Serum Cystatin C, a Sensitive Marker of Renal Function and Cardiovascular Disease, Decreases After Smoking Cessation

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Background: Smoking exerts detrimental effects during the progression of atherosclerotic vascular disease. Serum cystatin C is useful in the evaluation of early renal dysfunction and serves as a cardiovascular prognostic marker. This study measured changes in serum cystatin C after smoking cessation (SC).

Methods and Results: In this study, patients who visited the SC clinic for the first time and succeeded in SC for 1 year were enrolled. In the entire cohort of 86 patients, body mass index (BMI, $P<0.001$) and waist circumference (WC, $P<0.001$) increased significantly at 3 months after SC compared with baseline. These values were further increased significantly (BMI, $P<0.001$; WC, $P<0.001$) from 3 months to 1 year after SC. Serum cystatin C decreased significantly at 3 months ($P=0.045$) after SC, and remained unchanged ($P=0.482$) from 3 months to 1 year after SC. Percent change from baseline to 3 months after SC in serum cystatin C was correlated with the percent change in serum monocyte chemoattractant protein 1 ($P=0.047$).

Conclusions: Serum cystatin C, a marker of chronic kidney disease, was significantly reduced at 3 months after SC.

Key Words: Cardiovascular disease; Cystatin C; Monocyte chemoattractant protein 1; Smoking cessation

Smoking causes 1 of every 5 deaths in developed countries and is the world's leading preventable cause of death.^{1,2} Smoking causes a number of atherosclerosis vascular diseases and contributes to the progression of chronic kidney disease.^{3–6} Moreover, smoking is damaging to the kidneys of not only the patients with kidney disease but also healthy individuals.⁷ A cohort study of 40,000 individuals reported that smoking was associated with increased urine albumin, whereas the Multiple Risk Factor Intervention Trial that involved over 300,000 men reported that male smokers were at an increased risk for renal failure compared with non-smokers.⁸ Furthermore, the risk of renal failure for smokers increases in accordance with the number of cigarettes smoked, compared with non-smokers.⁹ This suggests that smoking is closely associated with the onset and progression of kidney disease.

Serum creatinine is used to calculate estimated glomerular filtration rate (eGFR), a primary clinical index of renal function.¹⁰ Serum creatinine, however, depends on the muscle mass of an individual; and eGFR is usually underestimated if muscle mass increases.^{11,12} Serum cystatin C is superior to serum creatinine for the assessment of early

renal dysfunction.^{13–16} Serum cystatin C has a low molecular weight of 13 kDa and can readily pass through the glomeruli; therefore, even slight renal dysfunction can be detected by an increase in serum cystatin C.^{15–17} Moreover, serum cystatin C is produced constantly and is not affected by a decrease in muscle mass; therefore, serum cystatin C is a sensitive marker for renal function.¹⁸

The aim of the current study was to examine time-dependent changes in serum cystatin C after smoking cessation (SC). The level of cystatin C is reported to be strongly correlated with inflammatory cytokine levels, blood pressure and blood coagulation factor tendency.^{19–21} In addition, cystatin C serves as a predictive biomarker of cardiovascular events.²² This suggests that cystatin C is not only a sensitive early predictor of kidney dysfunction, but also a powerful marker of cardiovascular disease. Nothing is known, however, about the change of serum cystatin C after SC, therefore the aim of the present study was to clarify the time-dependent changes in serum cystatin C after SC.

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Table 1. Changes in Clinical Parameters From Baseline After Smoking Cessation

	n	Baseline	3 months	1 year	P-value		
					Baseline vs. 3 months	Baseline vs. 1 year	3 months vs. 1 year
BMI (kg/m ²)	78	23.1 (21.5–25.3)	23.9 (22.0–26.0)	24.4 (22.2–26.7)	<0.001*	<0.001*	0.001*
WC (cm)	76	86.6±9.9	88.2±9.9	89.4±9.5	<0.001*	<0.001*	0.021*
SBP (mmHg)	80	131±16	126±17	127±18	0.011*	0.070	0.609
DBP (mmHg)	80	76±11	75±10	75±11	0.555	0.801	0.857
HbA1c (mg/dL)	67	5.8 (5.6–6.1)	5.8 (5.5–6.3)	5.8 (5.6–6.2)	0.209	0.364	0.709
LDL-C (mg/dL)	67	115.7±30.1	120.7±32.7	115.0±30.5	0.289	>0.999	0.119
HDL-C (mg/dL)	75	53.0 (45.0–63.0)	55.0 (45.0–72.0)	57.0 (45.0–68.0)	0.002*	0.073	0.042*
TG (mg/dL)	77	137 (99–201)	149 (112–210)	150 (119–213)	0.011*	0.088	0.807
Cystatin C (mg/L)	86	0.77 (0.7–0.9)	0.76 (0.6–0.9)	0.75 (0.6–0.9)	0.045*	0.135	0.482
MCP-1 (pg/mL)	86	409 (339–475)	422 (346–484)	404 (352–449)	0.380	0.706	0.218
hsCRP (mg/dL)	86	0.75 (0.3–2.5)	0.70 (0.3–2.7)	0.66 (0.3–1.8)	0.703	0.241	0.077
CO (p.p.m.)	84	12.0 (7.0–19.5)	1.0 (1.0–2.0)	2.0 (1.0–2.0)	<0.001*	<0.001*	0.423

Data given as n, mean±SD or median (IQR). *P<0.05. BMI, body mass index; CO, carbon monoxide; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; SBP, systolic blood pressure; TG, triglyceride; WC, waist circumference.

Methods

Cohort Description

The present subjects consisted of the cohort of patients who visited the SC clinic at Kyoto Medical Center, and who had successfully stopped smoking by the 3-month visit to the SC clinic, and who had continued successful SC at 1 year.

Patients and Public Involvement

The present subjects were the same as in past studies.²³ From June 2007 to January 2014, a total of 783 outpatients attended the SC clinic at the Kyoto Medical Center. Of these, 408 patients had successfully stopped smoking after 3 months. Of these who had stopped smoking for 3 months, 101 patients visited the SC clinic at 1 year after the first visit and 3 of them had restarted smoking at this time point. Of the remaining 98 patients, 86 patients whose serum data were available for the analysis were included in this study. Age, body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), expired carbon monoxide (CO) concentration, triglyceride (TG), monocyte chemoattractant protein 1 (MCP-1), high-sensitivity C-reactive protein (hsCRP) and cystatin C were evaluated at the time of initial consultation as well as at 3 months and 1 year after SC. Informed written consent was obtained from all patients, and none of the patients was coerced into taking part in this study. The study data were anonymized with no personal identifiers. This study is part of the clinical trial entitled “Study on the Effect of Smoking Cessation on Cardiovascular Risk”. The ethics review board of the National Hospital Organization at Kyoto Medical Center approved the study protocol.

SC Clinic and Data Collection

Anti-smoking treatment was conducted according to the Standard Procedures for Anti-Smoking Treatment (originally issued in March 2006 by the Japanese Circulation Society, Japan Lung Cancer Society, and Japanese Cancer Association). Patients were examined on their first visit and at 2, 4, 8, and 12 weeks thereafter. All patients were

treated with transdermal nicotine patches or oral varenicline. On subsequent visits, maintenance of SC was evaluated, and a nurse and a doctor gave specific advice related to the continuation of SC. At the end of the 3-month anti-smoking treatment, SC status was re-evaluated. Abstinence was confirmed on expired CO concentration <7 p.p.m. and by the patient's affirmation of no smoking. Attempt to quit smoking was determined as unsuccessful when the patient stopped visiting during the treatment period or continued visiting but failed to quit smoking.

BMI was calculated as weight in kilograms divided by square of the height in meters. WC was measured at a point midway between the lowest rib and iliac crest by the study staff at each visit. SBP and DBP were measured in a sitting position after resting >5 min, using an automatic electronic sphygmomanometer (BP-103iII; Nippon Colin, Komaki, Japan). A regular-sized cuff appropriate for Japanese subjects (arm length, 17–32 cm) was used as recommended. At each visit, expired CO concentration was measured with an EC50 Micro Smokerlyzer® (Bedfont Scientific, Kent, UK), which measures end-tidal CO electrochemically with a reported precision of <2%.²⁴ On initial consultation, nicotine dependence was assessed with the Fagerström test for nicotine dependence (FTND), a globally accepted standard test to assess physical dependence to nicotine.^{25–27} FTND scores range from 0 to 10, with higher scores indicating more severe nicotine dependence. Number of cigarettes smoked per day was determined by asking the patient the following question: “On average, in the past month, how many cigarettes did you smoke per day?”

Blood Sampling

Blood tests were conducted at first consultation as a screening and at 3 months and 1 year after the first visit to assess changes in biochemistry and hematology profiles. Blood samples were collected from the antecubital vein 2–3 h after lunch. Blood samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C. Serum MCP-1 was quantified on sandwich enzyme immunoassay for human MCP-1 (QuantikineE; R&D Systems, Germany) according to the manufacturer's protocol. Serum cystatin C was

measured on a latex immunoturbidimetric assay using an automated analyzer (JCA-BM8060; JEOL, Japan).²⁸ All assays were performed by an investigator blinded to the sample sources.

Statistical Analysis

All statistical analyses were performed by a professional statistician using SPSS version 17.0 (SPSS, Chicago, IL, USA). Normality was assessed using the Shapiro-Wilk test. Clinical data before treatment were compared with those 3 months and 1 year after SC therapy using paired t-test for parametric data and Wilcoxon signed-rank test for non-parametric data (**Table 1**). Linear regression analysis was performed to identify factors related to the percent change in serum cystatin C (**Table 2**; **Supplementary Table**).

Results

In the study cohort of 86 patients, none of the patients received dialysis. Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, 7 patients; Ca²⁺-blocker, 9 patients; diuretics, 6 patients; α -blocker, 1 patient; β -blocker, 2 patients; anti-diabetic drug, 4 patients; nitrates, 2 patients. **Table 1** lists clinical data at baseline, 3 months and 1 year after SC. Briefly, there were 56 men and 30 women, with a mean age of 61 $\pm$ 13 years. Patients smoked 23 $\pm$ 11 cigarettes per day for 39 $\pm$ 11 years. Mean FTND score of the final cohort was 6.5 $\pm$ 2.3. The concentration of expired CO decreased significantly at 3 months ($P<0.001$) and at 1 year ($P<0.001$) after SC compared with baseline. BMI ($P<0.001$), WC ($P<0.001$), high-density lipoprotein cholesterol (HDL-C; $P=0.002$) and TG ($P=0.011$) increased and SBP ($P=0.011$) decreased significantly at 3 months after SC compared with baseline. At 1 year after SC, BMI ($P<0.001$), WC ($P<0.001$) and HDL-C ($P=0.042$) were further increased significantly compared with those at 3 months after SC. Serum cystatin C at baseline ranged between 0.41 and 1.53 mg/dL, and it decreased significantly ($P<0.045$) at 3 months after SC, but it remained unchanged ($P=0.482$) from 3 months to 1 year after SC (**Table 1**).

Next, linear regression analysis for the entire cohort of 86 patients was performed to assess correlations between percent change in serum cystatin C from baseline to 3 months after SC and percent changes in clinical parameters during the same time interval, with adjustment for sex (**Table 2**).

Consequently, there was a significant correlation between percent change in serum cystatin C and percent change in serum MCP-1 ($P=0.05$). Additionally, a significant inverse correlation between percent change in serum cystatin C and percent change in WC ($P=0.05$) was noted. These findings were similar to the results of the linear regression analysis conducted without adjustment for sex (**Supplementary Table**).

Discussion

Serum cystatin C is a sensitive marker of early renal function and serves as a prognostic marker of cardiovascular disease. In the present study, serum cystatin C decreased significantly soon after SC. Cystatin C is shown to strongly correlate with the elasticity of blood vessel walls in type 2 diabetic patients and may be a useful marker for asymptomatic early arteriosclerosis.²⁹ SC is also associated with

Table 2. Indicators of Percent Change in Serum Cystatin C After SC†

	Sex-adjusted		
	SE	β	P-value
% change BMI	0.436	-0.176	0.131
% change WC	0.431	-0.235	0.036*
% change SBP	0.136	-0.141	0.214
% change DBP	0.129	-0.153	0.166
% change HbA1c	0.161	-0.083	0.454
% change LDL-C	0.066	0.033	0.774
% change HDL-C	0.079	0.021	0.848
% change TG	0.021	-0.029	0.795
% change MCP-1	0.084	0.216	0.047*
% change hsCRP	0.001	-0.194	0.078
% change CO	0.082	0.071	0.532

†Sex-adjusted linear regression analysis. * $P<0.05$. β , standardized regression coefficient. Other abbreviations as in Table 1.

improved vascular endothelial function and increased cerebral blood flow.^{30–32} Taken together, the significant decrease in serum cystatin C may indicate SC-induced improvement of vascular function and increase in blood flow.

Renal function naturally decreases with age. Serum cystatin C has been reported to increase by 0.55 mg/L in a span of 25–30 years, that is, from <65 years of age to >80 years old,³³ suggesting that serum cystatin C increases by 0.02 mg/L per year as part of the natural course. In the present study, however, median serum cystatin C was unchanged or decreased from that of baseline to 1 year after the start of SC therapy, suggesting that SC suppresses a natural tendency to increase in serum cystatin C.

It is well known that body weight increases after SC.³⁴ In this study, body weight increased from baseline to 3 months after the start of SC therapy. Serum cystatin C, however, significantly decreased during this same time period. In addition, from 3 months after the start of SC therapy to 1 year after the start of SC therapy, body weight further increased while serum cystatin C was unchanged or slightly decreased. These data suggest that the adverse effects of gaining weight have been canceled by the beneficial effects of SC.

Serum MCP-1 is highly chemotactic for monocytes and macrophages; it induces macrophage infiltration of vascular lesions and subsequent formation of foam cells. In addition, induction of various cytokines by MCP-1 contributes to the exacerbation of atherosclerosis through plaque formation.^{35,36} Serum MCP-1 plays a key role in arteriosclerosis progression as well.³⁷ In the present study the percent change in serum MCP-1 was significantly correlated with the percent change in serum cystatin C during the 3 months after SC. This raises the possibility that serum cystatin C might also be associated with the atherosclerotic process.^{38,39} Serum cystatin C has been shown to be a predictor of cardiovascular disease.^{40,41} Whether serum MCP-1 and cystatin C are directly related remains unclear. The change in serum level of both factors in the current study might reflect a reduction in vascular inflammation following SC. Association of both serum MCP-1 and cystatin C with cardiovascular disease with an arteriosclerosis component indicates that a decrease in serum cystatin C following SC might reflect a reduced risk of cardiovascular events.

Body weight increase leads to increase in muscle mass and serum creatinine. Actual renal function, however, may have improved after SC because cystatin C decreased in the present study. Cystatin C has been reported to be associated with inflammation markers such as CRP,^{42,43} and in the present study the percent change of serum cystatin C from baseline to 3 months after the start of SC therapy was positively correlated with that of the inflammation marker MCP-1. Therefore, it is possible that suppression of vascular inflammation by SC decreases serum cystatin C.

This study for the first time has determined that serum cystatin C, a sensitive marker of renal dysfunction, was decreased significantly at 3 months after SC. An association of the decrease in serum cystatin C after SC with a similar change in serum MCP-1, may reflect alleviation of vascular inflammation. Therefore, significant decrease in cystatin C at 3 months after SC might indicate a decrease of cardiovascular risk.

Study Limitations

The sample size of this study was small, and the follow-up period was 1 year. A large-scale, long-term study is needed to validate the present findings. Also, the endpoint of this study was the measurement of a biomarker of renal function and cardiovascular disease. A large-scale, long-term follow-up study with cardiovascular or renal events as the primary endpoint will clarify the clinical significance of the present data.

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Disclosures

The authors declare no conflicts of interest.

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Supplementary Files

Please find supplementary file(s);
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