Estimation of Glomerular Filtration Rate using Serum Cystatin C in Overweight and Obese Subjects

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SUMMARY

Background: Obesity and overweight are strong independent risk factors for chronic kidney disease (CKD). Using serum creatinine-based estimated glomerular filtration rate (eGFR) equations in these subjects may be inaccurate. On the other hand, cystatin C–based eGFR equations may overestimate CKD prevalence as recent findings suggest an association of cystatin C with obesity. The objective of this study was to assess the accuracy of a cystatin C–based eGFR equation compared to two creatinine –based eGFR equations in overweight and obese subjects.

Methods: This was a prospective cross-sectional study which recruited healthy volunteers aged 18-55 years with a body mass index (BMI) ≥ 23kg/m² (Asia Pacific Guidelines). Their renal profiles, serum cystatin C and 99m technetium diethylene triamine pentacetic acid (^{99}mTc-DTPA) scans were performed on the same day. The correlations and accuracy of the creatinine-based and cystatin C-based eGFR equations with the ^{99}mTc-DTPA GFR were determined.

Results: One hundred and one subjects with a median age of 30.0 (27.0-43.5) years and mean BMI of 28.7 ± 4.5 kg/m2 were recruited. The cystatin C-based eGFR equation showed the best correlation with the ^{99}mTc-DTPA GFR (r=0.526, p=0.001) and was more accurate in measuring abnormal GFR compared to the creatinine-based eGFR equations.

Conclusion: Our study showed that the cystatin C-based eGFR equation was more accurate, sensitive and specific in overweight and obese subjects compared to the creatinine-based eGFR equations.

KEY WORDS:
Chronic kidney disease, Creatinine, cystatin C, Estimated glomerular filtration rate, Obese, Overweight

INTRODUCTION

Early identification and treatment of chronic kidney disease (CKD) is vital so that prophylactic measures can be instituted to delay to delay its progression. The Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines have established a five stage classification of patients with CKD based on the level of glomerular filtration rate (GFR) and a clinical action plan based on the disease stage [1]. Table I.

Obesity has become a major public health problem and its prevalence has been rising worldwide [2]. According to the World Health Organization (WHO), the optimal BMI for Asians is 18.5 to 22.9 kg/m² based on the mortality outcome studies [3]. Several prospective studies have reported that obesity was associated with an increased risk for CKD and end stage renal disease (ESRD) [4-8]. The mechanisms of renal failure in obesity remain elusive and are largely speculative. In addition to haemodynamic factors, inflammatory and metabolic effects related to obesity have been implicated [9].

GFR provides an excellent measure of the filtering capacity of the kidneys and is considered the best index of renal function [10, 11]. Inulin clearance has long been regarded as the gold standard for measuring GFR but the procedure is costly, time consuming and difficult to perform [11]. Several equations have been developed to improve the accuracy of serum creatinine as a measure of estimated glomerular filtration rate (eGFR). The most widely used in adult populations are the Cockroft-Gault (CG) [12] equation and the four-variable Modification of Diet in Renal Disease equation (MDRD4) [13].

Calculating eGFR using these equations in overweight and obese individuals remains problematic as they are less accurate at extreme levels of kidney function. It is also affected by inter-laboratory and inter-methodology variations in the measurement of serum creatinine levels. Our earlier work have demonstrated that the CG equation was inaccurate in overweight and obese subjects [14]. Shara *et al* suggested that obesity in young subjects may significantly impact on eGFR calculations [15].

Serum cystatin C is a new and promising marker for kidney dysfunction [16-18]. Numerous studies have found cystatin C to be a better marker of GFR than creatinine. However, recent findings suggest that cystatin C is independently associated with obesity, body mass index (BMI) and waist circumference [19-22]. Hence, cystatin C–based eGFR equations may result in overestimation of CKD prevalence at greater BMI levels.

The objective of this study was to assess the accuracy of cystatin C–based eGFR equation in measuring abnormal GFRs in overweight and obese subjects compared to creatinine –based eGFR formulae.

The eGFR equations used were:

1. Larsson Cystatin C-based formula corrected to body surface area (BSA CysCbsa).
2. CG corrected to BSA (CGbsa).
3. MDRD4 (already expressed as BSA)

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The reference GFR in this study was GFR measured by 99m technetium diethylene triamine pentacetic acid (^{99}mTc-DTPA) scan.

Abnormal GFRs were defined as
1. GFR < 90ml/min/1.73m^2
2. GFR > 120ml/min/1.73m^2

SUBJECTS AND METHODS

This was a prospective cross-sectional, single centre study involving overweight and obese subjects. The study protocol was approved by the Medical Research and Ethics Committee of the Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre and adhered to the Declaration of Helsinki. Healthy volunteers aged 18-55 years with a BMI >23 kg/m^2 were eligible for this study. Subjects with the following conditions were excluded: acute and chronic medical illnesses, history of hospital admission within one month prior to the study, history of taking traditional medications and/or non-steroidal anti-inflammatory drugs and/or corticosteroids and/or angiotensin converting enzyme inhibitors and/or angiotensin receptor blockers, pregnant women and lactating mothers.

The study population was selected from subjects who fulfilled the study criteria and gave informed consent. A full history and physical examination including the subjects' height, weight and blood pressure were performed. Baseline blood investigations including serum creatinine and serum cystatin C were taken. The ^{99}mTc-DTPA radionuclide scan was performed on the same day as blood investigations. Serum creatinine measured using the latest generation of buffed kinetic Jaffe reaction without deproteinization. Blood for serum cystatin C was stored at -80^{o}C and sent to a certified private laboratory in a batch within six months of blood collection. Serum cystatin C was performed using the Particle enhanced nephelometric immunoassay (PENIA) principle with cystatin C kits (Dade Behring). Should any abnormality be detected, subjects were referred to the appropriate subspecialty clinics.

Definition of Terms:
The Asia Pacific Guidelines (3) define:
1. Obese as BMI ≥ 25.0 kg/m^2
2. Overweight as BMI between 23.0-24.9 kg/m^2

Equations:
1. *Cockckroft – Gault* [12]:
   $$\text{eGFR (ml/min)} = \frac{(140\text{-age}) \times \text{body weight (kg)} \times \text{constant}}{\text{Serum creatinine (umol/L)}}$$
   Constant: 1.23 for male and 1.04 for female.
2. *MDRD4* [13]:
   eGFR (ml/min/1.73m^2) = 186 x creatinine (mg/dL)$^{-1.154}$ x age (years)$^{-0.203}$ x constant
   Constant: 1 for male, 0.742 for female and 1.212 for African American.
   *creatinine (mg/dL) = creatinine (umol/L) / 88.4
3. Larsson Cystatin C-based equation [23]:
   GFR (mL/min) = 77.24 X Cys C$^{-1.2623}$
4. BSA calculation [24]
   BSA (m^2) = 0.007184 x Height (cm)$^{0.725}$ x Weight (kg)$^{0.425}$
5. *eGFR corrected to BSA* [15]
   eGFR (ml/min/1.73m^2) = eGFR (ml/min) x 1.73m^2/BSA
6. *BMI* [25]
   BMI (kg/m^2) = weight (kg)/ [height (m)]^2

Statistical Analysis
Based on the prevalence of impaired kidney function in an overweight and obese population of 12.4 % (4), 100 patients were needed for a power of study of 90% with a confidence interval (CI) of 95%. To provide a slight margin of error given the possibility of subject attrition, we targeted to recruit 130 subjects.

The Statistical Package for Social Science (SPSS) version 12.0 (SPSS Inc. Chicago, IL) was used for statistical analysis. Correlation (r) between any two parameters was determined by the Pearson coefficient for normally distributed data. Sensitivity, specificity and areas under the receiver operating characteristic (ROC) curves were calculated to evaluate the overall performance of the eGFR equations. A p value <0.05 was considered significant.

RESULTS

During the study period (August 2008 to February 2009) a total of 126 volunteers were screened. However, 25 subjects dropped out for various reasons – two became pregnant, three went for further studies, one had a knee injury, 19 withdrew consent after the baseline investigations. Therefore only 101 subjects completed the study. Their baseline characteristics and demographic data are as shown in Table II. Their blood investigations, reference GFR and eGFR results are as tabulated in Table III.

The eGFR from CGbsa, MDRD4 and CysCbsa correlated significantly with the reference GFR with r's of 0.297 (p=0.03), 0.456 (p<0.001) and 0.526 (p<0.001) respectively. (Figure 1)

There were 10 obese subjects (9.8%) with a GFR <90 ml/min/1.73m^2, nine in CKD stage 2 and one in CKD stage 3. There were 38 subjects (37.6%) with a GFR >120 ml/min/1.73m^2. Of these, 14 were overweight and 24 were obese. The sensitivity, specificity and area under the ROC curve for each equation for the measurement of GFR < 90 ml/min/1.73m^2 and GFR >120ml/min/1.73m^2 are as tabulated in Table IV . Figure 2 and Figure 3 displayed the ROC curve for each equation in the measurement of GFR < 90 ml/min/1.73m2 and GFR >120ml/min/1.73m^2 respectively.

DISCUSSION

WHO estimates that more than one billion people are overweight and of these, 300 million are obese 26. Obesity-related morbidity has imposed a heavy burden on health care systems and lowered the quality of life. Obesity is also a strong independent risk factor for CKD and ESRD Thus early and accurate assessment of renal function in overweight and obese patients is vital to ensure success of interventions[4-8].

We studied creatinine-based and cystatin C-based eGFR equations in overweight and obese subjects who were

Table I: Classification of Chronic Kidney Disease

Stage	Description	Creatinine Clearance (≈GFR) (ml/min/1.73m²)
1	Normal or increase GFR- people at risk or with early renal damage	> 90
2	Early renal insufficiency	60-89*
3	Moderate renal failure	30-59
4	Severe renal failure	15-29
5	End stage renal disease	< 15

GFR: Glomerular filtration rate
* may be normal for age

Table II: Baseline characteristics and demographic data of study subjects

Parameters	Results
Age (years) *	30.0(27.0-43.5)
Race (Malay : Chinese : Indian : Others)	85 : 7 : 6 : 3
Gender (male : female)	27 : 74
Height (cm)*	157.0 (153.3-166.0)
Weight (kg)	73.5 ± 14.5
Body Mass Index (kg/m²)	28.7 ± 4.5
Systolic Blood Pressure (mmHg)	126.1±17.0
Diastolic Blood Pressure (mmHg)	74.9±10.3

Values are given in mean ± SD or * median (interquartile range)

Table III: Blood investigations, ^{99}mTc-DTPA GFR and eGFR results

Parameters	Results
Creatinine (NR: 44-80 µmol/L)	59.0(54.0-68.0)
Cystatin C (NR:0.42-0.85 mg/L)	0.72 ± 0.12
Fasting blood sugar (NR: 3.0-6.7 mmol/L)	5.0 (4.7-5.3)
Total cholesterol (NR <5.7 mmol/L)	5.5 ± 1.0
Triglyserides(NR<1.40 mmol/L)	1.1(0.8-1.4)
^{99}mTc-DTPA GFR(ml/min/1.73m²)	115.6 ± 24.5
CGbsa eGFR (ml/min/1.73m²)	134.4 ± 27.5
MDRD4 eGFR(ml/min/1.73m²)	112.7 ± 20.7
CysCbsa eGFR(ml/min/1.73m²)	123.3 ± 35.3

Values are given in mean ± SD or * median (interquartile range)
NR: Normal range

Table IV: The sensitivity, specificity and area under the ROC curve for each equation for the measurement abnormal GFR.

Equation	GFR < 90 ml/min/1.73m²			GFR >120 ml/min/1.73m²		
	Sensitivity	Specificity	Area under ROC	Sensitivity	Specificity	Area under ROC
CGbsa	30.0%	97.8%	0.689 (95% CI 0.470-0.908)	78.9%	36.5%	0.632 (95% CI 0.524-0.741)
MDRD4	50.0%	91.2%	0.796 (95% CI 0.640-0.953)	52.6%	79.4%	0.734 (95% CI 0.633-0.834)
CysCbsa	60.0%	86.8%	0.884 (95% CI 0.802-0.965)	78.9%	63.5%	0.797 (95% CI 0.710-0.885)

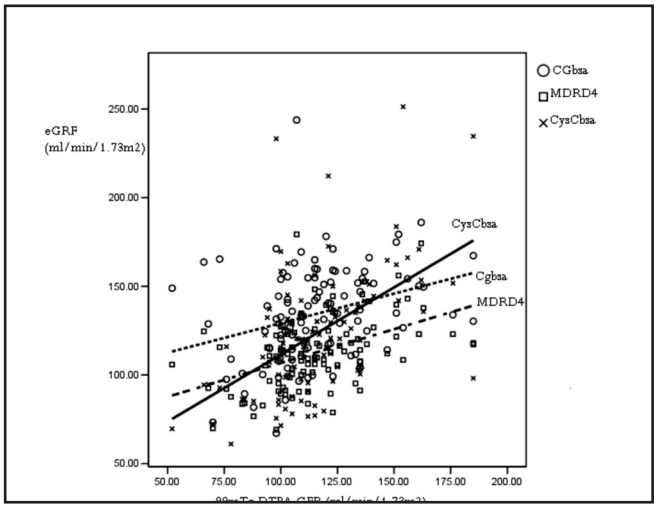

Fig. 1: Correlation between eGFR equations with ^{99}mTc-DTPA GFR.

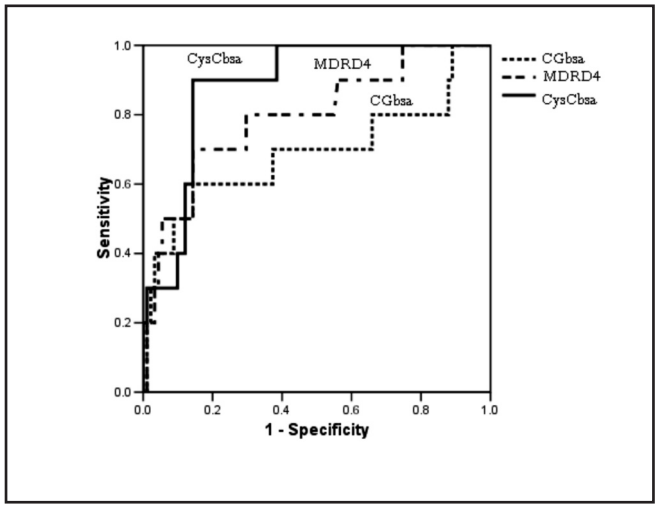

Fig. 2: ROC curve of the eGFR equations in measuring GFR < 90 ml/min/1.73m²

otherwise healthy and had no medical illnesses. As these equations were derived from mostly CKD patients, their use in subjects without CKD is questionable. Lin *et al*[27] reported that the MDRD equation consistently underestimates GFR, whereas the CG equation consistently overestimates GFR in subjects with normal renal function. Our earlier work has demonstrated that the CG equation was inaccurate in healthy overweight and obese subjects[14].

In this study, we found that the cystatin C-based eGFR equation had the highest correlation with ^{99}mTc-DTPA GFR compared to the creatinine-based eGFR equation in overweight and obese subjects. Furthermore, the cystatin C-based equation had better accuracy, sensitivity and specificity

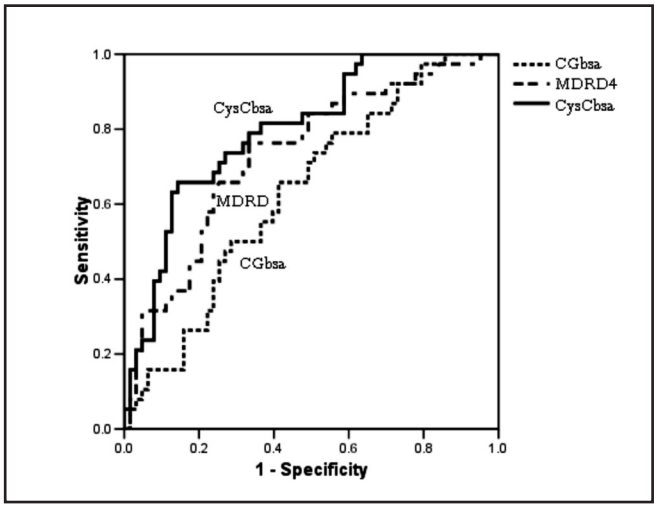

Fig. 3: ROC curve of the eGFR equations in measuring GFR >120 ml/min/1.73m^2.

in measuring abnormal GFRs defined as levels <90ml/min/1.73m^2 or >120ml/min/1.73m^2. Many recent studies have reported that cystatin C production increased during pre-adipocyte differentiation [28, 29]. Whereas other reports suggested an association of BMI and weight with serum cystatin C levels, hence raising the question whether cystatin C can be used for estimating GFR's in overweight and obese subjects [19-22]. Our finding was important as it supports the superiority of the use of cystatin C- based versus creatinine based equations to estimate GFR in overweight and obese subjects.

The better performance of the cystatin C-based equation may be attributed to the imperfect properties of serum creatinine as a marker of renal function. Serum creatinine concentration is affected by age, weight, muscle mass, race and various medications [30]. Furthermore serum creatinine levels are affected by inter-laboratory and inter-methodology variations. Contrary to previous beliefs, cystatin C levels are also affected by extra-renal factors such as age, gender, height, weight, tobacco and corticosteroids use and C-reactive protein levels albeit to a lesser extent [19-22, 31]. Thus, its use as a kidney marker under various clinical circumstances needs to be validated in larger prospective studies.

Creatinine-based equations are also inaccurate at the extremes of renal function and of BMIs. Our earlier study revealed that the CG equation overestimated GFR by 20 ml/min/1.73m^2 and lacked precision in overweight and obese individuals [14]. Saracino *et al* [32] suggested a correction factor for the CG equation in obese subjects whereas Fabbian *et al* [33] found that the CG equation was inaccurate when it was applied to obese or cachectic subjects and the MDRD equation underestimated renal function in normal-high GFR. Hence, these equations may overestimate the prevalence of CKD in overweight and obese populations.

We found that 9.8% of our otherwise healthy overweight and obese subjects had GFRs <90 ml/min/1.73m^2. The prevalence of CKD in overweight and obese subjects has been reported to vary between 2.5-13.1% depending on the population studied and guidelines used [14, 34]. There were 37.6% subjects with GFR >120 ml/min/1.73m^2 in our study. The presence of glomerular hyperfiltration in the overweight and obese subjects may represent glomerular hyperperfusion and hypertension which are the primary mechanisms hypothesized to cause structural changes in the kidney and subsequent kidney dysfunction [35-37]. Hence these subjects need to be counseled with regards weight loss and lifestyle modification as well as monitored at regular intervals to prevent deterioration of their kidney function.

Limitations of our study included over-representation by young Malay females (who work at the Hospital) and the Asia Pacific Guidelines were used to define overweight and obese. Hence, our findings may not be applicable to other populations.

In conclusion, we have shown that the cystatin C-based equation is more accurate, sensitive and specific compared to the creatinine-based equation for estimating GFR in overweight and obese subjects.

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