Comparison of cystatin C- and creatinine-based estimated glomerular filtration rate to predict coronary heart disease risk in Japanese patients with obesity and diabetes

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Abstract. The aim of this study is to determine which indicator of chronic kidney disease most closely correlates with 10-year Framingham coronary heart disease (CHD) risk among serum creatinine, serum cystatin C (S-CysC), urine albumin-creatinine ratio (UACR), estimated creatinine-based GFRs (eGFRcre), and estimated CysC-based GFRs (eGFRcys) in patients with obesity and diabetes. Serum creatinine, S-CysC, UACR, and cardio-ankle vascular index (CAVI) were examined in 468 outpatients with obesity and type 2 diabetes, free of severe renal dysfunction or previous history of cardiovascular disease, as a cross-sectional survey using baseline data from the multi-centered Japan Diabetes and Obesity Study. S-CysC and eGFRcys had significantly stronger correlations with the 10-year Framingham CHD risk than serum creatinine, eGFRcre, and UACR (creatinine, ρ = 0.318; S-CysC, ρ = 0.497; UACR, ρ = 0.174; eGFRcre, ρ = -0.302; eGFRcys, ρ = -0.521; P < 0.01 by Fisher’s z-test). S-CysC and eGFRcys had significantly stronger correlations with CAVI than serum creatinine, eGFRcre, and UACR (creatinine, ρ = 0.198; S-CysC, ρ = 0.383; UACR, ρ = 0.183; eGFRcre, ρ = -0.302; eGFRcys, ρ = -0.544; P < 0.05 by Fisher’s z-test). The receiver operating characteristic curves to distinguish the high-risk patients for CHD revealed significantly larger areas under the curve of S-CysC and eGFRcys than those of serum creatinine, UACR, and eGFRcre (serum creatinine, 0.64; S-CysC, 0.75; UACR, 0.56; eGFRcre, 0.63; eGFRcys, 0.76; P < 0.01). The data suggested that eGFRcys can be more predictive of the 10-year CHD risk than eGFRcre in Japanese patients with obesity and diabetes.

Key words: Cystatin C, eGFR, CKD, Framingham score, Cardio-renal association

CHRONIC KIDNEY DISEASE (CKD), which is defined as renal damage or glomerular filtration rate (GFR) < 60 mL/min/1.73 m² for at least 3 months, is known to be an independent risk factor for cardiovascular diseases (CVD) [1]. Several recent epidemiologic studies have revealed a close association of obesity and metabolic syndrome with CKD [2]. In patients with type 2 diabetes, microalbuminuria (urine albumin-creatinine ratio (UACR)) is an established biomarker that reflects glomerular damage and is closely
associated with the risk of all-cause and cardiovascular mortality and CVD events [3].

Cystatin C (CysC) has gained attention as a new biomarker to evaluate the progression of CKD and CVD. CysC, a 13-kd endogenous cysteine proteinase inhibitor, is ubiquitously expressed, filtered freely by the glomeruli, and catabolized in the proximal tubules [4]. Serum CysC (S-CysC) is a sensitive marker for detecting a reduced GFR and is also a stronger predictor of the risk of death and cardiovascular events in elderly American people than creatinine [4].

Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed GFR-estimating equations based on a standardized S-CysC [5]. The Japanese Society of Nephrology also developed new GFR-estimating equations based on a standardized S-CysC modified from the CKD-EPI equations for a Japanese population [6]. Japanese GFR equations based on serum creatinine (eGFRcre) and S-CysC (eGFRcys) are available and performed well in Japanese subjects with GFR under 60 mL/min/1.73 m² [7]. However, it is uncertain whether eGFRcys is superior in predicting CVD risk compared to eGFRcre and UACR in Japanese patients with obesity and diabetes. The patients with diabetes and obesity are at high-risk for CVD. Therefore, there is merit in inspecting the eGFRcre and eGFRcys of these patients.

In this study, we examined the relationships between each CKD marker (serum creatinine, S-CysC, UACR, eGFRcre, and eGFRcys) and 10-year Framingham coronary heart disease (CHD) risk [8], which has been widely adopted as a valid predictor of CHD in Western countries, and the application of which for predicting CHD in a Japanese population was validated by Suka et al. [9]. In addition, we examined the relationships between each CKD marker and cardio-ankle vascular index (CAVI), an index of arterial stiffness.

**Materials and Methods**

**Subjects**

The Japan Diabetes and Obesity Study (J-DOS) is a multi-center prospective cohort study to investigate markers for early detection of CKD and CVD in patients with obesity and diabetes in Japan. This study involved 15 hospitals associated with the National Hospital Organization in Japan as part of a study by the Policy Based Medical Service Network during the period from January 2011 to June 2012. Each institution’s ethical committee approved this study, and the study conformed to the Declaration of Helsinki guidelines. All patients gave their written informed consent before enrollment. The J-DOS has been registered in the University Hospital Medical Information Network system (UMINStudyID:000007358). We recruited patients with type 2 diabetes, based on the guidelines of the Japan Diabetes Society, and obese patients with a BMI of ≥ 25 kg/m², based on the guidelines of the Japan Society for the Study of Obesity. The candidates were those aged 20 to 79 years at enrollment. The exclusion criteria were: a previous history of CVD, other vascular diseases, severe renal disease (serum creatinine ≥ 3.0 mg/dL (265.2 μmol/L)), severe liver dysfunction, and secondary obesity due to endocrine disorders.

In the present study, we made a cross-sectional survey using baseline data from the J-DOS. Of the participants in J-DOS, we analyzed only patients whose 10-year CHD risk could be estimated based on Framingham CHD score sheets (i.e., patients aged from 30 to 74 years, in whom there was no missing value pertaining to risk factors on Framingham CHD score sheets) [8].

**Data collection and laboratory methods**

We measured the BMI, systolic and diastolic blood pressures, HbA1c, serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), creatinine, S-CysC, and CAVI as previously described [10]. The eGFRcre, eGFRcys, and UACR were calculated using the formulas in the guidelines of the Japanese Society of Nephrology [6]. An averaged value of eGFRcre and eGFRcys (eGFRave) was calculated as the average value of eGFRcre and eGFRcys [11].

**Assessment of 10-year Framingham CHD risk**

The individual’s CHD risk level was estimated based on the Framingham CHD score sheets [8]. Using this score, an individual’s estimated 10-year CHD risk can be calculated with some risk factors (gender, age, BP, TC, HDL-C, smoking status, and presence or absence of diabetes). The details of the algorithm for the determination of the risk score are described in the original report [8].

**Statistical analysis**

Data are presented as the mean ± standard deviation (SD) or median and interquartile range. Spearman’s rank correlation coefficients were employed to investigate the correlations between CKD indicators and the
Table 1 Baseline characteristics of participants (n=468)

<table>
<thead>
<tr>
<th>data</th>
<th>range</th>
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<tbody>
<tr>
<td>Men / women</td>
<td>234 / 234</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.3 ± 11.6</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
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<tr>
<td>HbA1c (mmol/mol)</td>
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<td>HbA1c (%)</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.2 ± 1.0</td>
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<tr>
<td>HDL-C (mmol/L)</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>65.3 ± 19.4</td>
</tr>
<tr>
<td>S-CysC (mg/L)</td>
<td>0.84 ± 0.22</td>
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<tr>
<td>UACR (mg/gCr)</td>
<td>13.9 [5.9 - 40.7]</td>
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<tr>
<td>eGFRcre (mL/min/1.73 m²)</td>
<td>79.8 ± 20.2</td>
</tr>
<tr>
<td>eGFRcys (mL/min/1.73 m²)</td>
<td>94.6 ± 26.8</td>
</tr>
<tr>
<td>eGFRave (mL/min/1.73 m²)</td>
<td>87.1 ± 21.2</td>
</tr>
<tr>
<td>CAVI</td>
<td>7.8 ± 1.4</td>
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<td>Framingham risk score</td>
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<tr>
<td>10-year Framingham CHD risk (%)</td>
<td>10 [5 - 16]</td>
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<tr>
<td>Obesity (n)</td>
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<tr>
<td>Diabetes (n)</td>
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<td>Taking lipid-lowering agents (n)</td>
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<tr>
<td>Statin (n)</td>
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</tr>
<tr>
<td>Fibrate (n)</td>
<td>22</td>
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</table>

Data are expressed as the mean ± SD or median [interquartile range].

BMI, body mass index; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; S-CysC, serum cystatin C; UACR, urine albumin-to-creatinine ratio; eGFRcre, estimated glomerular filtration rate based on serum creatinine; eGFRcys, estimated glomerular filtration rate based on serum cystatin C; eGFRave, average value of eGFRcre and eGFRcys; CAVI, cardio-ankle vascular index; CHD, coronary heart disease; RAS, renin-angiotensin system

10-year CHD risk or CAVI. Fisher’s z-test was used for comparing two correlation coefficients. Analyses of the receiver operating characteristics (ROC) curves were also performed to examine the sensitivity and specificity of values of each CKD indicator for detecting high-risk subjects for CHD. The threshold value of high-risk of CHD was set as the median 10-year CHD risk in this study’s subjects. The areas under the curve (AUCs) were compared by the DeLong test. The optimal cut-off points were obtained from the Youden index. $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Baseline clinical characteristics of participants**

A total of 468 Japanese outpatients (234 men and 234 women, age: 55.3 ± 11.6 years) were included in this cross-sectional survey. A summary of the clinical characteristics of participants in this study is shown in Table 1. There were 368 (78.6%) with obesity, 275 (58.8%) with type 2 diabetes, and 91 (19.4%) currently smoking; 227 were taking antidiabetic agents (82.5% of patients with diabetes), and 70 were taking insulin (25.5% of patients with diabetes); and 71 (15.2%) were exhibiting stage 3 or higher CKD.
Correlations between CKD indicators and the 10-year Framingham CHD risk as well as CA VI

Spearman’s correlation revealed that the 10-year CHD risk was significantly correlated with all CKD indicators (creatinine, $\rho = 0.318$ [95% confidence interval 0.234, 0.397]; S-CysC, $\rho = 0.497$ [0.426, 0.562]; UACR, $\rho = 0.174$ [0.085, 0.261]; eGFRcre, $\rho = -0.291$ [-0.372, -0.206]; eGFRcys, $\rho = -0.521$ [-0.584, -0.452]; $P < 0.01$, Table 2). S-CysC showed a significantly stronger correlation with the 10-year CHD risk than creatinine and UACR ($P < 0.01$ by Fisher’s z-test for comparing two correlation coefficients between i) S-CysC and creatinine, and ii) S-CysC and UACR). The eGFRcys exhibited a significantly stronger correlation with the 10-year CHD risk than eGFRcre ($P < 0.01$ by Fisher’s z-test). The correlation coefficient of eGFRave with the 10-year CHD risk was -0.469 [-0.537, -0.395]; the trends of correlations for the 10-year CHD risk were eGFRcys > eGFRave > eGFRcre (Table 2).

CAVI was also significantly correlated with all CKD indicators: serum creatinine, S-CysC, UACR, eGFRcre, eGFRcys and eGFRave (creatinine, $\rho = 0.198$ [0.109, 0.284]; S-CysC, $\rho = 0.383$ [0.303, 0.458]; UACR, $\rho = 0.183$ [0.094, 0.269]; eGFRcre, $\rho = -0.302$ [-0.382, -0.217]; eGFRcys, $\rho = -0.444$ [-0.514, -0.368]; eGFRave, $\rho = -0.439$ [-0.509, -0.363]; $P < 0.01$, Table 2). In addition, S-CysC and eGFRcys had significantly stronger correlations with CAVI than creatinine, eGFRcre, and UACR ($P < 0.05$ by Fisher’s z-test).

ROC curves of CKD indicators for the discrimination of high-risk subjects for CHD

In this study, the median 10-year CHD risk was 10%, therefore the subjects who showed a 10% or higher 10-year CHD risk were defined as high-risk subjects. The ROC curves of CKD indicators to discriminate the high-risk subjects are presented in Fig. 1A and 1B. The AUC of S-CysC was significantly larger than those of serum creatinine and UACR ($P < 0.01$ by the DeLong test, Fig. 1A). The AUC of eGFRcre was significantly larger than that of eGFRcys ($P < 0.01$ by the DeLong test, Fig. 1B). The cut-off values for serum creatinine, S-CysC, UACR, eGFRcre, and eGFRcys were 64.5 $\mu$mol/L, 0.77 mg/L, 17.0 mg/gCr, 70.0 mL/min/1.73 $m^{2}$, and 70.0 mL/min/1.73 $m^{2}$, respectively. There was a similar tendency in men and women concerning the ROC analyses of CKD indicators.

When we also calculated the eGFRave, the ROC curves of CKD indicators to discriminate the high-risk subjects are presented in Fig. 1A and 1B. The AUC of S-CysC was significantly larger than those of serum creatinine and UACR ($P < 0.01$ by the DeLong test, Fig. 1A). The AUC of eGFRcys was significantly larger than that of eGFRcre ($P < 0.01$ by the DeLong test, Fig. 1B). The cut-off values for serum creatinine, S-CysC, UACR, eGFRcre, and eGFRcys were 64.5 $\mu$mol/L, 0.77 mg/L, 17.0 mg/gCr, 70.0 mL/min/1.73 $m^{2}$, and 94.9 mL/min/1.73 $m^{2}$, respectively. There was a similar tendency in men and women concerning the ROC analyses of CKD indicators.

Discussion

It is an emerging topic to examine the clinical implication of eGFRcys and eGFRcre on assessment of CVD risk [5-7]. Here, we demonstrated for the first time that eGFRcys was correlated with the 10-year Framingham CHD risk and CA VI more strongly than eGFRcre in Japanese patients with obesity and type 2 diabetes.

S-CysC is not only an excellent predictor of the early stages of patients with type 2 diabetic nephropathy [12], but also a stronger predictor of the risk of death and CVD in elderly persons than creatinine [4]. A cohort study in Europe reported that not eGFRcre but eGFRcys...
eGFRcys and eGFRcre in obesity/diabetes

independently improved cardiovascular risk prediction of the Framingham Cardiovascular Risk Score in diabetic subjects with CKD [13]. This result appears to be compatible with that of our present study in Japanese patients with obesity and diabetes. However, our study is unique in that it demonstrated the superiority of eGFRcys over eGFRcre for predicting CVD in patients without severe renal dysfunction.

In regards to CAVI, we previously reported that S-CysC was significantly correlated with CAVI in obese patients in a multi-centered study [10]. Previous studies reported the correlation between cystatin C and CAVI in patients with cardiovascular risk factors [14], or in women in a non-CKD population (eGFR > 60 mL/min/1.73 m^2) [15]. These findings are compatible with that of our present study in Japanese patients with obesity and diabetes, who have mild renal dysfunction and have no history of CVD. However, this is the first report to demonstrate conclusively that eGFRcys had significantly stronger correlations with CAVI than eGFRcre.

Previous studies reported a significant difference in the distributions between eGFRcys and eGFRcre, especially at a higher eGFR range [7, 16]. This is similar with a significant difference between eGFRcys and eGFRcre observed in our study patients (without severe renal dysfunction). Additionally, there was a report revealing that eGFRcys and cystatin C-based eGFR with creatinine (eGFRcys-CysC) had a stronger association with CKD risk factors than did the gold-standard measured GFR (mGFR) and eGFRcre [17]. Furthermore, another study reported that, in subjects with diabetes, the prevalence of reduced kidney function by eGFRcys was higher than that using eGFRcre, and a lower eGFRcys was strongly associated with diabetic complications [18]. These studies are consistent with our results showing that S-CysC and eGFRcys were more strongly correlated with the 10-year CHD risk and CAVI than serum creatinine and eGFRcre. There are two possible explanations for our results. Firstly, it is due to the more accurate reflection of eGFRcys on the kidney function than eGFRcre [19, 20], since cystatin C is less influenced by the age factor and muscle mass than creatinine [21]. Secondly, cystatin C may include some non-GFR mechanisms that affect CHD risk factors. Indeed, it has been reported that cathepsin, a pro-atherogenic factor that promotes plaque rupture, is inhibited by cystatin C, a cathepsin inhibitor [22]. Our results are in line with these previous articles and support the notion that cystatin C is a better predictor of atherosclerosis risk.

Fig. 1A Receiver operating characteristic (ROC) curves of serum creatinine, S-CysC, and UACR for the discrimination of high-risk subjects for CHD.

Fig. 1B ROC curves of eGFRcre, eGFRcys, and eGFRave for the discrimination of high-risk subjects for CHD. The subjects who showed a 10% or higher 10-year Framingham CHD risk were defined as high-risk subjects for CHD. The 95% confidence intervals of the area under the curves are shown in parentheses. S-CysC, serum cystatin C; UACR, urine albumin-to-creatinine ratio; eGFRcys, estimated glomerular filtration rate based on serum creatinine; eGFRcre, estimated glomerular filtration rate based on serum cystatin C; eGFRave, average value of eGFRcre and eGFRcys; CHD, coronary heart disease; AUC, area under curve; CI, confidence interval.
than creatinine.

There was a recent report showing that the eGFRave could effectively estimate kidney function in Japanese renal transplant recipients with normal-to-mild reduction in kidney function [11]. Therefore, we also examined the association between eGFRave and the 10-year CHD risk. As a result, the overall trend of correlations for the 10-year CHD risk was eGFRcys > eGFRave > eGFRcre. Accordingly, eGFRcys may be more closely associated with the 10-year CHD risk compared to eGFRave and eGFRcre.

There are some limitations to our study. (1) We did not examine the measured GFR using the urinary clearance of inulin. We should be wary of the precision of cut-off values in ROC analyses of CKD indicators for the discrimination of high-risk subjects for CHD, because eGFRs sometimes elevate rather than diminish due to glomerular hyperfiltration in the early stage of diabetes. (2) Although the Framingham score has been widely adopted as a valid predictor of CHD in Western countries, its applicability in regards to each ethnicity is still being investigated. Since the mortality and morbidity of CHD differ by country and ethnicity, we must be guarded in our interpretation of the results. It is possible that we have overestimated the prediction of 10-year CHD risk via the Framingham score in the Japanese population, especially in subjects who have normal kidney function and normoalbuminuria. (3) The study was performed with a cross-sectional design. In the future, we will examine the precise CHD risk of this cohort in a prospective study. The 10-year Framingham CHD risk is an easy scoring system for evaluating a subject’s risk, and the cardio-renal association is important for the onset of CVD in patients with obesity and diabetes. Therefore, if we can determine the precise CHD risks with eGFRcys (a relatively simple measure), it would be useful in primary screening in general practice and public health situations.

Collectively, these observations suggest that eGFRcys may serve as a useful CKD indicator to predict the atherogenic CHD risk in patients with obesity and type 2 diabetes.

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Disclosure

None.

References


