Final Report of

Glomerular Filtration Rate Estimation in HIV infected Thai Patients

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GFR Study team

January 2012

Abstract

Background:

Understanding how best to measure renal function in HIV-infected patients is critical because estimated glomerular filtration rate (eGFR) in HIV-infected patients can be affected by ethnicity and body composition, particularly, lipodystrophy. This study validated all the currently available methods of renal function assessments and compared them to the gold standard of plasma ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) clearance in HIV-infected patients.

Methods:

196 HIV-infected patients underwent GFR measurement with ^{99}mTc-DTPA plasma clearance, 4 creatinine-based glomerular filtration rate estimation (eGFR) [re-expressed Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation], cystatin-C GFR and 24-hour urine creatinine clearance (CrCl). A single intravenous bolus of ^{99m}Tc-DTPA was injected into each patient, and blood specimens were collected for plasma radioactive activity at 5, 10, 20, 30, 60, 90, 120, 180 and 240 minutes post ^{99m}Tc-DTPA injection.

Results:

The mean (SD) age, body mass index and the body surface area were 43.6±7.8 years, 22.3±3.2kg/m^{2}and 1.63 ±0.18 m^2, respectively. Mean (SD) skeletal muscle mass and fat mass were 24.6 ±5.6 kg and 13.7 ±6.5 kg. Mean (SD) ^{99m}Tc-DTPA GFR was 117.7± 29.2 mL/min per 1.73 m^2. The re-expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation, cystatin-C GFR, and 24 hr urine CrCl, underestimated the reference GFR. The bias estimated by the mean of differences ± the limits of agreement for the re-expressed MDRD equation, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR, Cockcroft & Gault, cystatin C, and 24 hr urine CrCl can be expressed as 18.9±27.3, 11.1±25.5, 6.2±28.8, 13.5±27.0, 30.4±28.0, 3.2±36.1 and 5.0±12.1 mL/min per 1.73 m^2 respectively.

Conclusion:

The available eGFR equations underestimated GFR in HIV-infected adults. However, the eGFR by cystacin C GFR was the most precise and accurate. Among Cr-based eGFR, re-expressed MDRD formula with Thai racial correction factor was the most precise and accurate. The racial factor for each ethnicity is important and the existing eGFR equation should be validated before using it in the HIV population.

Keywords: Glomerular filtration rate, eGFR equation, HIV-infected patients

บทคัดย่อ

ที่มา:

วิธีการวัดการทำงานของไตที่แม่นยำในผู้ป่วยติดเชื้อเอชไอวีมีความสำคัญมาก เพราะอัตราการกรองของไตของผู้ที่ติดเชื้อเอชไอวีอาจแตกต่างจากผู้ไม่ติดเชื้อเอชไอวีเนื่องจากมีส่วนประกอบของร่างกายต่างกันโดยเฉพาะภาวะไขมันย้ายที่ การศึกษานี้มีจุดประสงค์เพื่อประเมินการทำงานของไตด้วยวิธีต่างๆได้แก่ การคำนวณจากการตรวจ serum creatinine[re-expressed Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation] การตรวจcystatin C การตรวจปัสสาวะ24ชั่วโมง และเปรียบเทียบกับประเมินการทำงานของไตด้วยวิธีมาตรฐานโดยการวัดจาก ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) clearance ในผู้ที่ติดเชื้อเอชไอวี

วิธีการ :

มีผู้ป่วยที่เป็นผู้ติดเชื้อเอชไอวีเข้าร่วมโครงการจำนวนทั้งสิ้น207 คนและประสบผลสำเร็จเป็นจำนวน 196 คน โดยจะได้รับการประเมินอัตราการกรองของไตจากการตรวจ serum creatinine cystatin C และ ปัสสาวะ 24 ชั่วโมง และผู้ป่วยทุกรายจะได้รับการฉีด ^{99m}Tc-DTPA และเจาะเลือดดูการขับถ่ายของ^{99m}Tc-DTPA นาทีที่ 5, 10, 20, 30, 60, 90, 120, 180 และนาทีที่ 240 หลังจากที่ฉีด ^{99m} Tc-DTPA เข้าไป

ผลการวิจัย :

ผู้ป่วยติดเชื้อเอชไอวีมีอายุเฉลี่ยของ 43.6±7.8 ปี และดัชนีมวลกายเท่ากับ 22.3±3.2kg/m^{2}and และ body surface เท่ากับ 1.63 ±0.18 m^2 มวลกล้ามเนื้อเท่ากับ 24.6 ±5.6 กิโลกรัมและมวลไขมันของร่างกายเท่ากับ 13.7 ±6.5 kg กิโลกรัม ค่าเฉลี่ยของการตรวจอัตราการกรองของไตด้วยวิธี^{99m}Tc-DTPA คือ

117.7± 29.2 mL/min/1.73 m^2. ค่าคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineด้วยวิธี re-expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation ,หรือการตรวจ cystatin-C, และการตรวจปัสสาวะ24 ชั่วโมงได้ค่าต่ำกว่าการตรวจอัตราการกรองของไตด้วยวิธี^{99m}Tc-DTPA ซึ่งแสดงด้วย ค่า bias estimated by the mean of differences ± the limits of agreement ของค่าต่างๆดังนี้ตามลำดับ re-expressed MDRD equation, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR, Cockcroft & Gault, cystatin C, และปัสสาวะ 24 ชั่วโมง 18.9±27.3, 11.1±25.5, 6.2±28.8, 13.5±27.0, 30.4±28.0, 3.2±36.1 และ 5.0±12.1 mL/min/ 1.73 m^2 .

สรุป:

การศึกษานี้แสดงให้เห็นว่าค่าคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineด้วยวิธี re-expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation ,หรือการตรวจ cystatin-C, และการตรวจปัสสาวะ24 ชั่วโมงได้ค่าต่ำกว่าการตรวจอัตราการกรองของไตด้วยวิธี ^{99m}Tc-DTPAอย่างไรก็ตามค่าที่แม่นยำและถูกต้องที่สุดคือการตรวจด้วยวิธี cystatin C แต่วิธีนี้ไม่สะดวก ราคาสูงกว่า และไม่สามารถทำได้ตามสถานพยาบาลทั่วไป ดังนั้นการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineเป็นวิธีที่ง่ายและสะดวกที่สุด ซึ่งใน4 สมการ (re-expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation) ของการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineพบว่าการใช้

Re-expressed MDRD formula with Thai racial correction factor เป็นค่าที่แม่นยำและถูกต้องที่สุดซึ่งการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineด้วยสมการ re-expressed MDRD formula with Thai racial correction factor นี้ได้มีการใช้ในผู้ป่วยคนไทยที่ไม่ติดเชื้อเอชไอวีด้วย การศึกษานี้แสดงให้เห็นว่าเชื้อชาติมีความสำคัญต่อการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinine ดังนั้นในแต่ละเชื้อชาติควรมีการประเมิน racial factor ก่อนนำไปใช้ โดยเฉพาะในผู้ป่วยติดเชื้อเอชไอวี

คำสำคัญ : อัตราการกรองของไต สมการ eGFR และผู้ป่วยที่ติดเชื้อเอชไอวี

Glomerular filtration rate, eGFR equation, HIV-infected patients

Table of Contents

List of Tables

List of Illustrations

Final Report

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1. Background

Antiretroviral therapy (ART) has significantly reduced mortality and progression to AIDS. Instead, complications of long-standing HIV infection and treatment, including renal disease, have become increasingly important. Aging, concomitant metabolic diseases, and use of potentially nephrotoxic ART can lead to higher risks for developing renal diseases in HIV-infected people; therefore, it is critical that physicians have the best tool to measure renal function in HIV-infected patients. However, there is no clear guidance which tool is the most appropriate for measuring the renal function in patients with HIV.

Physicians who treat HIV-infected patients are concerned whether the calculated eGFRs derived from non-HIV population are precise and accurate in assessing HIV-associated chronic kidney disease (CKD) because the body compositions of HIV and non-HIV patients vary. None of the methods used to date have been well validated in HIV-infected Asian patients. Since all of the GFR measuring tools were validated in HIV-negative caucasians and blacks (African Americans)[1-4], therefore its use in other ethnic groups casts doubt to its appropriateness. Even though ^{99m}Tc DTPA plasma clearance is highly accurate and is the gold standard for GFR assessment, it is impractical to scale up in resource-limited settings. The serum creatinine is the simplest method but its inability to detect early decline of the renal function is its major pitfall[5]. Serum cystatin C is more expensive than serum creatinine and the effect of HIV replication may limit its use in this population[6]. The calculated methods for GFR may be the best tool for use in resource-limited settings because it does not require the use of sophisticated equipments nor has any other additional expenses aside from serum creatinine tests. Each of the equations has its own pitfall but the most important is that it has not been well validated in HIV-infected patients, especially Asians. The Cockcroft-Gault (CG) method [7] is the most commonly used because it is easy to calculate.

Lately there is increasing evidence suggesting that the Modification of Diet in Renal Disease (MDRD)[1, 3] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)[4] may be more accurate in assessing GFR in caucasians and African Americans yet in Asians, the MDRD tends to overestimate the prevalence of renal disease[8]. As a result of this, Praditpornsilpa K et al[9] proposed a racial factor for Thais and Asians that appears to give a more accurate GFR measurement, at least in HIV-negative patients.

With this adjusted eGFR measurement, it will improve the detection of CKD and end stage renal failure (ESRD). Aside from that, this adjusted eGFR measurement can be used to monitor the deterioration rate of the renal function so physicians can change the dose of ART to prevent CKD/ESRD. Therefore, this study validated all of the available methods used to assess renal function (re-expressed MDRD formula, CKD-EPI equation, re-expressed MDRD formula with Thai racial factor correction, Thai eGFR equation, Cockcroft & Gault, and cystacin C GFR) in ARV drug experienced HIV-infected patients and compared the results to the gold standard of ^{99m}Tc DTPA plasma clearance.

Materials and Methods

Patients

The study was approved by the Ethical Committee for Research, Chulalongkorn University, Bangkok, Thailand. All patients have provided written informed consent. Stable HIV-infected adults > 18 years old, followed by HIV-NAT (The HIV Netherlands Australia Thailand Research Collaboration), Bangkok, Thailand, were recruited into the study. The study was conducted in an ambulatory setting and began at 08:00-09:00 AM to avoid the diurnal variations in the renal function. Patients with acute deterioration of the renal function, amputation, malnutrition (BMI < 18 kg/m^2), in a bed ridden state, with infection, in an edematous state, gastrointestinal bleeding, heart failure, or were hospitalized were excluded. Women of childbearing age without a reliable contraceptive method, patients on renal replacement therapy, patients taking methyldopa, levodopa, ascorbic acid, cimetidine, trimethoprim, antibiotics, steroids, or flucytosine were also excluded.

Clinical Data and Body Composition Assessment

Body composition was assessed by bioimpedance analysis (BIA) using Body Composition Analyzer (In Body S20, Biospace, Korea). Skeletal muscle mass, body fat mass, and total body water were analyzed. Body weight (BW), height, blood pressure were recorded.

Reference GFR Measurement

The reference GFR was determined by plasma collected at 10 different time points by using the ^{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 blood samples to determine the radioactivity background and for the serum creatinine assay. A single intravenous bolus of ^{99m}Tc-DTPA was injected into each patient. Blood specimens were drawn to assess plasma radioactivity at 5, 10, 20, 30, 60, 90, 120, 180, and 240 minutes post ^{99m}Tc-DTPA injection. Plasma radioactive activities were then plotted as a function of time to create a time-activity curve to calculate for GFR (Fig 1). The GFR equation was determined by using bi-exponential fitting method [10]:

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

D is the dosage of injected ^{99m}Tc-DTPA. The result was normalized by the body surface area which was calculated according to Dubois and Dubois [11].
Reference GFR by ^{99m}Tc-DTPA plasma clearance were read by a radiologist who was blinded to the clinical status and laboratory results of the patients.

Calibration for the Serum Creatinine Assay

Fasting serum creatinine was measured by using a Roche Diagnostics (Indianapolis, IN, US) CREA plus (11775642) enzymatic assay (Cr_{Enz}), on a COBAS, INTRGRA 400 plus analyzer. The measured Cr_{Enz} values were adjusted by using traceable high-level IDMS reference serum creatinine, as recommended by the National Kidney Disease Education Program. The IDMS

reference serum creatinine (SRM 967) was purchased from the National Institute of Standards and Technology. The certified concentration values for serum creatinine were 0.847 ± 0.018 mg/dL for level 1 and 3.877 ± 0.082 mg/dL for level 2.

Cystatin C

Serum for cystatin C levels was collected at the time of serum creatinine collection. Cystatin C was measured by a particle Enhanced Turbidimetric ImmunoAssay (PETIA) (ARCHITECT *c* Systems and AEROSET Cystatin C Reagent, Abbott Diagnostics)..

eGFR calculation for the Thai HIV population

The eGFR values were calculated by using the re-expressed MDRD equation, CKD-EPI equation, re-expressed MDRD equation with Thai racial factor correction, Thai eGFR equation, Cockcroft & Gault formula, and cystacin C GFR [12] (Table 1).

Urine 24-hour Collection

Urine was collected over a 24-hr period which included the morning when ^{99m}Tc DTPA plasma clearance GFR was measured. Verbal and written instructions on appropriate collection technique were provided to the patients beforehand. Container for urine collection was provided to each patient. Urine collection was performed at home 1 day prior the day of radioisotope GFR. Creatinine clearance (CrCl) was calculated by using this equation: CrCl = (Urine creatinine/Serum creatinine) x (urine volume / time of the actual collection). CrCl estimations were adjusted for BSA.

Statistical Analysis

Bland-Altman plots were used to assess the agreement between eGFR and reference GFR [13]. The regression of the average and the difference between the reference GFR and eGFR (reference GFR minus eGFR) were analyzed. Statistical analysis was performed by using MedCalc Software version 10 (Mariakerke, Belgium) and SAS/STAT software version 9.3 (SAS Institute, Cary, NC, USA).

Study Results

Characteristics of the Patients

A total of 208 HIV-infected cases were studied; 196 cases [10 anti-retrovirus (ARV) naïve and 186 well suppressed HIV; 43% were female] completed the study and were included in this analysis (Table 2). The average exposure to ARV was 8.6 **±** 3.5 years. The mean (SD) viral load was 2,647.9 ± 18,590.2 copies/ml, and only 10 subjects (ARV naïve) had VL > 50 copies/mL. The mean (SD) CD4 count was 610.3 ± 241.5 cells/μL; none of the patients had CD4 < 200 cells/μL. The averages of the body mass index (BMI) and body surface area (BSA) were 22.3 **±** 3.2 kg/m^2 and 1.63 ±0.18 m^2, respectively. Only 15% of the patients had a BMI in the overweight (>25kg/m^2) range. Fifty-five percent, 44% and 59% of them were classified as having low skeletal muscle mass, high body fat mass and high body fat percent, respectively. The mean Cr_{Enz} was 0.91 ±0.29 mg/dL (95% confidential interval (95% CI) of 0.86 to 0.95 mg/dL). The mean reference GFR (^{99m}TcDTPA) was 117.7± 29.2 mL/min/1.73 m^2 (95% CI of 113.6 to 121.8 mL/min /1.73 m^2). One hundred and sixty-seven patients (85%) had an isotope GFR of > 90 mL/min/1.73^2 and only 2% had low isotope GFR of < 60 mL/min/1.73 m^2. Diabetes Mellitus and hypertension were found in a minority of the patients (7% and 15%, respectively). None of them were on ganciclovir, adefovir, and cidofovir 6 months prior to this study.

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

To assess the agreement between eGFR values and the reference GFR, Bland-Altman plots were produced (Fig. 2). The agreement was compared by calculating the bias estimated on the mean of the differences ± the limits of agreement of each eGFR equation. The bias estimated by the mean of differences **±** the limits of agreement for the re-expressed IDMS traceable MDRD equation, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR, Cockcroft & Gault, cystacin C GFR and CrCL by 24 hr urine were expressed as 18.9±27.3, 11.1±25.5, 6.2±28.8, 15.4±27.0, 30.4±28.0,

3.2±36.1 and 5.0±12.1 mL/min per 1.73 m^2 respectively. The spread of the bias between the reference GFR and the eGFR by re-expressed MDRD equation with Thai racial correction factor was the most evenly distributed (Fig 2C) which can be interpreted that at each GFR, the bias for reference GFR and this equation was constant at 6.2 mL/min/1.73 m^2.

From the linear regression analysis between the reference GFR and different equations for eGFR, the slopes of the regression lines varied for each equation by 0.81 for the re-expressed MDRD equation, 0.85 for the CKD-EPI equation, 0.92 for the re-expressed MDRD equation with Thai racial correction factor, 0.86 for the Thai eGFR equation, 0.73 for the Cockcroft &Gault, 0.89 for the cysticin C GFR, and 0.96 for the CrCl by the 24-hr urine (Table 3). Compared to the other equations for serum Cr based eGFR, the slope from the re-expressed MDRD equation with Thai racial correction factor was almost identical to the reference GFR (slope=1.0).

Table 1. eGFR equations.

eGFR Methods	Gender	Serum Cr	Equations
Re-expressed MDRD equation [3]	-	Cr_{Enz}	$175 \times (Cr_{Enz})^{-1.154} \times (Age)^{-0.203}$ (x 0.742 if female)
CKD-EPI equation [4]	Female	$Cr_{Enz} \leq 0.7$ mg/dL	$144 \times (Cr_{Enz}/0.7)^{-0.329} \times (0.993)^{Age}$
	Female	$Cr_{Enz} > 0.7$ mg/dL	$144 \times (Cr_{Enz}/0.7)^{-1.209} \times (0.993)^{Age}$
	Male:	$Cr_{Enz} \leq 0.9$ mg/dL	$141 \times (Cr_{Enz}/0.9)^{-0.411} \times (0.993)^{Age}$
	Male:	$Cr_{Enz} > 0.9$ mg/dL	$141 \times (Cr_{Enz}/0.9)^{-1.209} \times (0.993)^{Age}$
Re-expressed MDRD equation with Thai racial factor [9]	-	Cr_{Enz}	$175 x Cr_{Enz}^{(-1.154)} x Age^{(-0.203)}$ x0.742 (if female) x1.129) if Thai
Thai eGFR equation [9]	-	Cr_{Enz}	$375.5x(Cr_{Enz})^{-0.848)}x(Age)^{-0.364}$ x0.712 (if female)
Cockcroft & Gault equation [7]	-	Cr_{Enz}	[(140-age)xBW/ Cr_{Enz} x72]/BSAx0.85 if female
Cystatin C GFR [12]	-	-	$86.7/\text{cystatin C}^{-4.2}$

Table 2. Characteristics of patients enrolled in the study.

	Mean ± SD	Median
• **Age (years)**	43.6±7.8	43.2
• **Body weight (kg)**	59.0 ±10.9	56.6
• **Height (meter)**	1.63 ±0.82	1.62
• **ARV vintage (years)**	8.6 ±3.5	8.7
• **HIV Viral load (copies/ml)**	2,647.9 ± 18,590.2	50.0
• **CD4 count (cells/uL)**	610.3 ± 241.5	585.5
• **CD4 (%)**	28.0 ±7.8	24.0
• **BMI (kg/m^2)**	22.3 ±3.2	21.9
• **BSA (m^2)**	1.63 ±0.18	1.60
• **Skeletal muscle mass (kg)**	24.6 ±5.6	23.9
• **Body fat mass (kg)**	13.7 ±6.5	13.1
• **Fat free mass (kg)**	45.0 ±9.2	44.2
• **Soft lean mass (kg)**	42.5 ±8.7	41.5
• **MAP (mmHg)**	91.0 ±11.5	90
• **BUN (mg/dL)**	12.41 ± 4.96	12.00
• **Serum creatinine (mg/dL)**	0.91 ±0.29	0.90
• **Urine protein (mg/day)**	439.6±1095.7	220.0
• **Total cholesterol (mg/dL)**	204.1 ±45.9	196
• **Serum triglyceride (mg/dL)**	152.0±96	119
• **HDL (mg/dL)**	50.9 ±16.6	49
• **Plasma glucose (mg/dL)**	92.1 ±25.1	87.0
• **Serum phosphate (mg/dL)**	3.52 ±0.53	3.50

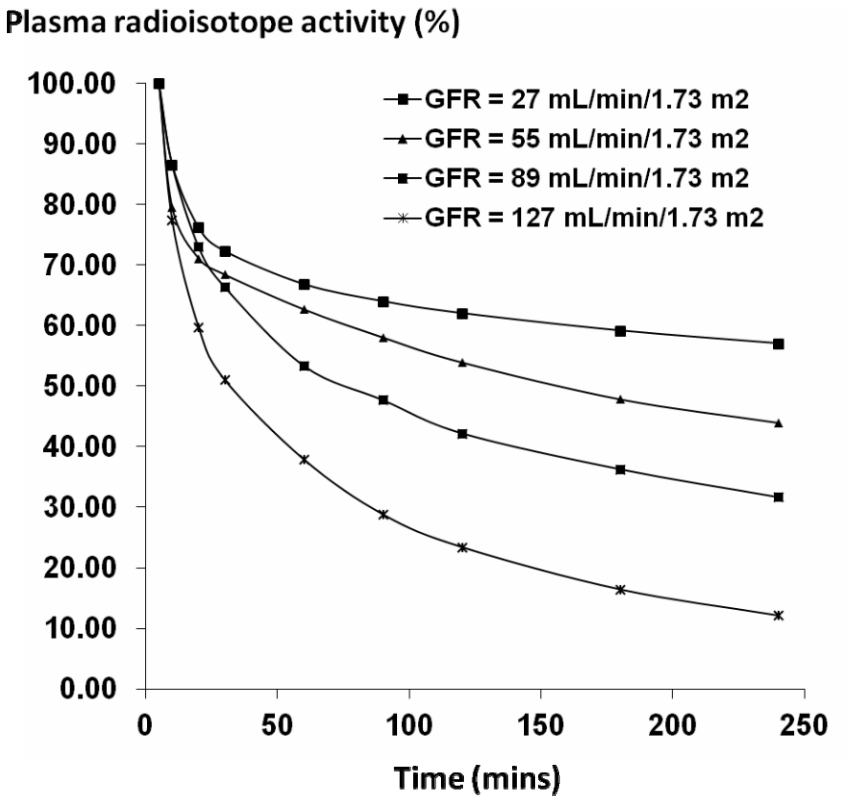
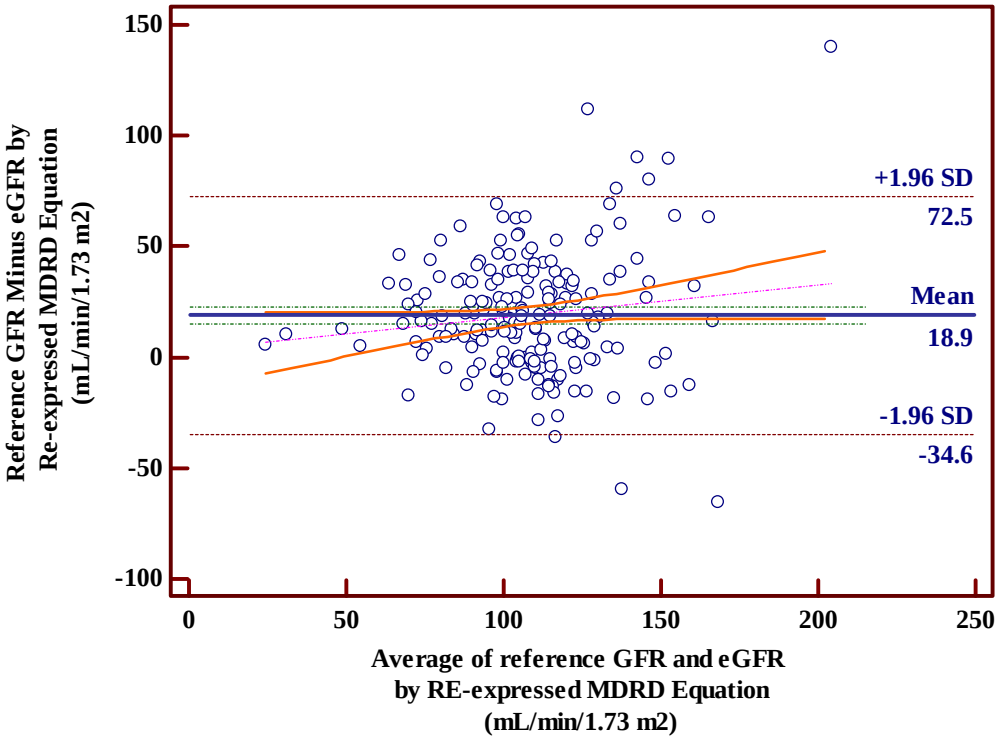
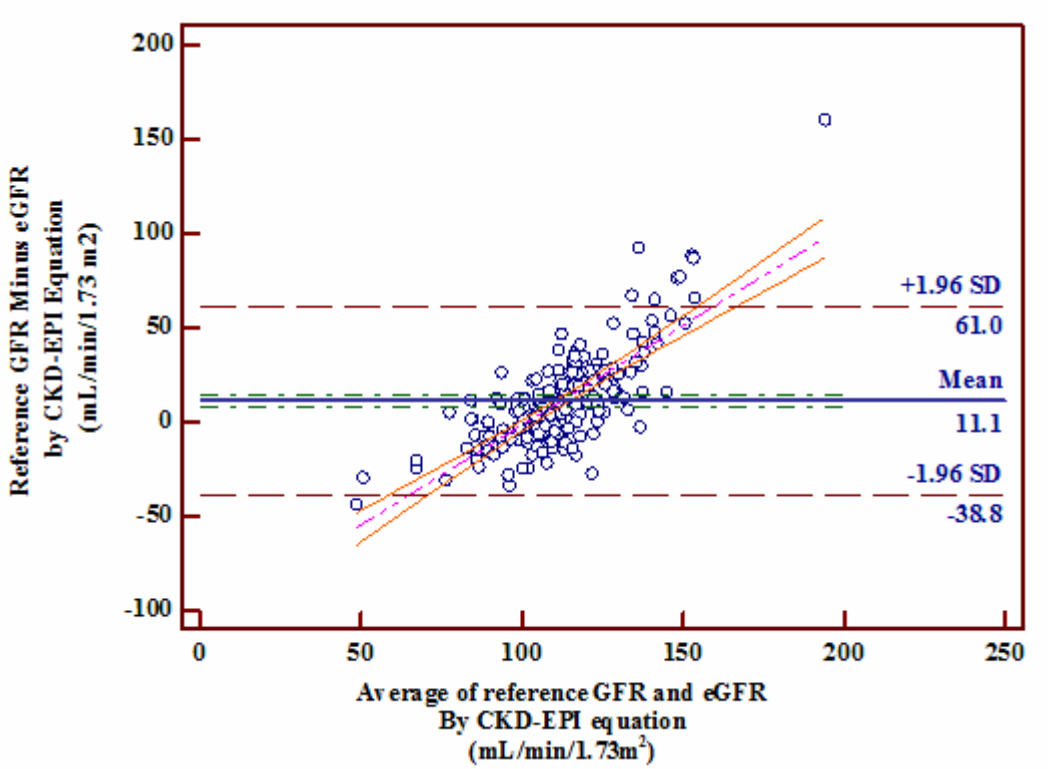


Figure 1. The plot of plasma radioisotope activities as a function of time for GFR in patients with GFR of 27 mL/min/1.73m^2 (CKD stage IV), 55 mL/min/1.73m^2 (CKD stage III), 55 mL/min/1.73m^2 (CKD stage IV), and 127 mL/min/1.73m^2 (CKD stage I)

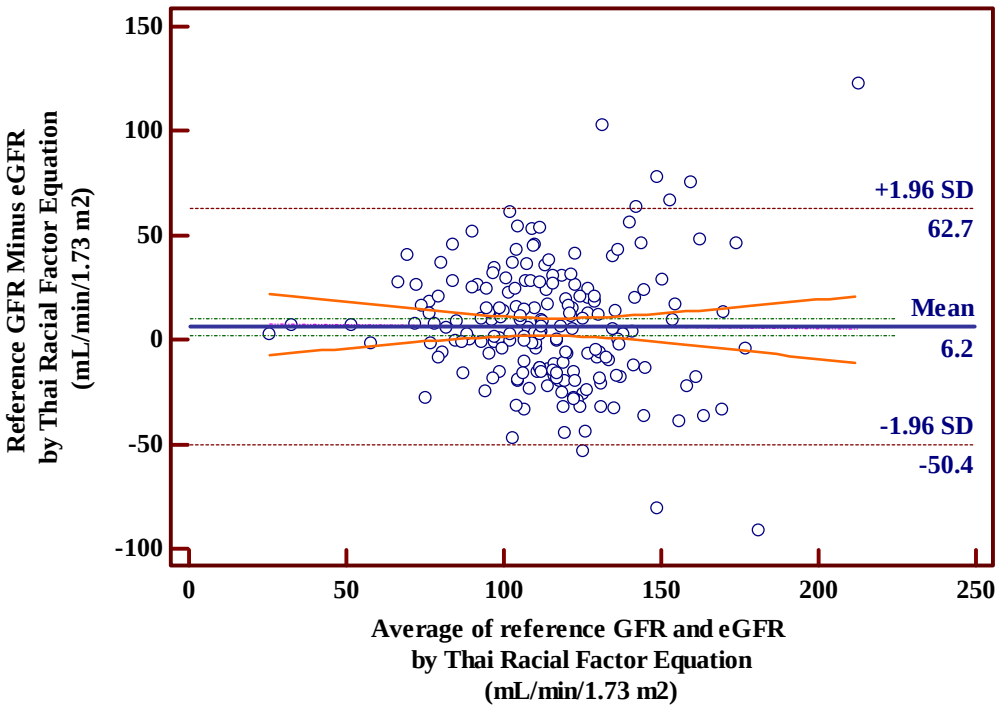
2A


Reference GFR Minus eGFR by
Re-expressed MDRD Equation
(mL/min/1.73 m2)
150
100
50
0
-50
-100
0
50
100
150
200
250
+1.96 SD
72.5
Mean
18.9
-1.96 SD
-34.6
Average of reference GFR and eGFR
by RE-expressed MDRD Equation
(mL/min/1.73 m2)

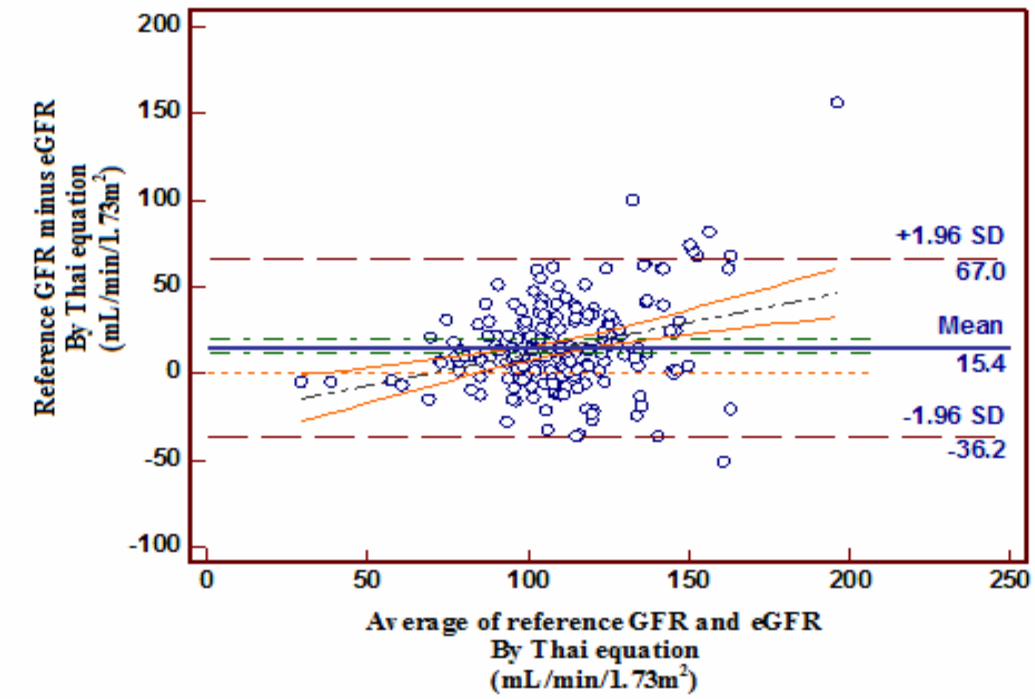
2B


Reference GFR Minus eGFR
by CKD-EPI Equation
(mL/min/1.73 m2)
200
150
100
50
0
-50
-100
0
50
100
150
200
250
+1.96 SD
61.0
Mean
11.1
-1.96 SD
-38.8
Average of reference GFR and eGFR
By CKD-EPI equation
(mL/min/1.73m2)

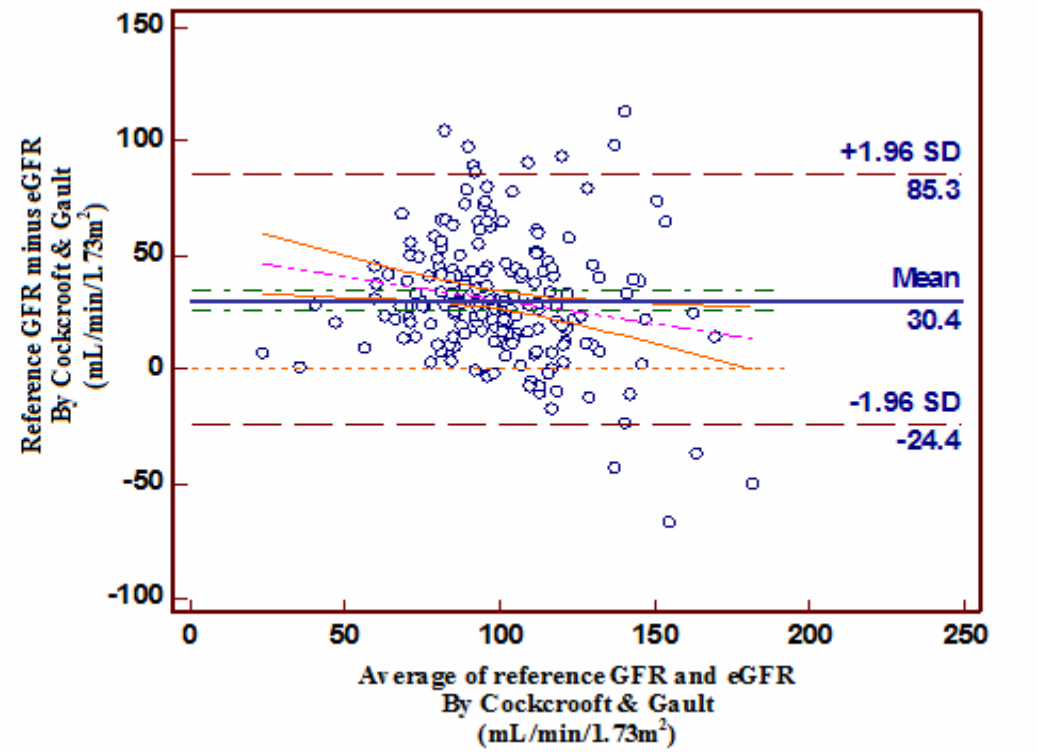
2C


Reference GFR Minus eGFR
by Thai Racial Factor Equation
(mL/min/1.73 m2)
150
100
50
0
-50
-100
+1.96 SD
62.7
Mean
6.2
-1.96 SD
-50.4
0
50
100
150
200
250
Average of reference GFR and eGFR
by Thai Racial Factor Equation
(mL/min/1.73 m2)

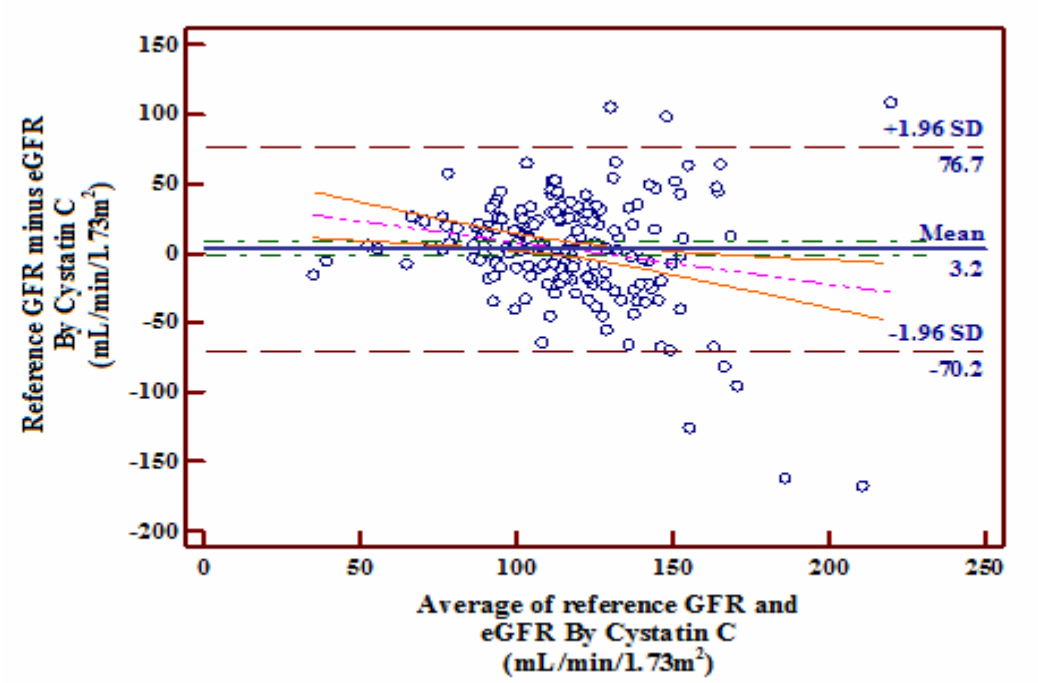
2D


Reference GFR minus eGFR
By Thai equation
(mL/min/1.73m2)
200
150
100
50
0
-50
-100
+1.96 SD
67.0
Mean
15.4
-1.96 SD
-36.2
0
50
100
150
200
250
Average of reference GFR and eGFR
By Thai equation
(mL/min/1.73m2)

2E


Reference GFR minus eGFR
By Cockcrooft & Gault
(mL/min/1.73m²)
+1.96 SD
85.3
Mean
30.4
-1.96 SD
-24.4
150
100
50
0
-50
-100
0
50
100
150
200
250
Average of reference GFR and eGFR
By Cockcrooft & Gault
(mL/min/1.73m²)

2F


Reference GFR minus eGFR
By Cystatin C
(mL/min/1.73m²)
+1.96 SD
76.7
Mean
3.2
-1.96 SD
-70.2
150
100
50
0
-50
-100
-150
-200
0
50
100
150
200
250
Average of reference GFR and
eGFR By Cystatin C
(mL/min/1.73m²)

2G

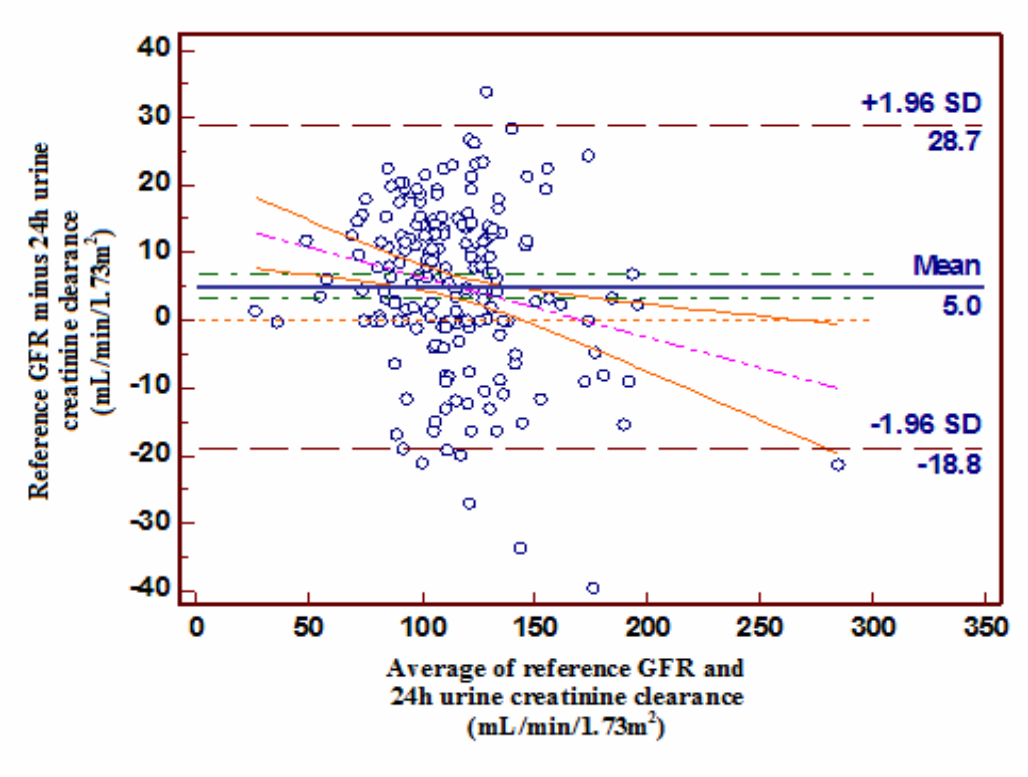


Figure 2. Bland-Altman plots of eGFR values calculated by different equations and the reference GFR which was used to show the disagreement between the equation and the reference. The mean bias and ± 1.96 SD are represented by the horizontal lines. The disagreement of different equations [**A**; re-expressed MDRD equation, **B**; CKD-EPI equation, **C**; re-expressed MDRD equation with Thai racial factor correction, **D**; Thai eGFR equation, **E**; Cockcroft & Gault, **F**; cystacin C GFR, and **G**; CrCL by 24 hr urine] are shown by the regression trend of the difference and the mean bias of the eGFRs towards the reference GFR.

Table 3. The means of reference GFR and eGFR calculated by the different eGFR equations. The disagreement between the mean bias of eGFR and reference GFR are shown. The regression showed a good correlation but the slope for eGFR by Thai racial factor had the best correlation to the reference GFR. The slope for eGFR by Thai racial factor was very close to 1.

	Mean GFR± SD ($mL/min/1.73m^2$)	Mean bias± SD ($mL/min/1.73m^2$)	Lower limit ($mL/min/1.73m^2$)	Upper limit ($mL/min/1.73m^2$)	Slope
Reference GFR	**117.7± 29.2**	**NA**	**NA**	**NA**	**NA**
eGFR by reexpressed MDRD	**98.7± 26.1**	**18.9±27.3**	**-34.8**	**72.5**	**0.81**
eGFR by CKD-EPI equation	**106.6± 11.9**	**11.1±25.5**	**-38.8**	**61.0**	**0.85**
eGFR by Thai racial factor	**111.5 ± 29.5**	**6.2±28.8**	**-50.4**	**62.7**	**0.92**
eGFR by Thai equation	**104.1 ± 23.4**	**13.5±27.0**	**-39.4**	**66.5**	**0.86**
Cockcroft & Gault	**86.4 ± 29.2**	**30.4±28.0**	**-24.4**	**85.3**	**0.73**
eGFR by Cystatin C	**114.0 ±36.1**	**3.6±36.1**	**-70.2**	**76.7**	**0.89**
CrCl by24 hr urine	**112.3 ± 31.4**	**5.0±12.1**	**-18.8**	**28.7**	**0.96**

Study Discussion

HIV infection remains incurable and indefinite ART is often limited by drug toxicity. Renal toxicity is a major cause for morbidity and mortality in HIV-infected patients. HIV infection is also a risk factor for CKD. Since kidney disease tends to be silent during the initial stages, therefore an accurate and reliable tool for measuring GFR in HIV-infected patients is urgently needed globally to properly monitor and manage HIV- and ART-related renal diseases. Studies showed high prevalence of CKD in HIV population [14]. The exposure to ARV is a unique risk factor of HIV population. Certain ARVs have been shown to be nephrotoxic and can cause renal stone disease as well as chronic tubulointerstitial disease. The expansion of HIV population and the success of HIV treatment can extend the lives of the patients that over time, some of them may develop CKD and progress to ESRD which will ultimately impact all health care services. It is important to find a reliable tool to calculate/measure GFR that will improve physician's clinical practice in detecting patients at risk for developing CKD if it can accurately monitor the deterioration rate of the renal function so doses of ART can be reduced to prevent the disease.

The re-expressed MDRD eGFR equation has been developed primarily for Caucasians and African-Americans with CKD [1-4]. Recent studies have shown that the calculation of eGFR derived from a race without prior validation will result in inaccurate estimations of GFR unless a racial factor is added to the equation to provide a more precise estimation [5-7]. Even though various eGFR equations have been studied in different races, the validation data has not been well studied in a large HIV population, especially in Asians. Our study has a large sample size (N=196) and is one of the first of its kind to compare various equations of estimated GFR against the radioisotope plasma clearance GFR in HIV-infected patients from Asia. Majority (95%) of the patients from the study's cohort are on ART and their HIV RNA are well suppressed (VL <50 copies/mL). Some of the patients are overweight but many have abnormal body compositions due to ART-related lipodystrophy, resulting in low skeletal mass and high body fat mass.

This study showed that the HIV status influenced the accuracy and precision of eGFR estimation. We demonstrated that the expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation, cystatin C GFR, and 24-hr urine CrCl underestimated the reference GFR. The application of re-expressed MDRD and CKD-EPI equations derived from non HIV-CKD population had a bias of 18.9 mL/min/1.73 m^2 and 11.1 mL/min/1.73 m^2 respectively. The spread of the bias between the reference GFR and the eGFR by CKD-EPI was not evenly distributed (Fig 2B). When GFR was less than 110 mL/min/1.73m^2 or more than 110 mL/min/1.73 m^2, the eGFR from the CKD-EPI overestimated or underestimated the reference GFR, respectively. From all of the

serum creatinine based eGFR equations, the re-expressed MDRD equation with Thai racial factor correction was the only equation that had the least bias of 6.2 mL/min/1.73 m^2 and an evenly distributed spread of bias. Therefore the re-expressed MDRD equation with Thai racial factor correction is more applicable to Thai HIV-CKD population. Our data agrees with Barraclough K et al's data [15] which showed that the MDRD formula was the most precise method for Caucasians infected with HIV.

Racial factor for each ethnicity is important. Recently, our group did a study in 350 HIV-uninfected patients with various CKD stages[16]. We found that differences in ethnicity significantly affected the results of the MDRD-based eGFR equation and racial factor for Thais should be 1.129. When we used the adjusted MDRD equation with Thai racial factor on our HIV-infected population with an abnormal body composition but well-preserved kidney function compared to the uninfected population, GFR estimation was precise and accurate. This study showed that re-expressed MRDR equation with Thai racial factor can precisely and accurately be used in Thais with or without HIV infection.

The performance of the MDRD with Thai racial factor suggests that this equation is suitable for GFR estimation in our HIV-infected population. However, this formulation may not be applicable for all HIV-infected Asians because other studies conducted in Chinese [17, 18]and Japanese [19-21] non-HIV-infected population have different racial factors of 1.23 and 0.88, respectively. This discrepancy within the Asian population makes it difficult to adopt a universal eGFR equation/racial corrected factor. It is unknown whether the body composition or the differences in determining the reference GFR method affected this disparity in eGFR equation and racial corrected factor for the MDRD-based GFR among Asians. The reference GFR from the Japanese study was obtained from using renal clearance of inulin whereas for the Chinese study, ^{99m}Tc DTPA was used. The techniques used in the Chinese study is similar to our group but we incorporated 10 time points within the 4 hours period instead of using only 2 time points in the Chinese study. Furthermore, we performed all isotopic measurements at the same time during the day for all patients to avoid diurnal GFR variation.

Our data supports Bonjoch A et al [22] who reported that cystatin C had the least bias compared to serum creatinine based eGFR equations in estimating isotopic GFR when used in 15 HIV-infected patients. The drawback of using cystatin C is that it is not standardized even though its use as a biomarker of renal function is increasing. It has been shown that there are systematic shifts in cystatin C levels [23] and standardization is necessary before it can be systematically and routinely used in the clinical setting. Following cystatin C GFR, the second less bias was CrCl by 24-hr urine collection. Unfortunately, 24-hr urine collection is the most impractical and difficult method to be used routinely in clinical practice; its precise collection of the urine has made this method unattractive.

The strong point of this study is its large sample size and intensive measurements of isotopic GFR (10 time points). This data can also be applied to females as 43% of the patients in the study were females. Aside from that, this data is representive for both HIV with and without lipodystrophy/abnormal body composition.

The primary limitation of this study is that the results may not be generalizable to non-Thais. In addition, most of the patients had high CD4 and undetectable VL or well-controlled HIV suppression so this data may not be applicable to patients with a more profound immunodeficiency or AIDS-related wasting.

In conclusion, we have proved that there need for the racial correction factor for the eGFR equation for both non-HIV CKD and HIV population. This study demonstrated that the eGFR by re-expressed MDRD formula with Thai racial correction factor of 1.129 was the most practical, precise and accurate in predicting the reference GFR. Therefore it is highly and strongly recommended that the existing eGFR equations should be validated before using it in both non-HIV CKD and HIV population in epidemiologic studies and in the clinical setting.

Summary and Suggestion

การศึกษานี้แสดงให้เห็นว่าค่าคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineด้วยวิธี re-expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation ,หรือการตรวจ cystatin-C, และการตรวจปัสสาวะ24 ชั่วโมงได้ค่าต่ำกว่าการตรวจอัตราการกรองของไตด้วยวิธี ^{99m}Tc-DTPAอย่างไรก็ตามค่าที่แม่นยำและถูกต้องที่สุดคือการตรวจด้วยวิธี cystatin C แต่วิธีนี้ไม่สะดวก ราคาสูงกว่า และไม่สามารถทำได้ตามสถานพยาบาลทั่วไป ดังนั้นการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineเป็นวิธีที่ง่ายและสะดวกที่สุด ซึ่งใน4 สมการ (re-expressed MDRD, CKD-EPI, re-expressed MDRD formula with Thai racial correction factor, Thai eGFR equation) ของการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineพบว่าการใช้ re-expressed MDRD formula with Thai racial correction factor เป็นค่าที่แม่นยำและถูกต้องที่สุดซึ่งการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineด้วยสมการ re-expressed MDRD formula with Thai racial correction factorนี้ได้มีการใช้ในผู้ป่วยคนไทยที่ไม่ติดเชื้อเอชไอวีด้วย การศึกษานี้แสดงให้เห็นว่าเชื้อชาติมีความสำคัญต่อการคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinine ดังนั้นในแต่ละเชื้อชาติควรมีการประเมินracial factorก่อนนำไปใช้ โดยเฉพาะในผู้ป่วยติดเชื้อเอชไอวี และการศึกษานี้แสดงให้เห็นว่าในผู้ป่วยติดเชื้อเอชไอวีคนไทยสามารถใช้การคำนวณอัตราการกรองของไตด้วยวิธีการคำนวณจากระดับserum creatinineด้วยสมการ re-expressed MDRD formula with Thai racial correction factorได้เช่นเดียวกับในผู้ป่วยคนไทยที่ไม่ติดเชื้อเอชไอวี ดังนั้นวิธีนี้ควรจะได้นำไปใช้กับคนไทยทั้งประเทศเพื่อให้เป็นมารตฐานเดียวกัน และเพื่อให้ได้ข้อมูลของการเกิดโรคไตเรื้อรังไปในทางเดียวกัน

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References

1. Cihlar T, Ray AS, Laflamme G et al. Molecular assessment of the potential for renal drug interactions between tenofovir and HIV protease inhibitors. *Antivir Ther* 2007; 12: 267-72.
2. Imaoka T, Kusuhara H, Adachi M et al. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. *Mol Pharmacol* 2007; 71: 619-27.
3. Izzedine H, Hulot JS, Villard E et al. Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy. *J Infect Dis* 2006; 194: 1481-91.
4. Kiser JJ, Aquilante CL, Anderson PL et al. Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. *J Acquir Immune Defic Syndr* 2008; 47: 298-303.
5. Ray AS, Cihlar T, Robinson KL et al. Mechanism of active renal tubular efflux of tenofovir. *Antimicrob Agents Chemother* 2006; 50: 3297-304.
6. DHHS. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. . Department of Health And Human Services December 29, 2008.: access online at www.aidsinfo.nih.gov June 14, 2009.
7. Kuller LH, Tracy R, Belloso W et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med* 2008; 5: e203.
8. Imai E, Horio M, Nitta K et al. Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. *Clin Exp Nephrol* 2007; 11: 41-50.
9. Zuo L, Ma YC, Zhou YH et al. Application of GFR-estimating equations in Chinese patients with chronic kidney disease. *Am J Kidney Dis* 2005; 45: 463-72.
10. Ma YC, Zuo L, Chen JH et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol* 2006; 17: 2937-44.
11. Imai E, Horio M, Iseki K et al. Prevalence of chronic kidney disease (CKD) in the Japanese general population predicted by the MDRD equation modified by a Japanese coefficient. *Clin Exp Nephrol* 2007; 11: 156-63.
12. PraditpornsilpaK T, Chawatanarat T, Wathanavaha A, Pansin P, Tiranathanagul K, Katawatin P, Trakarnvanich T, Kanjanabuch T, Avihingsanon Y, Tungsanga K, Eiam-Ong S Differences in the Racial Factor for MDRD-Based Glomerular Filtration Rate Estimation in Different CKD Populations. World Congress of Nephrology 2009 Milan Italy May 22-26, 2009. 2009.
13. Madero M, Sarnak MJ, Stevens LA. Serum cystatin C as a marker of glomerular filtration rate. *Curr Opin Nephrol Hypertens* 2006; 15: 610-6.
14. Knight EL, Verhave JC, Spiegelman D et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 2004; 65: 1416-21.
15. Mauss S, Berger F, Kuschak D et al. Cystatin C as a marker of renal function is affected by HIV replication leading to an underestimation of kidney function in HIV patients. *Antivir Ther* 2008; 13: 1091-5.
16. Levey AS. Measurement of renal function in chronic renal disease. *Kidney Int* 1990; 38: 167-84.
17. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 1992; 38: 1933-53.
18. Levey AS, Bosch JP, Lewis JB et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation.

Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; 130: 461-70.
19. Levey A, Greene T, Kusek J et al. A simplified equation to predict glomerular filtration rate from serum creatinine [Abstract]. *J Am Soc Nephrol* 2000; 11: A0828.
20. Eknoyan G, Levin N. NKF-K/DOQI Clinical Practice Guidelines: Update 2000. Foreword. *Am J Kidney Dis* 2001; 37: S5-6.
21. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39: S1-266.
22. Manetti L, Pardini E, Genovesi M et al. Thyroid function differently affects serum cystatin C and creatinine concentrations. *J Endocrinol Invest* 2005; 28: 346-9.
23. Risch L, Herklotz R, Blumberg A et al. Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem* 2001; 47: 2055-9.
24. Wasen E, Isoaho R, Mattila K et al. Serum cystatin C in the aged: relationships with health status. *Am J Kidney Dis* 2003; 42: 36-43.
25. Odden MC, Scherzer R, Bacchetti P et al. Cystatin C level as a marker of kidney function in human immunodeficiency virus infection: the FRAM study. *Arch Intern Med* 2007; 167: 2213-9.
26. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; 40: 221-6.

Appendix

1. Manuscript of Comparisons between validated estimated glomerular filtration rate (GFR) equations and isotopic GFR in an HIV-positive population

2. Abstract ที่ได้รับการคัดเลือกให้แสดงผลงานในการประชุม 19th Conference on retroviruses and opportunistic infections (CROI) ในระหว่างวันที่ 5-8มีนาคม 2012 ที่ Washington State Convention Center Seattle, Washington, USA