Original Article

Association between Cystatin C and Arteriosclerosis in the Absence of Chronic Kidney Disease

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Aim: Chronic kidney disease (CKD) is a known risk factor for cardiovascular disease (CVD). Cystatin C was recently reported to be an endogenous surrogate of kidney function, and a high level of cystatin C is reported to be a strong predictor of CVD; however, the association between cystatin C and arteriosclerosis in a non-CKD population is unclear. This study aimed to clarify the association between cystatin C and arteriosclerosis in a non-CKD population.

Methods: Of the 637 Japanese adults (264 men, 373 women) enrolled, we analyzed 446 participants with an estimated glomerular filtration rate (eGFR) > 60 mL/min and no proteinuria (177 men, 269 women) without a history of CVD. Kidney function was evaluated according to serum cystatin C levels and eGFR. Arteriosclerosis was evaluated on the basis of the cardio-ankle vascular index (CAVI) and carotid intima-media thickness (CIMT).

Results: The mean age of our subjects was 67.0 ± 10.0 years. No variables showed any significant differences according to gender. The results of multiple linear regression analysis showed a significant correlation between serum cystatin C and CAVI only in women, but not CIMT.

Conclusion: We observed a significant correlation between cystatin C and CAVI, which is a marker of early-stage arteriosclerosis, in women in a non-CKD population with no proteinuria and eGFR > 60 mL/min.

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Key words: Cystatin C, Cardiovascular disease, Cardio-ankle vascular index (CAVI), Carotid intima-media thickness (CIMT)

Introduction

Recently, chronic kidney disease (CKD) has become an important public health problem worldwide. In the United States, it is estimated that there are 25 million people with CKD¹⁾. In Japan, about 13% of the Japanese adult population (approximately

13.3 million people) was predicted to have CKD in 2005²⁾. CKD has been recognized as a risk factor not only for end-stage renal disease but also for the development of cardiovascular disease (CVD)^{3, 4)} and is a known predictor of cardiovascular mortality^{5, 6)}. In fact, an independent and graded association has been observed in a community-based population between renal dysfunction and the risk of death, cardiovascular events, and hospitalization⁷⁾. Therefore, it is necessary and important to detect CKD in its early stages to reduce cardiovascular disease events.

The glomerular filtration rate (GFR) is an important indicator of kidney function for detecting, evalu-

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ating, and managing CKD. The accurate and internationally accepted method of measuring GFR is by measuring inulin clearance; however, this is a complex procedure and is normally not used in clinical practice in Japan. Instead, creatinine clearance is often used as a surrogate marker of GFR. However, several factors other than GFR, such as muscle mass and dietary intake, affect the serum creatinine level. Recently, cystatin C has been proposed as an endogenous surrogate marker for GFR, because it can easily pass through the glomerular filtration barrier because of its size⁸. Cystatin C is a non-glycosylated 13-kDa basic protein that is a cysteine protease inhibitor; it is also a member of the human cysteine superfamily, functioning as a housekeeping gene protein that is stably produced by most nucleated cells⁹. Furthermore, cystatin C is filtered by the glomeruli and reabsorbed and catabolized by tubular epithelial cells, with only small amounts being excreted in the urine¹⁰. Further, in comparison to traditional markers of renal dysfunction, an advantage of cystatin C is that very small reductions in GFR cause a significant increase in cystatin C serum¹¹; cystatin C is therefore gaining attention as a marker of early-stage renal impairment.

It is very important to establish effective and accurate screening methods for arteriosclerosis to prevent CVD. Several non-invasive parameters, such as the ankle brachial index (ABI), carotid intima-media thickness (CIMT), and pulse wave velocity (PWV), have been useful for evaluating arteriosclerotic lesions in the clinical setting. CIMT is an established measure of subclinical arteriosclerosis that holds prognostic significance for cardiovascular events in the general population¹²⁻¹⁵. Among the several methods available for the evaluation of arterial stiffness, PWV is easy, fairly reproducible, and correlates well with arterial stiffness determined by an invasive method¹⁶. However, since PWV is influenced by blood pressure, severe fluctuations in blood pressure values at the time of measurement of PWV can cause clinical difficulties. Therefore, a novel arterial stiffness parameter that reflects the stiffness of the aorta, the cardio-ankle vascular index (CAVI)^{17, 18}, has been recently developed; this index is independent of blood pressure¹⁷.

To date, a large number of studies have demonstrated that CKD patients are at a high risk of arteriosclerosis-related CVD⁷. However, based on studies that have assessed the association between kidney function and arteriosclerosis in the non-CKD adult population, this relationship is considered controversial¹⁹⁻²⁶. Currently, CKD is defined by either GFR <60 mL/minute per 1.73 m² of body-surface area or the presence of kidney damage, regardless of the cause,

for 3 or more months⁵). Thus, in the present study, the “non-CKD population” included people with eGFR >60 mL/min and without proteinuria, and without an apparent past or present history of cerebral infarction or hemorrhage or ischemic heart disease. Thereafter, we measured cystatin C as an indicator of kidney function, and CIMT and CAVI as indicators of arteriosclerosis, and we investigated the association between cystatin C and arteriosclerosis in a Japanese non-CKD population.

Materials and Methods

Subjects

Ethics approval was obtained from the Special Committee of Nagasaki University (project registration number 08043068) before commencement of the study. The study population was chosen by using a medical screening program for individuals aged over 40 years living in Goto city (total population in 2007, 44,874), Nagasaki Prefecture, Japan. After obtaining informed consent, we enrolled 637 Japanese adults (264 men, 373 women) in the study. We excluded 164 participants based on eGFR <60 mL/min and proteinuria; patients with a urine albumin-to-creatinine ratio of 300 mg/gCr or higher were judged to be positive for proteinuria. In addition, we excluded 27 other participants because they had an apparent past or present history of cerebral infarction or hemorrhage or ischemic heart disease. Finally, 446 participants (177 men and 269 women) were included for further analysis. With regard to underlying diseases in the study participants, we adhered to the following definitions (based on values observed during physical examination or self-reported at an interview): hypertension was defined as blood pressure $\geq 140/90$ mmHg²⁷; dyslipidemia, as low-density lipoprotein-cholesterol (LDL-C) ≥ 140 mg/dL or high-density lipoprotein-cholesterol (HDL-C) <40 mg/dL²⁸; and diabetes mellitus (DM), as hemoglobin A1c (HbA1c) $\geq 6.4\%$ (HbA1c notation converted from the original Japan Diabetes Society (JDS) values to the National Glycohemoglobin Standardization Program (NGSP) values²⁹).

Data Collection and Laboratory Measurements

Sociodemographic characteristics, past medical history, and details regarding lifestyle behaviors (smoking) were obtained by means of a questionnaire. Height, weight, and waist circumference (WC) were measured, and body mass index (BMI: kg/m²) was calculated as an index of obesity. Systolic and diastolic blood pressure (SBP and DBP) were recorded at rest,

Table 1. Characteristics of the study participants

	Men (<i>n</i> =177)	Women (<i>n</i> =269)	<i>p</i> value	All participants (<i>n</i> =446)
Age (years)	66.5 ± 11.0	67.4 ± 9.2	n.s.	67.0 ± 10.0
BMI (kg/m ²)	23.5 ± 3.1	23.0 ± 3.5	n.s.	23.2 ± 3.4
WC (cm)	84.5 ± 9.3	82.1 ± 10.2	n.s.	83.1 ± 9.9
SBP (mmHg)	144 ± 18	144 ± 21	n.s.	144 ± 20
TG (mg/dL)	99 ± 71	94 ± 53	n.s.	96 ± 61
HDL-C (mg/dL)	57 ± 14	60 ± 13	n.s.	59 ± 14
LDL-C (mg/dL)	118 ± 27	126 ± 25	n.s.	122 ± 26
UA (mg/dL)	6.1 ± 1.3	4.7 ± 1.0	n.s.	5.2 ± 1.3
HbA1c (%)	5.6 ± 0.7	5.6 ± 0.5	n.s.	5.6 ± 0.6
U-Alb/Cre (mg/g·Cre)	18.7 ± 32.4	25.7 ± 36.7	n.s.	22.9 ± 35.2
Cre (mg/dL)	0.78 ± 0.09	0.59 ± 0.07	n.s.	0.67 ± 0.12
eGFR (mL/min/1.73 m ²)	78.0 ± 12.0	76.8 ± 11.6	n.s.	77.3 ± 11.8
CysC (mg/L)	0.78 ± 0.13	0.73 ± 0.11	n.s.	0.75 ± 0.12
CIMT (mm)	0.72 ± 0.12	0.69 ± 0.11	n.s.	0.70 ± 0.12
CAVI	8.4 ± 1.5	8.1 ± 1.4	n.s.	8.2 ± 1.4

with the simultaneous measurement of CAVI. Blood samples were collected from each participant after overnight fasting. Serum and plasma were separated and stored at -20°C and -80°C , respectively, until the assay. Serum concentrations of total cholesterol (TC), triglyceride (TG), and HDL-C were measured by standard laboratory procedures, and LDL-C values were calculated by the Friedewald equation. In addition to fasting blood sugar and HbA1c, serum creatinine (Cre) and uric acid (UA) were measured by standard laboratory procedures. Serum cystatin C was measured in plasma specimens by the latex immunoturbidimetric method using OLYMPUS AU600 (Ikagaku Co. Ltd., Kyoto, Japan).

We also analyzed random urine samples from each patient to measure urinary albumin and creatinine excretions.

eGFR was calculated by the following formula³⁰:

$$\text{eGFR} = 194 \times \text{standardized Scr}^{-1.094} \times \text{Age}^{-0.287} (\times 0.739 [\text{if female}])$$

Measurement of CAVI and CIMT

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in the supine position. The principles underlying CAVI have been described by Yambe *et al.*¹⁸) ECG electrodes are placed on both wrists, a microphone for detecting heart sounds is placed on the sternum, and cuffs are wrapped around both the arms and ankles. After automatic measurements, the obtained data were analyzed using VSS-10 software (Fukuda Denshi), and the values for right

and left CAVI were calculated. Averages of the right and left CAVI were used for analysis.

Measurement of CIMT by ultrasonography of the left and right carotid arteries was performed using a LOGIC Book XP with a 10-MHz linear array transducer (GE Medical Systems, Milwaukee, WI, USA).

Statistical Analysis

Results are expressed as the mean ± standard deviation. Differences between women and men in laboratory values were evaluated using the *t*-test. Simple linear regression analysis was conducted to determine the correlation between cystatin C and the other variables measured. Variables that showed a significant association were subjected to multiple linear regression analysis. Probability values <0.05 were considered significant. All statistical analyses were performed using SPSS v. 11.0 software (SPSS Japan, Tokyo, Japan).

Results

The characteristics of the study participants are shown in **Table 1**. The mean age was 67.0 ± 10.0 years. With regard to the presence of underlying diseases among the study participants, 30 (6.7%) had DM, 159 (35.7%) had dyslipidemia, and 291 (65.2%) suffered from hypertension. Further, 49 participants (11.0%) had a positive history of smoking.

No variables showed any significant differences according to gender. Simple linear regression analysis revealed that cystatin C was significantly correlated with CAVI in all the participants (men: $r=0.24$, $p<0.01$; women: $r=0.247$, $p<0.01$; total population:

$r=0.256$, $p<0.01$). Furthermore, cystatin C was significantly correlated with CIMT in men and with BMI, SBP, and LDL-C in women. In all the participants, cystatin C was significantly correlated with age, WC, TG, HDL-C, UA, Cre, eGFR, and CAVI as well as CIMT according to the results of simple linear regression analysis (**Table 2**).

By multiple linear regression analysis adjusted for confounding factors associated with arteriosclerosis, cystatin C was found to be significantly correlated with CAVI in women, but not in men (**Table 3A**); however, cystatin C was not correlated with CIMT in any participants, unrelated to gender (**Table 3B**). In addition, we analyzed the association between eGFR, which is routinely used as a surrogate marker of kidney function, and CAVI or CIMT using multivariate analysis, but did not find any association between eGFR, and CAVI or CIMT in any participants (**Table 4A** and **4B**).

Discussion

In this study, we investigated the association between cystatin C and arteriosclerosis in adult Japanese subjects who underwent a physical examination, who were determined not to have CKD based on the absence of proteinuria and eGFR >60 mL/min. In consideration of the effects of age, BMI, diabetes mellitus, dyslipidemia, hypertension, and smoking, we found a significant correlation between cystatin C and CAVI in women.

The association between progressive impairment of kidney function and arteriosclerosis with CVD has been previously demonstrated^{3, 4}; however, in people without CKD, the association between cystatin C and arteriosclerosis has been unclear. Although a few studies have investigated the association between arteriosclerosis and serum cystatin C, which is considered more sensitive in detecting early-stage renal impairment as compared to serum creatinine³¹⁻³³, their results have differed due to variations in the targets and indicators used for arteriosclerosis. Thus, in the present study, we simultaneously measured CAVI, CIMT, and serum cystatin C levels in a non-CKD population; to the best of our knowledge, this is the first report to demonstrate that cystatin C was associated with CAVI in female non-CKD subjects with no proteinuria and eGFR >60 mL/min.

These findings indicate that a slight decrease in kidney function even within the normal range may promote arteriosclerosis, similar to the report by Verhave *et al.* that arteriosclerotic risk factors, such as increasing diastolic blood pressure and serum triglyc-

Table 2. Simple correlation analysis of CysC and other variables

	Men	Women	All participants
Age	0.374**	0.347**	0.343**
BMI	0.145	0.287**	0.239**
WC	0.212**	0.320**	0.291**
SBP	0.092	0.160**	0.134**
TG	0.226**	0.199**	0.209**
HDL-C	-0.190*	-0.208**	-0.222**
LDL-C	0.098	0.129*	0.085
UA	0.244**	0.312**	0.332**
HbA1c	0.042	0.106	0.076
U-Alb/Cre	-0.009	0.086	0.005
Cre	0.420**	0.426**	0.426**
eGFR	-0.516**	-0.513**	-0.495**
CIMT	0.228**	0.108	0.181**
CAVI	0.240**	0.247**	0.256**

* $p<0.05$ and ** $p<0.01$

erides, were associated with a decrease in kidney function in the population who had normal kidney function³⁴. On the other hand, our results also showed that very mild dysfunction caused by arteriosclerosis might be detected by cystatin C.

After adjustment for confounding factors, we could not find a significant association between cystatin C and CIMT. A number of studies have investigated the association between cystatin C and CIMT as a marker of arteriosclerosis; these studies targeted a healthy Chinese population²⁰, middle-aged adults in the Seychelles²³, and cardiovascular disease-free people²²; however, no relationship was observed between cystatin C and CIMT in these studies, as in our study. On the other hand, a study that targeted hypertensive patients reported a positive correlation between cystatin C and CIMT^{24, 26}. One reason for this result might be the insufficient number of participants. Furthermore, our results can be explained by a report that CAVI could detect arterial stiffness prior to evident carotid arteriosclerosis and high CAVI might imply the progression of carotid arteriosclerosis³⁵; that is, CAVI may be more sensitive than CIMT for detecting arteriosclerosis.

Furthermore, the difference between men and women in the smoking rate might explain why a significant association between cystatin C and CAVI was not observed in men. In our subjects, the smoking rates were 23.2% in men and 3.0% in women. Previously, it was reported that elevated cystatin C was independently associated with smoking³⁶. Cystatin C is the most widespread and potent inhibitor of cathepsins B and L and it has been considered that a cathep-

Table 3A. Multiple linear regression analysis of CysC with relevant factors

	All		Men		Women	
	β	<i>p</i> value	β	<i>p</i> value	β	<i>p</i> value
Age	0.319	<0.001	0.404	<0.001	0.306	<0.001
UA	0.116	0.018	0.134	0.051	0.154	0.005
BMI	0.169	<0.001	0.136	0.042	0.194	<0.001
Diabetes mellitus	-0.006	0.886	0.012	0.847	0.016	0.748
Dyslipidemia	0.033	0.417	0.029	0.661	-0.029	0.573
Hypertension	-0.042	0.336	-0.031	0.635	-0.070	0.245
Smoking	0.052	0.205	0.202	0.002	-0.030	0.559
Cre	0.349	<0.001	0.397	<0.001	0.392	<0.001
CAVI	0.075	0.101	0.042	0.569	0.117	0.049

β : regression coefficient; CI: confidence interval.

Table 3B. Multiple linear regression analysis of CysC with relevant factors

	All		Men		Women	
	β	<i>p</i> value	β	<i>p</i> value	β	<i>p</i> value
Age	0.363	<0.001	0.394	<0.001	0.384	<0.001
UA	0.129	0.008	0.150	0.030	0.182	0.001
BMI	0.162	<0.001	0.145	0.029	0.164	0.003
Diabetes mellitus	-0.001	0.987	0.010	0.874	0.027	0.586
Dyslipidemia	0.038	0.341	0.027	0.675	-0.032	0.541
Hypertension	-0.032	0.456	-0.033	0.611	-0.039	0.510
Smoking	0.060	0.144	0.202	0.002	-0.003	0.961
Cre	0.353	<0.001	0.394	<0.001	0.395	<0.001
CIMT	0.015	0.736	0.089	0.206	-0.062	0.280

β : regression coefficient; CI: confidence interval.

sin-cystatin C imbalance causes tissue destruction; therefore, elevated cystatin C influences the state of lung tissue destruction, such as emphysema. We consider that cystatin C in smokers might be possibly elevated not only as a result of early renal dysfunction but also as a direct response to smoking. In our study, smoking might have influenced our results since cystatin C was significantly related to smoking in men. In addition, our subjects were comparatively older. It is well known that kidney function is correlated negatively with age. In fact, a strong correlation has been reported between cystatin C and age, regardless of the presence of impaired renal function³⁷⁻⁴⁰. Since the average age of our subjects was 67.0 years, there is a possibility that age influenced the serum cystatin C values. Moreover, it is known that women develop arteriosclerosis and cardiovascular disease typically after the menopause. As the mean age of women in our study was 67.4 years, ovarian hormone deprivation might also have affected our result that cystatin C

was related to CAVI only in women.

In this study, we adopted cystatin C and eGFR as surrogate markers of renal function. Currently, eGFR is accepted as a surrogate marker of kidney function worldwide; however, Coll *et al.* reported that serum cystatin C levels began to increase beyond the normal limit when the GFR was 88 mL/min/1.73 m², whereas serum creatinine levels began to increase when the GFR was 75 mL/min/1.73 m²⁴¹. These data suggest that the measurement of serum cystatin C may be useful for estimating GFR, especially for detecting mild reductions in GFR, and may therefore be important in the detection of early renal insufficiency in a variety of renal diseases for which early treatment is critical.

In this study the subjects had eGFR >60 mL/min and relatively intact renal function; therefore, we considered that cystatin C was a more sensitive marker than eGFR for detecting slight decreases in GFR. We subsequently focused upon the correlations of cystatin

Table 4A. Multiple linear regression analysis of eGFR with relevant factors

	All		Men		Women	
	β	<i>p</i> value	β	<i>p</i> value	β	<i>p</i> value
Age	-0.010	0.841	-0.099	0.229	0.037	0.586
UA	-0.090	0.052	-0.199	0.003	-0.126	0.333
BMI	0.066	0.147	0.110	0.105	0.036	0.565
Diabetes mellitus	0.027	0.513	0.015	0.864	0.016	0.762
Dyslipidemia	-0.114	0.006	-0.136	0.037	-0.062	0.264
Hypertension	-0.049	0.278	-0.034	0.600	-0.066	0.301
Smoking	0.154	<0.001	0.203	0.002	-0.041	0.451
CysC	-0.462	<0.001	-0.444	<0.001	-0.462	<0.001
CAVI	-0.030	0.529	0.016	0.829	-0.060	0.343

β : regression coefficient; CI: confidence interval.

Table 4B. Multiple linear regression analysis of eGFR with relevant factors

	All		Men		Women	
	β	<i>p</i> value	β	<i>p</i> value	β	<i>p</i> value
Age	-0.008	0.876	-0.067	0.398	0.032	0.635
UA	-0.097	0.036	-0.200	0.004	-0.133	0.025
BMI	0.066	0.145	0.116	0.087	0.038	0.531
Diabetes mellitus	0.026	0.523	0.019	0.766	0.013	0.813
Dyslipidemia	-0.117	0.005	-0.129	0.047	-0.065	0.238
Hypertension	-0.044	0.322	-0.029	0.658	-0.063	0.314
Smoking	0.157	<0.001	0.212	0.001	-0.043	0.432
CysC	-0.464	<0.001	-0.445	<0.001	-0.469	<0.001
CIMT	-0.035	0.456	-0.032	0.653	-0.059	0.330

β : regression coefficient; CI: confidence interval.

C with CAVI and CIMT, although it is unfortunate that our results only led to the conclusion that cystatin C is superior to eGFR with regard to the association with arteriosclerosis. In contrast, although the urinary albumin-to-creatinine ratio (UACR) is also known to be a sensitive marker of kidney injury, no significant association was observed between UACR and cystatin C or eGFR in our study. UACR mainly reflects glomerular injury or increased intra-glomerular pressure, but not GFR, while cystatin C is a sensitive marker that reflects GFR⁴²⁾. In other words, while cystatin C and UACR are both markers of kidney dysfunction, we consider that they reflect fundamentally different conditions. One such example is nephrosclerosis, which is an underlying disease of chronic kidney failure; even when proteinuria is not observed, this entity leads to decreased kidney function, wherein cystatin C or eGFR and UACR do not necessarily display identical changes. Therefore, although no correlation was observed between cystatin C and UACR, we consider

that our result is helpful for subjects whose eGFR is >60 mL/min with no evident proteinuria.

Apart from renal function, classical risk factors for arteriosclerosis, such as hypertension, DM, dyslipidemia, and smoking, may also affect the level of CAVI or CIMT; this may have affected our results. The relationship of DM and LDL-C with kidney function and arteriosclerosis has been previously demonstrated^{43, 44)}; however, no association between DM and cystatin C was observed in our study and we believe that this was because the overall proportion of DM patients was only 6.7%. With regard to the association between cystatin C and dyslipidemia, although univariate analysis found no correlation between LDL-C and cystatin C, a significant negative correlation was observed between cystatin C and HDL-C. Low HDL-C is also defined as dyslipidemia and is believed to be a risk factor for arteriosclerosis⁴⁵⁾; therefore, we consider that our results are consistent with previous studies. In this study, the mean LDL-C (143

mg/dL) was relatively low, which may have influenced the lack of a significant association between cystatin C and LDL-C.

There were some limitations of our study. First, our subjects were selected within a confined geographical area. Thus, our results might have been affected by this selection bias. We therefore hope for future large-scale studies. Second, there is no standard assay system or cutoff value for cystatin C⁴⁶⁾. The possibility that the lack of such standards affected the results of not only our study but also previous research cannot be ruled out. It is therefore necessary to define a cutoff value for cystatin C and to establish a universal assay system for cystatin C.

In conclusion, we observed a significant correlation between cystatin C and CAVI in women in a non-CKD population with no proteinuria and eGFR >60 mL/min. This may imply that patients with a high level of cystatin C, even within the normal range, have the potential to develop CKD or CVD. Therefore, patients with high levels of cystatin C or CAVI in medical screening studies should be carefully monitored for kidney function and arteriosclerosis severity in order to prevent CKD or CVD.

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Conflicts of Interest

None.

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