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Original Article

Time-dependent Changes of Atherosclerotic LDL Complexes after Smoking Cessation

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***Aim*: The α1-antitrypsin−low-density lipoprotein complex (AT-LDL) and serum amyloid A-LDL complex (SAA-LDL) are oxidatively modified LDL complexes that promote atherosclerosis. The serum levels of AT-LDL and SAA-LDL are suggested to be increased by obesity and smoking. We have previously demonstrated that larger weight gain after smoking cessation (SC) perturbs a decrease in the serum level of AT-LDL at 3 months after SC. However, changes of these atherosclerotic makers >3 months after SC are unknown. This study investigated post-SC time-dependent changes in two atherogenic lipoproteins, AT-LDL and SAA-LDL, and in the extent of abdominal obesity.**

***Methods*: In 50 outpatients who had continued SC for 1 year, we measured serum AT-LDL and SAA-LDL levels by the enzyme-linked immunosorbent assay before SC, and at 3 months and 1 year after SC.**

***Results*: Both body mass index and waist circumstance significantly increased from pre-SC to 3 months after SC and from 3 months after SC to 1 year after SC. Although the serum levels of AT-LDL and SAA-LDL were unchanged from pre-SC to 3 months after SC, these levels decreased significantly from 3 months after SC to 1 year after SC.**

***Conclusions*: The extent of abdominal obesity and levels of two atherogenic lipoproteins time-dependently change after SC. Although abdominal obesity progressively worsened after SC, the beneficial effect of non-smoking overcomes the potential vascular risks by cessation-associated obesity at 1 year after SC.**

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Key words: Smoking Cessation, Obesity, Oxidative stress, Atherosclerosis, Prevention

Introduction

Although cardiovascular risks decrease within 2 years of smoking cessation (SC), it takes >10 years for the risk levels to decline to those of non-smokers[1)]. Furthermore, it takes 20 years SC for high-sensitivity C-reactive protein (CRP) levels to significantly decrease to those of non-smoker levels[2)]. Although it is unclear why it takes such a long time for cardiovascular risks to reduce after SC, one of the reasons is thought to be the increase in oxidative stress due to weight gain after SC. It is often very typical for body weight to increase, at least for a few years, after quitting smoking[3)]. The increase in weight after SC should preferentially be <5 kg in order to sufficiently reduce the risk of cardiovascular events, compared with smokers[4)].

The α1-antitrypsin−low-density lipoprotein complex (AT-LDL) and serum amyloid A−LDL complex (SAA-LDL) are oxidatively modified LDL complexes

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that promote atherosclerosis[5-8]. Serum AT-LDL and SAA-LDL levels are closely associated with inflammation[5, 9]. The serum SAA-LDL level reflects intravascular inflammation directly and can be a more sensitive prognostic marker than CRP in patients with stable coronary artery disease[5, 10]. Serum AT-LDL and SAA-LDL levels have also been suggested to be associated with obesity and smoking[5, 11, 12]. We have previously reported that the serum level of AT-LDL, is higher in current smokers than in both former smokers and non-smokers[13]. This fact indicates that AT-LDL has a close relationship with smoking states and that it may be a useful indicator of oxidative stress in smokers. In addition, we have found a significant decrease in serum AT-LDL values among patients with a BMI increase smaller than the median. However, no significant changes in serum AT-LDL values were found in patients with a BMI increase greater than the median[9]. Thus, large weight gain after SC perturbs the decrease in AT-LDL at 3 months after cessation. However, it is unknown how the relationship between the beneficial SC effect (the decrease in cardiovascular risk) and vascular risk increase by post-SC weight gain will change over a longer period of time. This study investigated time-dependent changes for two atherosclerotic LDL complexes, AT-LDL and serum SAA-LDL, at 1 year after SC as well as the relationships of these changes with weight gain.

Aim

This study investigated post-SC time-dependent changes in two atherogenic lipoproteins, AT-LDL and SAA-LDL, and in the extent of abdominal obesity.

Methods

Participants

This is a prospective study which was conducted at the National Hospital Organization, Kyoto Medical Center during the period of September 2009 to January 2014. Patients who consulted the SC clinic to receive treatment and successfully quit smoking for 1 year were enrolled in this study. Various parameters were evaluated in these patients at the time of initial consultation and after SC (at 3 months and 1 year after the initial consultation). Informed written consent was obtained from all participants. They were not coerced into taking part in this study. The study data was anonymized with no personal identifiers. The Ethical Review Board, National Hospital Organization, Kyoto Medical Centre 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)[14]. The patients were examined on their first visit and 2, 4, 8, and 12 weeks (3 months) thereafter and treated with transdermal nicotine patches or oral varenicline. On their repeated visits, maintenance of SC was checked, and specific advice regarding the continuation of cessation was given by a nurse and a doctor. At the end of the 3-months anti-smoking treatment, whether or not SC had been maintained was evaluated. In addition, 1 year after SC treatment, the maintenance of SC was re-evaluated. Abstinence was confirmed by an expired carbon monoxide (CO) concentration of <7 parts per million (ppm) and by the patient's affirmation of no smoking. The attempt to quit smoking was judged to have been unsuccessful when the patient stopped visiting during the treatment period or continued visiting but failed to quit smoking.

BMI was calculated as the weight in kilograms divided by the square of the height in meters. The waist circumference (WC) was measured at a level midway between the lowest rib and iliac crest by the study staff at each visit[15]. Systolic blood pressures (SBP) and diastolic blood pressures (DBP) were measured in a sitting position after resting for more than 5 min using an automatic electronic sphygmomanometer (BP-103iII; Nippon Colin, Komaki, Japan)[16]. The regular-sized cuff that is appropriate for Japanese (the arm length: 17−32 cm) was used as recommended. At each visit, a nurse measured expiratory CO concentration with the EC50 Micro Smokerlyzer^{R} (Bedfont Scientific Ltd., Kent, UK), which measures the end-tidal CO electrochemically, with a reported precision of $<2\%$[17]. On initial consultation, nicotine dependence was assessed with the Fagerström Test for Nicotine Dependence (FTND), a world standard test to assess physical dependence of nicotine[18-20]. Scores range from 0 to 10, with higher scores indicating more severe nicotine dependence. The number of cigarettes smoked per day was determined by asking the smoker the following question: "On average, in the past month, how many cigarettes did you smoke per day?" The Brinkman index was calculated as the daily number of cigarettes multiplied by smoking years.

Blood Sampling

Blood tests were conducted three times at their first consultation as a screening and at 3 months and 1 year after their first visit to assess the change of the biochemical and hematological profile of patients.

Blood samples were taken from their antecubital vein 2-3 h after lunch to determine hemoglobin A1c (HbA1c), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and high sensitivity C-reactive protein (hsCRP) levels. Blood samples were immediately centrifuged at 3,000 revolutions per minute (rpm) for 10 min at 4°C. Plasma levels of HbA1c and serum levels of HDL-C, LDL-C and hsCRP were measured using an automatic analyzer (LABOSPECT 008; Hitachi High-Technologies Co., Ltd., Tokyo, Japan) with enzyme-based reagents (Kyowa Medex Co., Ltd., Tokyo, Japan)[9]. Serum levels of AT-LDL and SAA-LDL were measured using specific sandwich enzyme-linked immunosorbent assays (Ikagaku Co., Ltd., Kyoto, Japan), and the details are provided elsewhere[13]. Mixed solutions of very-low-density lipoprotein and LDL isolated from serum by polyanion precipitation[21] were measured using anti-human polyclonal antibodies against SAA or AT (DAKO Denmark A/S, Glostrup, Denmark) as the primary antibodies and anti-human apoB monoclonal antibody (SAA-LDL, clone No. 427; AT-LDL, clone No. 27) as the secondary antibody. The intra-assay coefficients of variation at low and high levels of SAA-LDL were 2.6% and 4.7%, respectively. The intra-assay coefficients of variation at low and high levels of AT-LDL were 1.8% and 1.6%, respectively. The inter-assay coefficients of variation at low and high levels of SAA-LDL were 5.0% and 6.7%, respectively. The inter-assay coefficients of variation at low and high levels of AT-LDL were 5.9% and 5.4%, respectively. The intra-assay and inter-assay coefficients of variation of SAA were 7.4% and 7.8%, respectively. The intra-assay and inter-assay coefficients of variation of AT were 4.1% and 7.0%, respectively. These assays were performed by an investigator blinded to the sources of the samples.

Statistical Analysis

All statistical analyses were performed by a professional statistician using the Statistical Package for Social Sciences (SPSS) Statistics 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). The hsCRP values were logarithmically transformed in the statistical analysis. The normality was assessed using the Shapiro–Wilk test. Clinical data were compared between all time points (pre-, 3 months, and 1 year after SC therapy) using one-way repeated measures ANOVA for parametric data or the Friedman test for non-parametric data. In cases where statistically significant differences were observed between time points using ANOVA or the Friedman test, pairwise comparisons using paired *t*-tests (parametric data) or Wilcoxon signed rank test (non-parametric data) were conducted to identify specific differences between time points.

Results

Participants

Among the 99 patients who successfully quit smoking, 49 patients were excluded because of the lack of blood sampling data. Therefore, we analyzed the findings of 50 patients [31 males and 19 females, aged between 35 and 80 years, (mean 61 ± 13 years)]. During the SC program, four patients (8%) received anti-hypertensive agents, two (4%) received statins and two (4%) received medications for diabetes mellitus. Various parameters were evaluated in these patients at the time of initial consultation and at 3 months and 1 year after the initial consultation. The average FTND score of participants was 6.2 ± 2.5, the daily number of cigarettes smoked was 22.0 ± 4.9, and the Brinkman index was 870 ± 20.

Expired CO concentrations significantly decreased from the initial visit to 3 months after SC (from 16.2 to 1.4, $P<0.0001$).

Time-dependent Changes of AT-LDL and SAA-LDL and Metabolic Parameters

Table 1 compares data collected at the time of first examination and 3 months and 1 year after the first examination. Compared with their baseline values, 3 months after SC, patients experienced a significant increase in BMI (from 22.8 to 23.0 kg/m^2, $P=0.046$), WC (from 85.9 to 87.6 cm, $P<0.001$), HDL-C (from 55.3 to 58.4 mg/dL, $P=0.047$), and TG (from 148 to 187 mg/dL, $P=0.010$). Serum AT-LDL and SAA-LDL levels did not change significantly 3 months after cessation (AT-LDL: from 1.8 to 1.8 μg/mL, SAA-LDL: from 9.0 to 9.0 μg/mL).

BMI and WC further increased from 3 months to 1 year after SC (BMI: from 23.0 to 23.8 kg/m^2, $P=0.028$; WC: from 87.6 to 89.2 cm, $P=0.019$). In contrast, both AT-LDL and SAA-LDL levels significantly decreased from 3 months to 1 year (AT-LDL: from 1.8 to 1.5 μg/mL, $P=0.006$; SAA-LDL: from 9.0 to 7.5 μg/mL, $P=0.008$). HDL-C and TG did not change significantly from 3 months to 1 year. No significant change was found in LDL-C level throughout the 1 year, from baseline to 1 year after SC.

The serum hsCRP levels demonstrated no significant differences between baseline and 3 months after SC and between 3 months and 1 year after SC.

Discussion

Smoking is the largest preventable cause of death and disease worldwide, and SC is one of the most

Table 1. Patient data before and after successful smoking cessation (N = 50)

	Baseline	After 3 months	After 1 year	*P*-value		*P*-value			
				Overall		0-3M	3M-1Y	0M-1Y	
BMI (kg/m^2)	22.8 [21.0, 23.4]	23.0 [21.2, 24.2]	23.8 [21.9, 25.0]	**0.001**	a	**0.046**	**0.028**	**0.013**	c
WC (cm)	85.9 ± 9.0	87.6 ± 8.9	89.2 ± 8.4	**<0.001**	b	**<0.001**	**0.019**	**<0.001**	d
SBP (mmHg)	131 ± 17	128 ± 18	129.5 ± 18.7	0.244	b	0.085	0.434	0.386	d
DBP (mmHg)	76 ± 10	75 ± 10	76 ± 11	0.434	b	0.117	0.329	0.893	d
HbA1c (%)	5.8 [5.6, 6.6]	5.9 [5.6, 6.7]	5.9 [5.6, 7.0]	0.206	a	0.361	0.188	0.052	c
LDL-C (mg/dl)	112 ± 29	119 ± 36	114 ± 34	0.187	b	0.093	0.166	0.595	d
HDL-C (mg/dl)	55.3 ± 14.2	58.4 ± 17.2	56.3 ± 16.4	0.095	b	**0.047**	0.106	0.508	d
TG (mg/dl)	148 ± 64	187 ± 97	178 ± 93	**0.013**	b	**0.010**	0.547	**0.022**	d
AT-LDL (μg/ml)	1.8 [1.4, 2.2]	1.8 [1.4, 2.1]	1.5 [1.1, 1.9]	**<0.001**	a	0.177	**0.006**	**<0.001**	c
SAA-LDL (μg/ml)	9.0 [6.0, 13.0]	9.0 [6.0, 12.0]	7.5 [4.0, 10.0]	**0.010**	a	0.280	**0.008**	**0.014**	c
hsCRP (mg/dL)	0.6 [0.3, 2.0]	1.0 [0.3, 2.7]	1.1 [0.4, 1.9]	0.408	a	0.357	0.364	0.365	c

Data are presented as the mean ± standard deviation or median [interquartile range]
p-value: a, Friedman Test; b, One-way repeated measures ANOVA; c, Wilcoxon signed rank test; d, paired *t*-test
0-3M, baseline vs 3 months; 3M-1Y, 3 months vs 1 year; 0M-1Y, baseline vs 1 year; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressures; DBP, diastolic blood pressures; HbA1c, hemoglobin A1c; ; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SAA-LDL, serum amyloid A-LDL; AT-LDL, α1-antitrypsin−low-density lipoprotein; hsCRP; high sensitivity C-reactive protein

effective ways to reduce the likelihood of diseases such as stroke and cardiovascular diseases[22]. However, body weight gain and abdominal obesity generally occur after quitting smoking[23, 24]. The increase in oxidative stress from obesity results in an increase in various inflammatory markers that are linked with an increased cardiovascular risk[25]. Therefore, it is hypothesized that body weight gain after SC could perturb the merits of SC[9]. However, the relationship between weight gain after SC and cardiovascular risk is intricately intertwined, and how the relationship will change over time is not well known.

Serum AT-LDL and SAA-LDL levels have been postulated to be increased by obesity and smoking[5, 11, 12], and the elevation of these markers is reported to predict prognosis in patients with stable coronary artery disease[5]. The serum level of AT-LDL, an oxidatively modified LDL that promotes atherosclerosis, accurately reflects current smoking states[6-8]. We have previously reported that body weight gain may attenuate the decrease (improvement) in AT-LDL at 3 months after SC[9]. This study followed up patients for a longer period (1 year). As a result, at 3 months after SC, BMI and WC increased significantly, and the decrease (improvement) in serum AT-LDL and SAA-LDL levels was unclear. However, during the period of 3 months to 1 year after SC, a further increase was observed in both BMI and WC; nevertheless, serum AT-LDL and SAA-LDL levels significantly decreased. These results indicate that cardiovascular risks by weight gain may outweigh the beneficial effects of SC during an early period after cessation, e.g., during 3 months. In contrast, at 1 year after SC, the beneficial effect of SC certainly overcomes the potential vascular risks through cessation-associated obesity. The prevention of abdominal obesity may lead to a further decrease in cardiovascular risk. However, the results of this study suggest that the benefits of SC increase over time and outweigh the risks associated with body weight at least at the time of 1 year after SC. Thus, the continuation of SC is very important.

No significant differences in the serum hsCRP levels were observed between baseline and 3 months after SC or between 3 months and 1 year after SC. The CRP levels reportedly gradually reduced over a 5-year period after cessation of smoking[25]. Thus, inflammatory markers may slowly improve over a long period. The number of cardiovascular events is reportedly reduced by continuation of SC for 4 years despite increases in body weight[4]. We believe further studies evaluating the association between changes in inflammatory markers and weight gain after SC in individuals who maintain SC are required in the future.

There are a few limitations to this study. First, the focus of this study was AT-LDL and SAA-LDL, modified LDL complexes that promotes atherosclerosis. However, no investigation was made to examine the endpoints of cardiovascular diseases per se. Second, we employed blood samples obtained 2-3 h after a meal. Serum levels of most markers of lipids and adipocytokines can be affected by a meal. However, in our previous report employing similar blood samples,

we showed that the AT-LDL level sensitively reflected smoking states. Third, this study included only 50 patients. In the future, it will be necessary to increase the number of participants and to conduct long-term observations on cardiovascular events.

In addition, the baseline values of metabolic parameters, such as BMI, TG, and oxidized LDL, in this study were better than those in our previous study[9]. This difference may be attributable to variations in the patient background between the two studies because the participants in this study included those who had successfully maintained SC for >1 year. That is, all patients who completed 3 months of SC program were asked to revisit our SC clinic for medical examinations at 1 year after the initial visit. Unfortunately, a proportion of patients did not return to our clinic. Patients who revisited the SC clinic at 1 year after their initial examination may be considered to have good self-management skills. Differences in patient background may explain the varying results of the two studies.

Conclusions

BMI and the levels of two atherogenic lipoproteins, AT-LDL and SAA-LDL, change time-dependently after SC. BMI progressively increases until 1 year after quitting smoking. In contrast, a decrease in AT-LDL and SAA-LDL levels is obvious at 1 year after SC. These findings suggest that the beneficial effect of SC overcomes potential cardiovascular risks by cessation-associated obesity, 1 year after SC.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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