Original Article

Association of cystatin C with leptin and TNF-α in elderly Japanese women

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Background and Objectives: Determinants of cystatin C, a novel marker of mortality in the elderly, have not been extensively studied in Asian elderly population. **Methods and Study Design:** Associations of cystatin C with anthropometric, cardiometabolic, hematological, nutritional variables and inflammatory markers were examined in 159 community-living elderly Japanese women whose BMI averaged 22.6±2.9 (SD) kg/m^2. **Results:** Serum creatinine and cystatin C averaged 0.73±0.16 mg/dL and 0.85±0.20 mg/L, respectively. Creatinine-based estimated glomerular filtration rate (standardized β, -0.538, p<0.001), age (standardized β, 0.274, p<0.001), serum leptin (standardized β, 0.218, p<0.001) and tumour necrosis factor-α (TNF-α, standardized β, 0.165, p=0.002) emerged as significant predictors of serum cystatin C independent of percentage body fat, homeostasis model assessment of insulin resistance, high-sensitivity C-reactive protein, systolic blood pressure and HDL cholesterol (cumulative R^2=0.674). **Conclusions:** Elevated serum levels of leptin and TNF-α contributed to elevated cystatin C independent of kidney function, fat mass, insulin resistance and inflammation in community-living elderly women and may represent confounders of associations between cystatin C and mortality in this population.

Key Words: cystatin C, leptin, TNF-α, kidney function, elderly

INTRODUCTION

Cystatin C is a novel measure of kidney function that appears to be more sensitive than creatinine for determining changes in glomerular filtration rate (GFR).[1] Serum cystatin C as compared to creatinine was less influenced by diet and reduced muscle mass and hence, cystatin C-based estimated GFR appeared to be more accurate in assessing kidney function than creatinine-based GFR in the elderly population.[2] Cystatin C has recently been found to be a predictor of cardiovascular disease in elderly Western populations.[3,4] The impact of elevated cystatin C on cardiovascular disease risk was larger in the elderly versus the general population.[2] However, the relationship between cystatin C and traditional and non-traditional CV risk factors has not been well characterized, particularly in community-dwelling elderly persons of Asian origin.

Recently, cystatin C has been shown to be associated with insulin resistance, components of metabolic syndrome and inflammation.[5-8] However, many of those studies were performed in patients with type 2 diabetes, dyslipidemia and hypertension,[6-8] and data are limited in the general population, specifically in elderly population.[9] Therefore, we evaluated associations of cystatin C with anthropometric, cardiometabolic, hematological, nutritional variables and inflammatory markers in community-living elderly Japanese people.

PARTICIPANTS AND METHODS

We examined 159 free-living elderly women whose details have been reported elsewhere.[10] They were residents in Nishinomiya City and were recruited as volunteers by local welfare commissioners from the city of Nishinomiya, Hyogo, Japan. Although 8 men participated in the study, we reported data on women only. Although 43, 9 and 58 women (27.0%, 5.7%, and 36.5%, respectively) reported to be receiving statins, anti-diabetic and anti-hypertensive drugs, respectively, detailed drug information was not available. Subjects with clinical diagnosed acute or chronic inflammatory diseases, cancer, cardiovascular, hepatic and renal diseases, unusual dietary habits were excluded from the study. This research followed the tenets of the Declaration of Helsinki. The design of this study was approved by the Ethical Committees of Mukogawa Women's' University (No. 11-7) and written informed consents were obtained from all partici-

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Manuscript received 23 July 2014. Initial review completed 09 October 2014. Revision accepted 14 December 2014.
doi: 10.6133/apjcn.2015.24.4.18

pants.

Anthropometric indices and blood pressure were measured after an overnight fasting. Thereafter, blood samples were obtained from the cubital vein. Fat mass was measured using an impedance method (InBody 430, Biospace, Tokyo, Japan).

We evaluated routine chemical parameters, including plasma glucose, insulin, serum lipids and lipoproteins as previously reported.[11] Insulin resistance was evaluated using homeostasis model assessment (HOMA-IR).[12]

Metabolic syndrome was defined using the modified criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP) guidelines.[13] Because waist circumference was not available in all participants, obesity (a cardinal feature of the metabolic syndrome) was defined using Asian criteria as BMI ≥25.0 kg/m^2.[14] Elevated blood pressure was defined as systolic/diastolic blood pressures of 130/85 mmHg or greater and/or current use of antihypertensive medicine. Hypertriglyceridemia was defined as a serum triglyceride level of 150 mg/dL or greater. Low HDL cholesterol level was defined as less than 50 mg/dL for all participants are women. Elevated blood glucose level was defined as fasting blood glucose level of 100 mg/dL or greater and/or current use of anti-diabetic medicine. Metabolic syndrome was defined as the presence of 3 or more of these components.

High-sensitivity C-reactive protein (hsCRP) was measured by an immunoturbidometric assay with the use of reagents and calibrators from Dade Behring Marburg GmbH (Marburg, Germany; interassay CV <5%). Tumor necrosis factor-α (TNF-α) were measured by immunoassays (R&D Systems, Inc., Minneapolis, MN, interassay CV <6%). Interleukin-6 (IL-6) was measured by a commercially available kit (IL-6, Human, ELISA Kit, QuantiGlo, 2nd Generation, Funakoshi Co, Ltd, Tokyo, Japan). Plasminogen activator-inhibitor-1 (PAI-1) was measured by an ELISA method (Mitsubishi Chemicals, interassay CV <8%). Complete blood cell count, total and differential peripheral leukocytes was analyzed using an automated blood cell counter (Sysmex XE-2100, Sysmex, Kobe, Japan).

Serum creatinine concentrations were measured enzymatically using an autoanalyzer (AU 5200, Olympus, Tokyo, Japan) and cystatin C was measured by latex immunoassay using a commercially available kit (IatroCys-C, Mitsubishi Chemical Medience, Tokyo, Japan). The eGFR was calculated using the equation recommended by the Japanese Society for Nephrology.[15,16]

Data are presented as mean±SD unless otherwise stated. Due to deviation from normal distribution, hsCRP, insulin and HOMA-IR were logarithmically transformed for analysis. Differences between 2 groups were analyzed by t test and frequencies of conditions by chi-square tests. Differences among 3 groups were analyzed using analysis of variance. When p values in analysis of variance were $p<0.05$, Bonferroni's multiple comparison procedure was performed. Correlations of cystatin C were evaluated by Pearson correlation analysis. Stepwise multiple regression analyses were performed to further indentify the most significant variables contributing to the variation of cystatin C. Potential confounders were forced into the model and standardized β coefficients were calculated. The explanatory power of the model was expressed as adjusted R^2 values. A two-tailed $p<0.05$ was considered statistically significant. All calculations were performed with SPSS system 15.0 (SPSS Inc, Chicago, IL).

RESULTS

As previously reported,[10] participants were relatively healthy, community-living, ambulatory elderly women (Table 1). Cystatin C and creatinine averaged 0.85±0.20 mg/L and 0.73±0.16 mg/dL, respectively (Table 1). Creatinine-based and cystatin C-based eGFR averaged 62±14 and 80±20 mL/min/1.73m^2, respectively. The prevalence of renal dysfunction (eGFR <60 mL/min/1.73m^2) was 46.5% (74 women) and 12.6% (13 women) when diagnosed using creatinine-based and cystatin C-based eGFR, respectively.

Of 159 elderly women, only 18 (11.3 %) had metabolic syndrome. There was no difference in serum cystatin C between women with and without the syndrome (0.95±0.32 and 0.84±0.18 mg/L, p=0.183). However, cystatin C increased as the number of components of metabolic syndrome increased [0 (n=21); 0.76±0.15, 1 (n=80); 0.85±0.17, 2 (n=40); 0.87±0.20, 3-5 (n=18); 0.95±0.32 mg/L, $p<0.01$ for trend].

In univariate analysis (Table 1), age, percentage body fat and serum leptin correlated positively with cystatin C. In addition, cystatin C showed a positive association with systolic blood pressure, pulse pressure, fasting glucose and insulin, and hence HOMA-IR. Further, cystatin C showed positive associations with uric acid and transthyretin and a negative association with HDL cholesterol. Finally, cystatin C was correlated positively with TNF-α and leukocyte count, but not with log hsCRP and IL-6. After adjustment for creatinine-based eGFR, an association with triglyceride became significant and associations with age, TNF-α, leptin, systolic blood pressure, HDL cholesterol and HOMA-IR remained significant.

In women with renal dysfunction (eGFR <60 mL/min/1.73m^2) diagnosed using creatinine-based eGFR (Table 2), associations of cystatin C with HOMA-IR, triglyceride, HDL cholesterol and leptin were stronger than in women without renal dysfunction. Associations with age, creatinine-based eGFR and TNF-α were consistent in women with and without renal dysfunction.

We have done multiple regression analysis with cystatin C as a dependent variable (Table 3). The model included creatinine-based eGFR and variables which showed significant associations with cystatin C after adjustment for eGFR (Table 1) as independent variables. Creatinine-based eGFR, age, serum TNF-α and leptin emerged as significant determinants of cystatin C independently of systolic blood pressure, pulse pressure, HOMA-IR, HDL cholesterol and serum TG. These 4 variables explained 68.2% of variability of serum cystatin C.

Because participants with high cystatin C (≥1.0 mg/dL)[5] were small in number (n=28), we decided to divide participants according to cystatin C tertiles in order to confirm associations of cystatin C with cardiometabolic and inflammatory variables (Table 4). Higher concentrations of cystatin C were associated with older age and higher leptin. In addition, higher cystatin C concentrations were associated with higher log TNF-α and leu-

Table 1. Anthropometric and biochemical features of 159 elderly women studied and correlation coefficients of serum cystatin C before (simple) and after (partial) adjustment for creatinine-based estimated glomerular filtration rate.

	Mean±SD	Serum cystatin C			
		Simple		Partial	
Age (years)	75.6±8.0	0.527	***	0.354	***
BMI (kg/m^2)	22.6±2.9	0.076		0.103	
Body fat percentage (%)	33.0±6.9	0.165	*	0.159	
SBP (mmHg)	143±19.0	0.249	**	0.230	**
DBP (mmHg)	84.4±10.4	0.095		0.134	
Pulse pressure (mmHg)	59.0±12.7	0.295	***	0.236	**
Albumin (g/dL)	4.4±0.3	-0.299	***	-0.191	*
Transthyretin (mg/dL)	28.2±4.8	-0.163	*	-0.211	*
Plasma glucose (mg/dL)	100±29	0.176	*	0.103	
Insulin (μU/mL)	8.3±7.5	0.220	**	0.127	
HOMA-IR	1.23±1.08	0.270	***	0.164	*
Total cholesterol (mg/dL)	219±31	-0.153		-0.038	
HDL-cholesterol (mg/dL)	64±14	-0.208	**	-0.234	**
Non-HDL-cholesterol (mg/dL)	155±33	-0.056		0.079	
Triglyceride (mg/dL)	119±64.9	0.019		0.169	*
Serum uric acid (mg/dL)	4.8±1.0	0.343	***	0.098	
Serum creatinine (mg/dL)	0.73±0.16	0.770	***	0.343	***
Cystatin C (mg/L)	0.85±0.20	1.000	***	1.000	***
eGFRcreat (mL/min/1.73m^2)	62±14	-0.742	***	adjusted	
Leptin (ng/mL)	7.7±4.7	0.293	***	0.267	**
Adiponectin (μg/mL)	14.1±7.8	0.097		0.021	
hsCRP (μg/dL)	85±109	0.075		0.049	
TNF-α (pg/mL)	1.6±1.0	0.481	***	0.310	***
PAI-1 (ng/mL)	26.5±16.5	-0.004		0.129	
IL-6 (pg/mL)	5.5±12.0	0.134		0.043	
Leukocytes (×10^3/μL)	6.1±1.6	0.161	*	0.135	

BMI: body mass index; SBP: Systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model insulin resistance; eGFRcreat: creatinine-based estimated glomerular filtration rate; hsCRP: high-sensitivity C-reactive protein; TNF: tumour necrosis factor; PAI-1: plasminogen activator inhibitor-1; IL-6: interleukin-6.
$^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Table 2. Associations of cystatin C in women with and without renal dysfunction (estimated glomerular filtration rate <60 mL/min/1.73m^2) diagnosed using creatinine-based equation

	Renal dysfunction			
	No (n=85)		Yes (n=74)	
	r	*p*	r	*p*
Age (years)	0.552	**	0.456	**
BMI (kg/m^2)	0.026		0.074	
Body fat percentage (%)	0.138		0.170	
SBP (mmHg)	0.176		0.216	
DBP (mmHg)	0.092		0.105	
Pulse pressure (mmHg)	0.204		0.223	
Albumin (g/dL)	-0.412	**	-0.259	*
Transthyretin (mg/dL)	-0.240	*	-0.146	
Plasma glucose (mg/dL)	-0.097		0.228	
Insulin (μU/mL)	-0.120		0.249	*
HOMA-IR	-0.131		0.309	**
Total cholesterol (mg/dL)	-0.169		-0.121	
HDL-cholesterol (mg/dL)	-0.044		-0.295	*
Non-HDL-cholesterol (mg/dL)	-0.157		0.014	
Triglyceride (mg/dL)	-0.113		0.246	*
Serum uric acid (mg/dL)	0.674		-0.271	
Serum creatinine (mg/dL)	0.392	**	0.714	**
Cystatin C (mg/L)	1		1	
eGFRcreat (mL/min/1.73m^2)	-0.964	**	-0.947	**
Leptin (ng/mL)	0.159		0.296	*
Adiponectin (μg/mL)	0.210		0.102	
hsCRP (μg/dL)	0.262	*	0.005	
TNF-α (pg/mL)	0.323	**	0.456	**
PAI-1 (ng/mL)	-0.074		0.103	
IL-6 (pg/mL)	0.259	*	0.086	
Leukocytes (×103/μL)	0.117		0.123	

Abbreviations are the same as in Table 1. $^*p<0.05$, $^{**}p<0.01$

Table 3. Stepwise multiple regression analysis with serum cystatin C as a dependent variable in community-dwelling elderly Japanese women

	Standardized β	*p* values	Cumulative R^2
eGFRcreat	-0.538	<0.001	0.545
Age	0.274	<0.001	0.602
Leptin	0.218	<0.001	0.654
TNF-α	0.165	0.002	0.674

The model included eGFRcreat, age, serum leptin, TNF-α, systolic blood pressure, pulse pressure, homeostasis model insulin resistance, HDL cholesterol and triglycerides as independent variables. Abbreviations are the same as in Table 1.

Table 4. Anthropometric and biochemical characteristics of elderly women stratified by tertiles of serum cystatin C.

	Low (n=50)	Medium (n=55)	High (n=54)
Age (years)	70.0±7.4[a]	75.3±7.1[b]	81.1±5.5[c]
BMI (kg/m^2)	22.6±2.8	22.5±2.4	22.6±3.5
Body fat percentage (%)	32.3±7.3	32.4±5.8	34.1±7.4
SBP (mmHg)	136±17.2[a]	144±17.1[ab]	149±20.6[b]
DBP (mmHg)	82.1±10.5	85.9±9.7	84.9±10.8
Pulse pressure (mmHg)	54.1±10.1[a]	57.6±10.8[a]	64.3±14.5[b]
Albumin (g/dL)	4.5±0.2[a]	4.4±0.2[b]	4.3±0.3[c]
Transthyretin (mg/dL)	29.4±4.3[a]	28.4±4.4[ab]	26.8±5.5[b]
Plasma glucose (mg/dL)	88±10	87±12	88±15
Insulin (μU/mL)	5.3±3.3	5.3±3.9	5.9±4.8
HOMA-IR	1.17±0.82	1.15±0.88	1.37±1.44
Total cholesterol (mg/dL)	228±33	219±34	216±31
HDL-cholesterol (mg/dL)	69±17	65±16	65±14
Non-HDL-cholesterol (mg/dL)	158±30	154±33	151±31
Triglyceride (mg/dL)	128±80	122±65	107±46
Serum uric acid (mg/dL)	4.4±0.7[a]	4.7±1.2[a]	5.2±1.0[b]
Serum creatinine (mg/dL)	0.62±0.09[a]	0.70±0.09[b]	0.86±0.18[c]
Cystatin C (mg/L)	0.67±0.06[a]	0.82±0.03[b]	1.06±0.20[c]
eGFRcreat (mL/min/1.73m^2)	73.3±11.1[a]	63.0±9.5[b]	50.4±10.4[c]
Leptin (ng/mL)	8.1±5.0[a]	8.8±5.1[ab]	10.6±7.9[b]
Adiponectin (μg/mL)	14.3±7.5	16.0±7.0	17.0±7.6
hsCRP (μg/dL)	158±232	182±316	297±493
TNF-α (pg/mL)	1.8±0.9[a]	2.1±0.8[a]	3.0±1.6[b]
PAI-1 (ng/mL)	30.7±13.1	28.6±8.9	27.8±10.5
IL-6 (pg/mL)	4.0±6.5	4.1±4.7	8.4±18.9
Leukocytes (×10^3/μL)	5.5±1.1	5.8±1.4	6.1±1.7

Data are mean±SD. Abbreviations are the same as in Table 1. Means not sharing common alphabetical letters are statistically significant each other at $p<0.05$ or less.

kocyte count. Further, higher cystatin C concentrations were associated with higher systolic blood pressure, pulse pressure and uric acid. After controlling for creatinine-based eGFR, associations with TNF-α, systolic blood pressure, pulse pressure and age remained significant (data not shown).

DISCUSSION

The current study demonstrates that higher serum levels of leptin and TNF-α are associated with higher cystatin C in community-living elderly women. These associations remained significant after adjustment for age, creatinine-based eGFR, a conventional measure of renal function, percentage body fat measured using an impedance method and hsCRP, a marker of systemic low-grade inflammation. We confirmed the previous findings that high cystatin C is associated with some components of metabolic syndrome in elderly women, specifically in women with renal dysfunction, as previously reported in patients at higher risk,[6-8] and demonstrated that high cystatin C is associated with high TNF-α and leptin in community-living elderly women independent of age, fat mass, systemic low-grade inflammation and kidney function.

Naour et al[17] reported that serum cystatin C is elevated in obese subjects and that adipose tissue expression of the protein is increased in obesity. Although several prior studies have reported the relationship between cystatin C and BMI,[18-20] a crude marker of fat mass, the present study is the first to demonstrate an independent relationship between serum cystatin C and leptin, a sensitive marker of fat mass.[21] However, this association was independent of percentage body fat measured using bioelectrical impedance method, which is a good alternative for dual-energy X ray absorptiometry, a gold standard for estimating percentage body fat, when subjects are within a normal body fat range.[22] In this context, it has recently been shown even in the elderly that high levels of leptin are associated with arterial stiffness, hypertension and low endothelial-dependent vasodilation,[23] all of which are known CV risk factors. These associations were attenuated after adjusting for body mass index suggesting that leptin may be the mediator between obesity and im-

paired vascular function.[23]

Although TNF-α is an inflammatory cytokine produced mainly by monocytes and macrophages, TNF-α produced by adipose tissue may play an important role in obesity-associated insulin resistance and diabetes.[24] In the Insulin Resistance Atherosclerosis Study,[25] increased TNF-α levels were predominantly associated with insulin resistance.[18] In our study of elderly women, TNF-α was correlated with cystatin C independently of hsCRP and IL-6, hallmarks of systemic inflammation. Taken together, these findings suggest that TNF-α- may be a biomarker of insulin resistance rather than systemic inflammation in our elderly women. It is probable that locally produced TNF-α may act synergistically with circulating TNF-α on fatty and muscular tissues to induce insulin resistance although the serum levels of TNF-α found in the present study were relatively low and circulating TNF-α may not be biologically active at such low concentrations.

Previous studies have reported positive associations between cystatin C and CRP,[6,21,22,26,27] but most of these studies analyzed a single biomarker. The present study examined associations between cystatin C and a broad range of inflammatory markers and found a significant association of cystatin C with serum TNF-α independent of hsCRP and IL-6. Although it is known that TNF-α stimulates the synthesis of CRP in the liver, correlation coefficient between circulating levels of TNF-α and CRP was 0.173 in the present study and 0.27 in a large, multiethnic population of the Insulin Resistance Atherosclerosis Study.[18] As mentioned above, TNF-α may be a biomarker of insulin resistance rather than systemic inflammation in the present study. Associations between serum cystatin C and TNF-α independent of eGFR and CRP were also reported in well-functioning older population of white and black subjects[28] and in patients with essential hypertension[29] or acute heart failure.[30]

Cystatin C levels progressively increased in association with the number of MS components in community-living elderly women in the present study as previously reported in patients with type 2 diabetes, hypertension and dyslipidemia.[6-8] Although cystatin C was higher in patients with MS than in patients free of MS,[6-8] there was no significant difference in cystatin C between elderly women with and without MS in the present study. This may be due in part to small sample size in women with MS (n=18, 11.3%).

Our study has several limitations. We are unable to determine either the direction of association or the causal pathway given the cross-sectional design of our study. The recruitment procedure may also have had an impact on the results. As participation was voluntary, women who pay more attention to their health may have been more likely to participate. Biochemical parameters, including cystatin levels, were measured only once. Although 43, 9 and 58 women (27.0%, 5.7%, and 36.5%, respectively) reported to be receiving statins, anti-diabetic and anti-hypertensive drugs, respectively, detailed drug information was not available. These drugs may have effects on serum cystatin C levels.[31] In addition, a more direct measurement of GFR, such as inulin clearance, was not used in this study as a gold standard for comparison. We also lacked measures of urine albumin excretion, clinical outcomes and fat distribution. Finally, participants were relatively small in number and were all females.

In conclusion, elevated levels of serum leptin, a mediator between obesity and impaired vascular function,[23] and TNF-α, a marker of insulin resistance,[25] were associated with higher cystatin C in ambulatory elderly women. Higher body fat and insulin resistance may represent important confounders of the relationship between serum cystatin C and mortality in the elderly population.

ACKNOWLEDGEMENTS

We are indebted to all the participants for their dedicated and conscientious collaboration. This study was supported by KAKENHI (21300260) of a Grant -in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science.

AUTHOR DISCLOSURES

No potential conflicts of interest were disclosed.

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Original Article

Association of cystatin C with leptin and TNF-α in elderly Japanese women

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日本老年女性胱蛋白 C 與瘦體素及 TNF-α 的相關性

背景與目的：胱蛋白 C 為一個老年死亡的新標記，其決定因子在亞洲老年族群尚未被大規模的探討。**方法與研究設計：**評估 159 名 BMI 平均值為 22.6±2.9 (SD) kg/m^2 的日本社區老年女性，其胱蛋白 C 與體位測量值、心血管代謝、血液資料、營養狀況及發炎標記之相關。**結果：**血清肌酸酐與胱蛋白 C 平均值分別為 0.73±0.16 mg/dL 及 0.85±0.20 mg/L。肌酸酐評估腎絲球過濾率（標準化迴歸係數-0.538，p<0.001）、年齡（標準化迴歸係數 0.274，p<0.001）、血清瘦體素（標準化迴歸係數 0.218，p<0.001）及腫瘤壞死因子-α（TNF-α 標準化迴歸係數 0.165，p=0.0021）顯示為血清胱蛋白 C 的顯著預測因子，且此結果獨立於體脂肪百分比、胰島素抗性之恆定模式評估、高敏感度 C 反應蛋白、收縮壓及高密度脂蛋白膽固醇（累積 R^2=0.674）。**結論：**社區老年女性血清瘦體素及 TNF-α 的增加導致胱蛋白 C 的提高，此相關獨立於腎功能、體脂質量、胰島素阻抗性及發炎反應。這個族群之胱蛋白 C 與死亡率的相關，可能是干擾因子造成的相關。

關鍵字：胱蛋白 C、瘦體素、腫瘤壞死因子-α、腎功能、老人