Original Article

dmj

DIABETES & METABOLISM JOURNAL

Serum Cystatin C Reflects the Progress of Albuminuria

Jeong Seon Yoo[1], Young Mi Lee[1], Eun Hae Lee[1], Ji Woon Kim[1], Shin Young Lee[1], Ki-Cheon Jeong[2], Shin Ae Kang[1], Jong Suk Park[1,3], Joo Young Nam[2], Chul Woo Ahn[1,3], Young Duk Song[2], Kyung Rae Kim[1]

[1]Department of Internal Medicine, Yonsei University College of Medicine, Seoul,
[2]Department of Internal Medicine, National Health Insurance Corporation Ilsan Hospital, Goyang,
[3]Severance Institute for Vascular and Metabolic Research, Yonsei University, Seoul, Korea

Background: Research on the relationship between urinary albumin excretion and serum cystatin C in diabetes is restricted to cross-sectional studies. In this study, we investigated how well serial measurements of serum cystatin C level reflect changes in the urinary albumin excretion rate.

Methods: We enrolled and retrospectively collected data on 1,058 participants with type 2 diabetes who were older than 18 years and who had more than 3 years of follow-up with serial measurements of albuminuria and serum cystatin C at an outpatient clinic.

Results: With the use of a linear mixed model, we found that the albuminuria level for each patient over time corresponded with the annual change in serum cystatin C-based estimated glomerular filtration rate (cysC-eGFR) but did not correspond with the creatinine-based eGFR calculated by the modification of diet in renal disease formula (MDRD-eGFR). The discrepancy in the direction of the trend was smaller with cysC-eGFR than with MDRD-eGFR.

Conclusion: Serum cystatin C level reflects the trend in albuminuria level more accurately than serum creatinine level in Korean type 2 diabetes mellitus patients.

Keywords: Albuminuria; Cystatin C; Creatinine; Diabetes mellitus, type 2; Diabetic nephropathies

INTRODUCTION

Most cases of end stage renal disease (ESRD) are caused by diabetes, and the number of cases has increased numerically and proportionally during the last few decades. Diabetic nephropathy, which causes the adverse outcomes of kidney failure, cardiovascular disease and premature death, is now the single most common cause of ESRD in both Western and Eastern countries [1]. However, our ability to assess early renal impairment in diabetic nephropathy, when active management is important, is still poor in comparison to biomarker-based approaches for other diseases, such as serum troponin concentration in acute myocardial infarct [2].

Serum creatinine level is the most commonly used surrogate measurement of glomerular filtration rate (GFR) in a clinical setting. In 2002, the Kidney Disease Outcomes Quality Initiative (K/DOQI) organization published guidelines for chronic kidney disease [3]. This classification, based on the modification of diet in renal disease (MDRD) study estimation equation for GFR using serum creatinine concentration, was immediately widely embraced [4]. However, serum creatinine concentration does not increase until renal function decreases to less than 50% of the normal value [5]. The variances according to age, sex, physical exercise, and lean body mass are also important limitations [6]. Gold standard tests using radioisotopes such as ^{51}Cr-labeled EDTA, ^{99m}Tc-labeled DTPA, and

[125]I-labeled iothalamate or iohexol [7,8] are most reliable for measurement of GFR but are too difficult to use in the clinic setting [9]. Therefore, an accurate and available marker of GFR for patients with diabetic nephropathy is needed.

In the past three decades, urinary albumin excretion has assumed a central role in the diagnosis and management of kidney disease among people with diabetes mellitus [2]. This led investigators during the early 1980s to search for early predictors of diabetic nephropathy through the measurement of low concentrations of albumin in the urine, especially in type 1 diabetic patients [10,11]. Despite a great deal of controversy [4,12], microalbuminuria is now widely accepted as a predictive marker of overt proteinuria [13] and increased cardiovascular disease and mortality [14] not only in type 1 diabetes mellitus [15], but also in type 2 diabetic mellitus [13]. The importance of albuminuria has increased in practice as well as in laboratory research.

However, urinary albumin excretion can be affected by several factors including plasma concentrations of atrial natriuretic peptide, arginine vasopressin, angiotensin II, aldosterone and fasting blood glucose, glycated hemoglobin, and mean arterial blood pressure [16] and albumin can be degraded in a manner consistent with the activity of endogenous urinary proteases [17]. Furthermore, the intraindividual variation is as high as 47%. Because of these problems, all results that are initially positive for albuminuria need to be confirmed with a second sample collected on a different day, and in cases of discrepancies between the first and second sample, a third sample is necessary. Despite the convenience of urinary albumin/creatinine ratio (uACR) measurement in random urine samples compared to 24-hour urine collection, it incommodes clinicians who provide primary care to diabetic patients and causes a delay in the recognition and initial treatment for diabetic nephropathy. It is a fundamental problem because its main purpose is the detection of diabetic nephropathy at an early stage. Therefore, while continuing to encourage the use of the K/DOQI classification, which provides a well-established clinical guideline, the diabetology community should eagerly seek other methods to measure or better estimate early kidney damage of diabetic nephropathy, while maintaining the virtue of uACR. Further, changes in urinary albumin excretion are dynamic, whereas changes in GFR are usually progressive. Although increases in urinary albumin excretion generally precede a decline in GFR, some patients follow a non-albuminuric pathway to renal impairment. Therefore, in the assessment of dia-

betic nephropathy, the roles of albuminuria and GFR are complementary rather than competitive [18].

Recently, cystatin C was introduced as a good marker of early renal function decline [19,20], particularly in diabetic [21] or elderly [22] patients. Cystatin C is being considered as a potential replacement for serum creatinine because it appears to be less affected by muscle mass [23], and most studies have shown that it is more highly correlated with GFR than is serum creatinine [20,24]. Furthermore, there is no interference from metabolites, like bilirubin, ketones, and various drugs like antidiabetic medications [25]. Cystatin C is a non-glycosylated protein belonging to the super family of cysteine protease inhibitors, and it is produced by all nucleated cells at a constant rate [26]. Serum cystatin C fulfills a number of the criteria that would make it a suitable marker of GFR. It can be freely filtered at the glomerular membrane because of a low molecular weight (Mr=13359) [9] and reabsorbed and catabolized by renal tubular cells [27]. Furthermore, its concentration is stable in stored plasma, unlike urinary albumin, probably due to the high concentration of other proteinase inhibitors [28]. Perkins et al. [29] reported longitudinal data demonstrating that the change in 100/cystatin C was more accurately correlated with the change in iothalamate-GFR than with the change in estimated GFR (eGFR) as calculated from serum creatinine.

Some studies have investigated the correlation between albuminuria and serum cystatin C level, but they evaluated serum cystatin C and albuminuria in cross-sectional datasets [30,31]. Therefore, we analyzed the longitudinal correlation between cystatin C and albuminuria in Korean patients with type 2 diabetes mellitus.

METHODS

Subjects

We collected and retrospectively reviewed the medical records of 1,058 subjects with type 2 diabetes mellitus who visited the out-patient department of the Endocrinology and Metabolism Division at Yonsei University, Gangnam Severance Hospital, Seoul, South Korea from March 2006 to March 2010 (Fig. 1). Inclusion criteria were as follows: 1) previously known type 2 diabetic mellitus; 2) more than 3 years of follow-up for serum creatinine, cystatin C, and uACR; and 3) age between 18 and 85 years. Subjects were excluded if they had 1) significant hepatic disease (alanine aminotransferase or aspartate aminotransferase >2.5-fold the normal value); 2) thyroid disease or
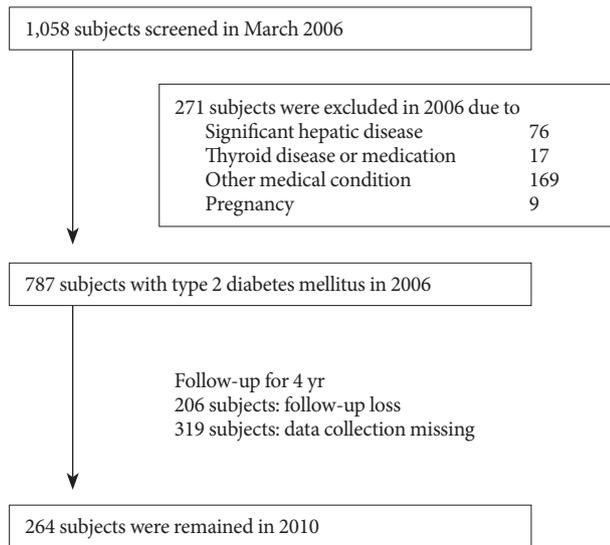
Fig. 1. Selection of study participants.

medication due to thyroid disease during the previous six months; 3) any other medical condition that could alter the level of serum cystatin C or uACR; or 4) if they were pregnant. The subjects were classified into three groups, according to uACR: 1) normoalbuminuria group (uACR <30 μg/mg); 2) microalbuminuria group (30 μg/mg≤uACR<300 μg/mg); and 3) overt albuminuria group (uACR≥300 μg/mg Cr). Obesity was defined according to 2000 WHO criteria, the Asia-Pacific perspective, as follows; 1) normal weight (body mass index [BMI] <23 kg/m^2); 2) overweight (23 kg/m^2≤BMI<25 kg/m^2); 3) obese (BMI≥25 kg/m^2). This study was approved by our human research ethics committee.

Study design

BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). Hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg and/or reported treatment with antihypertensive medications in the previous 2 weeks. SBP and DBP values were classified by the Joint National Committee (JNC) 7 report in 2003.

Blood was sampled from all of the subjects after fasting for more than 10 hours, and random urine was collected. Serum cystatin C was measured by latex particle-enhanced turbidimetric immunoassays (Cobas 6000 analyzer; Roche Diagnostics, West Sussex, UK). Serum and urine creatinine levels were measured by automatic picric colorimetry (Hitachi 7600-110 automatic analyzer; Hitachi Co., Tokyo, Japan). Hemoglobin

A1c (HbA1c) was determined by means of high-performance liquid chromatography (Variant II; Bio-Rad, Hercules, CA, USA). Total cholesterol (TC), triacylglyceride (TG), and high density lipoprotein cholesterol (HDL-C) levels were measured by an enzymatic method using an enzymatic color test (747 automatic analyzer; Hitachi Co., Tokyo, Japan), and low density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula.

The estimated GFR level was calculated using the MDRD formula (MDRD-eGFR) [32]:

$$MDRD\text{-}eGFR = 186 \times (\text{serum creatinine [mg/dL]})^{-1.154} \times age^{-0.203} \times (0.742 \text{ if female})$$

The cystatin C-based estimated GFR (CysC-eGFR) equation [33]:

$$CysC\text{-}eGFR = 78 / (\text{serum cystatin C in mg/L}) + 4$$

To compare the change in variables over three years, we calculated the initial and last value of cysC-eGFR and MDRD-eGFR using two different approaches, as follows:
1) Simple subtraction
   A. ΔcysC-eGFR=last cysC-eGFR–initial cysC-eGFR
   B. ΔMDRD-eGFR=last MDRD-eGFR–initial MDRD-eGFR
2) Declining slope
   A. cysC-eGFR change rate=ΔcysC-eGFR/initial cys-eGFR
   B. MDRD-eGFR change rate=ΔMDRD-eGFR/initial MDRD-eGFR

Statistical analysis

All statistical analyses were performed using SPSS version 18.0 (IBM, Chicago, IL, USA), except for the generalized linear mixed model for longitudinal analysis, which was performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA). Serum cystatin C and creatinine concentration were transformed to the reciprocal by multiplying by 100 for analysis. This transformation was performed to assure that changes over time had the same direction as the changes in estimated GFR calculated using the MDRD formula. Descriptive data are expressed as mean±standard deviation, but in the generalized linear mixed model, we used standard error instead of standard deviation. P values less than 0.05 were considered statistically significant.

RESULTS

Clinical and biochemical characteristics of subjects

The clinical characteristics of the study subjects are shown in Table 1. The group of 264 subjects was male-dominant, with an average age of 61.8 ± 10.6 years in the baseline period. The subjects were followed for three years. The proportion of elderly subjects, defined as older than 65 years, constituted almost half of the study group. The mean BMI of these subjects, 24.2 ± 3.0 kg/m^2, was in the overweight range, although fewer than 10% of the subjects were obese. Two-thirds of the subjects were prescribed angiotensin-converting enzyme inhibitors and/or angiotension receptor blocker as antihypertensive drugs, and their blood pressures were under control. The mean fasting glucose level of the subjects, 131.1 ± 65.7 mg/dL, was relatively high, but only one-sixth of the patients had an HbA1c greater than 9%, indicating that, overall, the blood sugar levels were under control in this study group.

The mean uACR of subjects was in the microalbuminuria range, and all stages of albuminuria were included. Patients in the nephritic range of proteinuria greater than 2 g/day were excluded because the possibility of other kidney disease could not be eliminated. The portion of patients with renal failure, defined as an eGFR lower than 60 mL/min/1.73 m^2, was almost half of the study group. However, if eGFR was calculated using a cystatin C-based equation rather than the MDRD formula, the portion decreased to 34.3%. Patients with end stage renal failure were not included because their status of albuminuria could not be interpreted properly since their urinary albumin was not only glomerular in origin, but also tubular, and some of them had an anuric status.

Longitudinal trends in uACR and renal function marker

As a result of longitudinal analysis using a general linear mixed model, only 100/cystatin C significantly affected uACR ($P=$ 0.0407), while 100/serum creatinine and MDRD-eGFR did not. Serum cystatin C and creatinine values were transformed to the reciprocal multiplied by 100 to assure that changes over time had the same direction as changes in MDRD-eGFR. CysC-eGFR was not involved in this analysis because, unlike MDRD-eGFR, its equation has no other variables except serum cystatin C. uACR decreased to 4.8321 mg/g when 100/cystatin C increased to 1 L/mg (Table 2). Even after adjustment for age, sex, serum uric acid, and HbA1c, these results did not change ($P=0.0106$); however, the other two renal

Table 1. Baseline clinical and biochemical characteristics of the 264 subjects

Characteristic		Initial, %	After 3 yr, %
Age, yr		61.8 ± 10.6	
	Older than 65 yr, %	44.3	
Gender, male		72.2	
Duration of diabetes		12.4 ± 10.1	
	≤10 yr	45.3	
	>10 yr	54.7	
BMI, kg/m^2		24.2 ± 3.0	
	Normal weight (BMI<23 kg/m^2)	44.7	
	Overweight (23 kg/m$^2\leq$BMI<25 kg/m^2)	48.0	
	Obesity (BMI≥25 kg/m^2)	8.3	
SBP, mm Hg		125.4 ± 16.8	
DBP, mm Hg		75.0 ± 12.5	
ACEi and/or ARB user, %		69.1	
Serum uric acid, mg/dL		5.7 ± 1.5	
Total cholesterol, mg/dL		169.1 ± 37.4	
Triacylglyceride, mg/dL		161.3 ± 127.7	
HDL-C, mg/dL		45.0 ± 12.8	
LDL-C, mg/dL		97.5 ± 32.8	
Fasting glucose, mg/dL		131.1 ± 65.7	
HbA1c, %		7.7 ± 1.5	
	HbA1c$<7.0\%$	56.1	
	$7.0\%\leq$HbA1c$<9\%$	27.4	
	HbA1c$\geq9\%$	16.5	
uACR, mg/gCr		251.4 ± 421.1	277.4 ± 441.0
	Normoalbuminuria, %	37.6	39.2
	Microalbuminuria, %	38.9	36.1
	Macroalbuminuria, %	23.6	24.7
Serum creatinine, mg/dL		1.53 ± 1.4	1.49 ± 1.3
MDRD-eGFR, mL/min/1.73 m^2		61.5 ± 24.8	60.5 ± 24.0
	eGFR≥60 mL/min/1.73 m^2	51.3	55.4
	eGFR<60 mL/min/1.73 m^2	48.7	44.6
Serum cystatin C, mg/dL		1.32 ± 0.72	1.26 ± 0.61
CysC-eGFR		74.6 ± 28.3	1.26 ± 0.61
	eGFR≥60 mL/min/1.73 m^2	65.6	71.5
	eGFR<60 mL/min/1.73 m^2	34.3	28.5

Values are presented as mean$\pm$standard deviation or percentage.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEi, angiotension-converting enzyme inhibitors; ARB, angiotension receptor blocker; uACR, urine albumin/creatinine ratio; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MDRD-eGFR, estimated glomerular filtration rate calculated using the modification of diet in renal disease formula; CysC-eGFR, estimated glomerular filtration rate calculated using serum cystatin C.

function markers did not show any effect at all (Table 3). This result was consistent, regardless of the stage of chronic kidney disease stage.

Additionally, age and serum uric acid concentration showed a significant correlation with uACR in serial measurement (*P* value=0.0023 and 0.027, respectively), although gender and HbA1c did not (*P*=0.1137, 0.4063, and 0.7372, respectively).

Accordance between uACR and cysC-eGFR or MDRD-eGFR

CysC-eGFR showed better direction accordance (39.4%) with uACR in comparison to MDRD-eGFR (23.8%) (Fig. 2) when analyzing declining slope. When we used the simple subtraction value (Fig. 3), the accordance with uACR increased in both cases (52.3% and 36.8%, respectively), although that of cysC-eGFR was superior. When we categorized the linear variables according to the K/DOQI classification, the accordance rate with uACR was 46.5% in the case of cys-eGFR and

Table 2. The effect of each variable on urinary albumin/creatinine ratio change analyzed by generalized linear mixed model

Parameter	Estimate	SE	*P* value
Time, day	0.2358	0.1400	0.0957
100/serum cystatin C	-4.8321	2.3309	0.0407
100/serum creatinine	-11.1506	5.8248	0.0577
MDRD-eGFR	10.7033	7.0592	0.1318

Unadjusted for confounding variables.
SE, standard errors; MDRD-eGFR, estimated glomerular filtration rate calculated by the modification of diet in renal disease formula.

36.1% in the case of MDRD-eGFR. A close look at the discordance portion revealed that cysC-eGFR was worse than MDRD-eGFR at detecting aggravation of albuminuria, and that MDRD-eGFR was worse than cysC-eGFRat detecting improvement in albuminuria or preservation status.

DISCUSSION

In our 3-year follow-up of a hospital cohort of type 2 diabetic patients, neither serum creatinine nor MDRD-eGFR corresponded with the trend in uACR change (Tables 1 and 2). This result indicates that the underlying pathophysiology of albu-

Table 3. The effect of each variable on urinary albumin/creatinine ratio change analyzed by generalized linear mixed model

Parameter	Estimate	SE	*P* value
Time, day	0.2437	0.1522	0.1137
Age, yr	-31.4470	9.4526	0.0023
Gender, male	-333.55	395.97	0.4063
Serum uric acid	-75.8817	23.6904	0.0273
HbA1c	-9.6286	28.4305	0.7372
100/serum cystatin C	-7.3863	2.8093	0.0106
100/serum creatinine	-14.1730	16.1013	0.3857
MDRD-eGFR	6.5659	18.3472	0.7229
CKD stage	-151.51	149.94	0.3204

Adjusted for age, sex, serum uric acid, HbA1c.
SE, standard errors; MDRD-eGFR, estimated glomerular filtration rate calculated by the modification of diet in renal disease formula; CKD, chronic kidney disease.
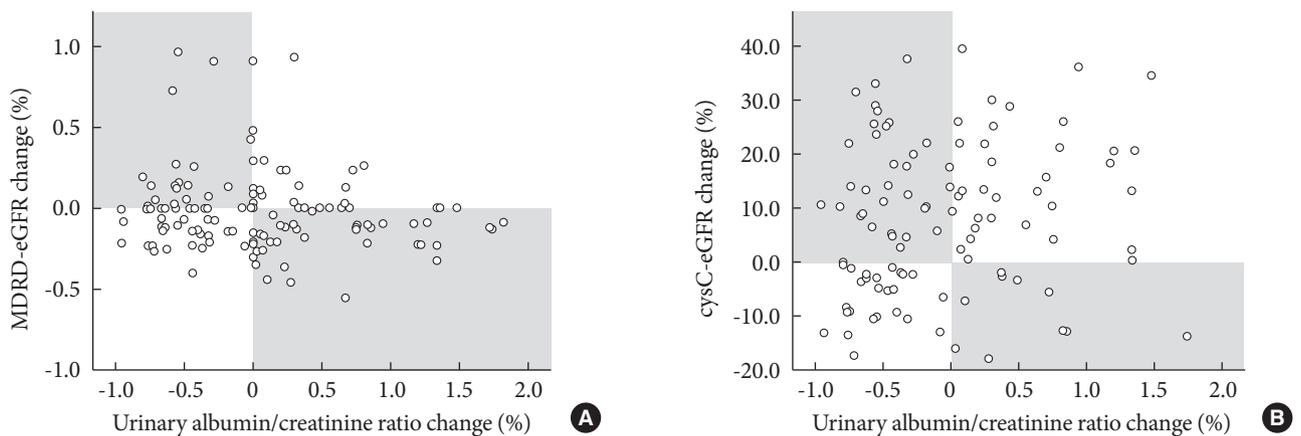

Fig. 2. Accordance of change rate between urinary albumin/creatinine ratio and estimated glumerular filtration rate (eGFR) calculated by (A) modification of diet in renal disease (MDRD) formula or (B) serum cystatin C. Trend accordance (percent of subjects in white box) is 23.8% in (A) and 39.4% in (B).
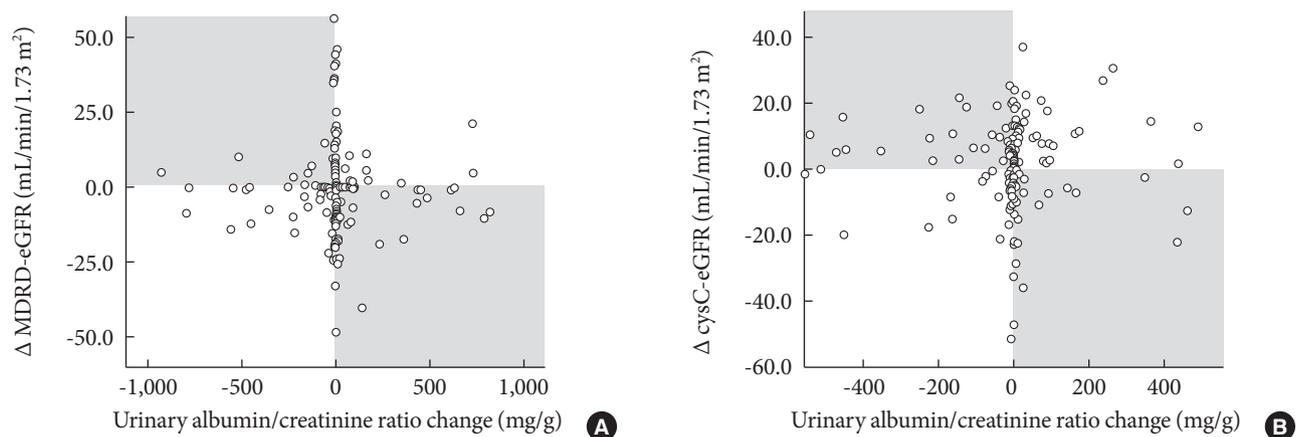
Fig. 3. Accordance of change between urinary albumin/creatinine ratio and estimated glomerular filtration rate (eGFR) calculated by (A) modification of diet in renal disease (MDRD) formula or (B) serum cystatin C. Trend accordance (percent of subjects in white box) is 36.8% in (A) and 52.3% in (B).

minuria and GFR loss are different in the early stages of diabetic kidney disease [27], and overt albuminuria does not always precede a significant loss of GFR in the setting of diabetes. Therefore, measuring albuminuria alone does not fully capture the scope of early diabetic kidney disease [10,12]. Instead, albuminuria and GFR loss may represent complementary, if overlapping, manifestations of kidney damage [2].

Most recent studies that have assessed serum cystatin C as a measure of GFR have been cross-sectional in nature, but longitudinal studies are needed to further define the potential role of cystatin C in clinical care [29]. Serum cystatin C has great advantages compared to inconvenient uACR measurements, as mentioned above. Furthermore, there is evidence that cysC-eGFR is more valuable than MDRD-eGFR in the prediction of microalbuminuric stage in a cross-sectional clinical study of Korean type 2 diabetic patients [31]. To our knowledge, this is the first study to suggest a correlation between serum cystatin C and uACR in longitudinal studies for Korean type 2 diabetic patients. In contrast to serum creatinine and MDRD-eGFR, 100/serum cystatin C showed a statistically significant correlation with the trend of albuminuria (estimate -4.8321 ± 2.2339, $P=0.0407$) (Table 2). Rather, the effect of 100/serum cystatin C on albuminuria increased after adjustments for age, sex, serum uric acid concentration and glycated hemoglobin (estimate -7.6667 ± 2.8110, $P=0.0106$). However, the effects of 100/serum creatinine ($P=0.3857$) and MDRD-eGRF ($P=0.7229$) on uACR remained insignificant (Table 3).

In our study, we demonstrated the superiority of cysC-eGFR in reflecting the trend of albuminuria. But, the discordance

Table 4. Accordance of stage change between uACR and cysC-eGFR or MDRD-eGFR using simple subtraction

	Accordance, %	Discordance, %	
		Improved or preserved albuminuria stage	Aggravated albuminuria stage
cysC-eGFR	46.5	20.6	32.9
MDRD-eGFR	36.1	44.5	19.4

uACR, urine albumin/creatinine ratio; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; cysC-eGFR, eGFR calculated using serum cystatin C; MDRD-eGFR, eGFR calculated using MDRD formula.

portion was nearly half of all subjects (Table 4), so serum cystatin C alone might have limited value as a replacement for uACR measurement.

There are some controversies about the usefulness of serum cystatin C. Many studies have shown that serum cystatin C is a better predictor of cardiovascular disease, renal function [31] and mortality than is the serum creatinine-based eGFR formula [34], although some researchers disagree with this conclusion [21]. The discordance comes from the difference in subject characteristics, mostly with regard to the mean age of the population or proportion of renal failure patients. Our study population was evenly distributed in terms of age and chronic kidney disease stage, thus reinforcing the reliability of our data. It is critical that the ideal marker of diabetic nephropathy not be limited to a certain stage of kidney disease or specific age group because diabetic nephropathy progresses throughout the entire life of diabetic patients and has a broad spectrum

from incipient nephropathy to end stage renal disease, which requires dialysis.

The limitations of our study are its retrospective design, the relatively small number of subjects and the fact that the data were collected from a single institute. Additional well-designed, prospective, and multicenter studies with a large population are required in the future.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. United States Renal Data System. USRDS 2010 annual data report: atlas of chronic kidney disease and end-stage renal disease in the United States. Bethesda: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2010.

2. de Boer IH, Steffes MW. Glomerular filtration rate and albuminuria: twin manifestations of nephropathy in diabetes. J Am Soc Nephrol 2007;18:1036-7.

3. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39(2 Suppl 1):S1-266.

4. Gansevoort RT, de Jong PE. Challenges for the present CKD classification system. Curr Opin Nephrol Hypertens 2010;19: 308-14.

5. Viberti G, Walker JD. Natural history and pathogenesis of diabetic nephropathy. J Diabet Complications 1991;5:72-5.

6. 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.

7. Brown SC, O'Reilly PH. Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard. J Urol 1991;146:675-9.

8. Krutzen E, Back SE, Nilsson-Ehle I, Nilsson-Ehle P. Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. J Lab Clin Med 1984; 104:955-61.

9. Swan SK. The search continues: an ideal marker of GFR. Clin Chem 1997;43(6 Pt 1):913-4.

10. Perkins BA, Ficociello LH, Ostrander BE, Silva KH, Weinberg J, Warram JH, Krolewski AS. Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. J Am Soc Nephrol 2007;18:1353-61.

11. Ficociello LH, Perkins BA, Roshan B, Weinberg JM, Aschengrau A, Warram JH, Krolewski AS. Renal hyperfiltration and the development of microalbuminuria in type 1 diabetes. Diabetes Care 2009;32:889-93.

12. Kravaritou M, Thanopoulou A, Karamanos B, Kofinis A, Noutsou M, Spanou E, Varsou A, Archimandritis A. Evidence that even "normal" albuminuria may denote incipient GFR reduction in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract 2009;85:317-21.

13. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med 1984;310:356-60.

14. Gosling P. Microalbuminuria and cardiovascular risk: a word of caution. J Hum Hypertens 1998;12:211-3.

15. Perkins BA, Ficociello LH, Roshan B, Warram JH, Krolewski AS. In patients with type 1 diabetes and new-onset microalbuminuria the development of advanced chronic kidney disease may not require progression to proteinuria. Kidney Int 2010; 77:57-64.

16. Pedersen MM, Christiansen JS, Pedersen EB, Mogensen CE. Determinants of intra-individual variation in kidney function in normoalbuminuric insulin-dependent diabetic patients: importance of atrial natriuretic peptide and glycaemic control. Clin Sci (Lond) 1992;83:445-51.

17. Kania K, Byrnes EA, Beilby JP, Webb SA, Strong KJ. Urinary proteases degrade albumin: implications for measurement of albuminuria in stored samples. Ann Clin Biochem 2010;47(Pt 2):151-7.

18. Jerums G, Panagiotopoulos S, Premaratne E, MacIsaac RJ. Integrating albuminuria and GFR in the assessment of diabetic nephropathy. Nat Rev Nephrol 2009;5:397-406.

19. Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. Nephrol Dial Transplant 2006;21:1855-62.

20. Madero M, Sarnak MJ, Stevens LA. Serum cystatin C as a marker of glomerular filtration rate. Curr Opin Nephrol Hypertens 2006;15:610-6.

21. Oddoze C, Morange S, Portugal H, Berland Y, Dussol B. Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with diabetes. Am J Kidney Dis 2001;38:310-6.

22. Fontsere N, Esteve V, Saurina A, Pou M, Barba N, Deulofeu R,

Di Gregorio S, del Rio LM, Rios J, Torres F, Maduell F, Campistol JM, Ramirez de Arellano M. The search for a new marker of renal function in older patients with chronic kidney disease stages 3-4: usefulness of cystatin C-based equations. Nephron Clin Pract 2009;112:c164-70.

23. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD 3rd, Zhang YL, Greene T, Levey AS. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis 2008;51:395-406.

24. Christensson AG, Grubb AO, Nilsson JA, Norrgren K, Sterner G, Sundkvist G. Serum cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. J Intern Med 2004;256:510-8.

25. Yanagawa T, Araki A, Sasamoto K, Shirabe S, Yamanouchi T. Effect of antidiabetic medications on microalbuminuria in patients with type 2 diabetes. Metabolism 2004;53:353-7.

26. Turk V, Bode W. The cystatins: protein inhibitors of cysteine proteinases. FEBS Lett 1991;285:213-9.

27. Russo LM, Sandoval RM, Campos SB, Molitoris BA, Comper WD, Brown D. Impaired tubular uptake explains albuminuria in early diabetic nephropathy. J Am Soc Nephrol 2009;20:489-94.

28. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function: a review. Clin Chem Lab Med 1999;37:389-95.

29. Perkins BA, Nelson RG, Ostrander BE, Blouch KL, Krolewski AS, Myers BD, Warram JH. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study. J Am Soc Nephrol 2005;16:1404-12.

30. Jeon YK, Kim MR, Huh JE, Mok JY, Song SH, Kim SS, Kim BH, Lee SH, Kim YK, Kim IJ. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. J Korean Med Sci 2011;26:258-63.

31. Lee BW, Ihm SH, Choi MG, Yoo HJ. The comparison of cystatin C and creatinine as an accurate serum marker in the prediction of type 2 diabetic nephropathy. Diabetes Res Clin Pract 2007;78:428-34.

32. Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann Intern Med 2004;141:929-37.

33. Herget-Rosenthal S, Bokenkamp A, Hofmann W. How to estimate GFR-serum creatinine, serum cystatin C or equations? Clin Biochem 2007;40:153-61.

34. Shlipak MG, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, Siscovick DS, Stehman-Breen C. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049-60.