reference GFR in identifying CKD stages

By using the K/DOQI classification, we validated the sensitivity and specificity of the newly derived eGFR equations against the existing eGFR equations in categorizing the stages of CKD (n = 100) (Table 4). The newly derived eGFR equations correctly identified CKD across Stages II and III and were more sensitive than the existing eGFR equations. The specificity of the newly derived eGFR equations for CKD Stages II and III identification was >82%.

Discussion

As a tool for CKD diagnosis and to determine the efficacy of novel treatments to delay CKD progression, having an accurate and validated GFR measurement is extremely important in the clinical practice and epidemiologic studies in handling the global CKD pandemic. Serum Cr can be problematic as a marker for GFR. Serum Cr does not only represent a wide range of GFR but also varies depending on the assay method used that needs to regularly validate the standard material for serum Cr [16, 17]. The National Kidney Disease Education Program has recommended that C_{Cr} should be adjusted and standardized through
calibration and use of traceable high-level IDMS reference serum Cr, which is now available.

The MDRD equation for eGFR has been developed primarily for the Caucasian and African-American population with renal disease [1, 2]. Recent studies have shown that the calculation of eGFR derived from non-Asian populations for Asian populations without prior validation could result in inaccurate estimations of GFR [5, 6, 18]. Such inaccuracy could be caused by the diversity of anthropometry and dietary intake in different ethnic groups. It is automatically assumed that the currently available eGFR equation derived from the Asian populations would provide a better estimation for the Thai population than the MDRD equation because of the similarity in anthropometry among Asian ethnic groups. However, the studies from two Asian populations yielded two different eGFR equations: the racial correction factor for the MDRD equation for the Chinese population was >1 (1.23) [5], whereas in the Japanese population, it was <1 (0.88) [6, 7]. This discrepancy within the Asian population makes it difficult to adopt a universal eGFR equation or universal racial coefficient correction for Asian CKD populations.

This conflicting data can also further exacerbate results obtained from epidemiologic studies. For example, an epidemiologic study conducted in Thailand showed a much higher prevalence of CKD as compared to other countries known to have high prevalence of the disease such as in the USA and Taiwan [8]. This information contradicts our findings; our data showed that the reexpressed MDRD and CKD-EPI equations underestimated GFR. This incorrect estimation can lead to inappropriate strategy to alleviate or monitor the burden of CKD in the country. Our study has shown that, when compared with other equations from other Asian countries, the equation from the Japanese population study had the widest bias, whereas the equation from the Chinese population study had the least bias with the reference GFR (Figure 2). Although the Chinese eGFR equation had the least bias (1.9 mL/min/1.73 m²), the adoption of the Chinese eGFR equation in the Thai population can be problematic. The spread of the bias between the reference GFR and the eGFR by the Chinese study was not evenly distributed (Figure 2). When GFR was <50.4 mL/min/1.73m², the eGFR from the Chinese equation underestimated the reference GFR, whereas when the GFR was >50.4 mL/min/1.73m², the eGFR from the Chinese equation overestimated the reference GFR. In the reexpressed MDRD and CKD-EPI equations, the spread of bias was evenly distributed. Therefore, the application of reexpressed MDRD or CKD-EPI equations with a Thai racial factor is more applicable to the Thai population.

Our data showed that the Thai racial coefficient factor for the reexpressed IDMS-traceable MDRD equation was 1.129 (95% CI: 1.09–1.16) and the Thai eGFR can be expressed as 375.5 $\times$ CrEnz $\times$ (Age $^{-0.364}$) $\times$ (0.712 if female). The derived Thai eGFR equation provided the best accuracy and precision for GFR estimation and should be recommended for eGFR in Thai CKD population. Thai Renal Replacement Therapy Registry Data [19] showed the steep increase of renal replacement therapy prevalence from 302.6 patients per million population (p.m.p.) in 2006
to 419.95 p.m. in 2007 and to 496.93 p.m. in 2008. The derived Thai eGFR equation will provide more precise and more accurate CKD staging to fight the pandemic of CKD in Thailand.

The discrepancies of eGFR equation and racial coefficient factor for the MDRD-based equations among Asian populations can be caused by the differences in determining the reference GFR method. The reference GFR in the Japanese population study [6, 7] was performed by using renal clearance of inulin. Renal inulin clearance was measured from samples taken from three different time points during 2 h of fasting by continuous infusion method. Unlike the Japanese study, the reference GFR from the Chinese study [5] was obtained from plasma clearance of a radioisotope. This was done by using dual plasma sampling isotope dilution method at 2 and 4 h post-intravenous injection of the radioisotope. Moreover, the reference GFR in the MDRD study was obtained by renal clearance of 125I-iothalamate. Systemic differences in the eGFR equations derived from the statistical regression model cannot be avoided when two distinct methods were used to obtain the reference GFR. A previous study has shown that renal clearance of 99mTc-DTPA exceeded the inulin clearance in a CKD population [20]. This systemic bias provides an explanation as why there is a discordance of racial factor was 1.129 for the reexpressed MDRD equation in various races/ethnicities. Our study demonstrated that the Thai racial coefficient closer to the Chinese even though different protocols and that used by the Chinese may make the Thai racial factor closer to the Chinese even though different protocols of plasma isotope clearance were used.

In conclusion, we have proved that there are differences in racial coefficients for the MDRD-based eGFR equation for various races/ethnicities. Our study demonstrated that the Thai racial factor was 1.129 for the reexpressed MDRD equation using IDMS-traceable CrTmax. Therefore, it is highly and strongly recommended that people of different race/ethnicity should validate the existing eGFR equation before applying it to epidemiologic studies and in the clinical setting.

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