Dose-dependent Effects of Cigarette Smoke on Blood Biomarkers in Healthy Japanese Volunteers: Observations from Smoking and Non-smoking

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Cigarette smoking is one of the avoidable causes for diseases such as lung cancers, but biomarkers for health condition by cigarette cessation are expected. Biochemical data in plasma from 3053 Japanese in Tokyo were investigated who underwent an annual health check-up from 2003 to 2007 with amount and duration of cigarette smoking. Dose-dependent effects of cigarette smoking as indicated by Brinkman index (daily number of cigarettes × years) were observed on increased circulating white blood cell counts in men and women, red blood cell in women, and plasma triglycerides concentrations in men. Higher levels of hemoglobin and hematocrit in females were seen with ≤ 400 of Brinkman index, but these increases were observed in the male group with ≤ 800 of index. Decreased high-density lipoprotein cholesterol levels in blood were seen in men (> 800 of index). Decreased low-density lipoprotein cholesterol levels and enhanced glucose levels were only seen in a pooled heavy smoker group. Since increased ratios for the white blood cell counts in men and women by cigarette smoking were high among the markers tested in this study, the recovery of these parameters by cigarette cessation was investigated. The white blood cell counts were recovered time-dependently after quitting smoking for 1–3 years as evident with 38 other former smokers. These results suggest white blood cell counts would be sensitive biomarkers for smoking exposures and time-dependent recovery in hearty volunteers in monitoring and educating cigarette smoking and cessation.

Key words —— Brinkman index, white cell count, smoking cessation

INTRODUCTION

Smoking is considered to cause cancer, stroke, and heart disease and also have close relationship with gastric ulcer, periodontal disease, sudden infant death syndrome, and metabolic syndrome.[1–5] Cigarette smoking is one of the greatest avoidable causes of premature death and disability in the world.[6] Because cigarette smoking has become an important public health challenge in the world,[7] helping smokers to stop smoking is one of the most cost effective interventions available in clinical practice. Promoting smoking cessation should therefore be a major priority in all countries and for all health professionals in all clinical settings. However, it has been reported that 39.9% of Japanese men and 10.0% of women are current smokers in 2007 (http://www.health-net.or.jp/tobacco/product/pd100000.html).[8] Nicotine gum or patch has been developed for a cigarette cessation medicine,[9] but most United States smokers do not use behavioral or pharmacologic treatments for quitting attempts.[10] Whether a smoker succeeds in stopping smoking depends on the balance between that individual's motivation to stop smoking and his or her degree of dependence on cigarettes.[11] Dependence is especially important in smokers who do want to stop smoking, as it influences the choice of intervention. Although the white blood cell counts have been reported to increase by smoking,[12,13] some familiar biomarkers for changes of health condition by cigarette cessation is generally expected to be developed for

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healthy volunteers to continue quitting smoking in a tailoring smoking-cassation programs.[14)]

The aim of this study was to clarify the link between changes in blood biomarkers and cigarette smoking, with detailed information for smoking index, in a large number of healthy volunteers in a Japanese population. Recovery in increased white blood cell counts by cigarette cessation was also significantly indicated in an analysis of 38 volunteers in Japan.

MATERIALS AND METHODS

Subjects —— We used data of 3053 Japanese (817 men and 2236 women), aged 21–95 years, who underwent an annual health check-up from 2003 to 2007 at Kume Clinic, Fuchu, Tokyo, Japan, with informed consents. Since regular health check-ups are legally mandated, the majority of these subjects from Fuchu Citizens did not have serious health problems. The study was approved by the local ethical committee. Subject age and cigarette smoking outcome data were collected in a structured interview concerning the amount and the duration of smoking, and concerning how long since they had stopped smoking at the time of the general health check when they were former smokers. Out of smokers, 38 subjects (22 men and 16 women) who quit smoking were followed up during 1–3 years. The analysis was conducted in 2008.

Laboratory Tests —— Blood samples were taken from our subjects after an overnight fasting. Blood cell counts (white blood cell, red blood cell, hemoglobin, and hematocrit) and biochemical data [triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and glucose] were determined by Mitsubishi Chemical BCL (Tokyo, Japan).

Statistical Analysis —— The mean and median Brinkman index from all smokers in this study were 586 ± 413 (S.D.) and 500, respectively. In terms of smoking habits, a unit of cigarette pack-year has also been used for dealing with the amount of cigarette smoking.[4)] We adapted one-pack for 20 years as a cut-off value for smoking amount in this study. Therefore the smoking groups were divided into three groups, namely ≤ 400, the double units of ≤ 800, and more (> 800) in the present analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests using computer software (Prism ver. 5, GraphPad Software, La Jolla, CA, U.S.A.). A value of $p < 0.05$ was taken to be statistically significant.

RESULTS

Effects of cigarette smoking on the blood levels of cells, hemoglobin, lipids, and glucose were investigated in a total of 3015 healthy volunteers (Table 1). The biomarkers in current male and female smokers were compared with non-smokers. White blood cell counts per μl in both male and female smokers were 1.2-fold higher than those in the non-smokers. Similarly hemoglobin and hematocrit values in smokers were 1.1-fold high than those in non-smokers. Red blood cell counts in female smokers, but not in men, were significantly higher than those in non-smokers. In pooled men and women, 1.1-fold high values of red blood cell counts in smokers were observed.

On the other hand, concentration of triglycerides in male smokers was 1.4-fold higher than those in non-smokers, but this increase was not seen in women. Concentrations of HDL cholesterol and LDL cholesterol were slightly decreased in a smoker group of pooled men and women. Levels of cholesterol or glucose were not apparently affected in the present total smoker population.

The mean and median Brinkman index from all the smokers in this study were 586 ± 413 (S.D.) and 500, respectively. The range of mean ± one S.D. was from 173 to 999 in this study group. In terms of smoking habits, a unit of cigarette pack-year has been also used for dealing with the amount of cigarette smoking; 38.3 pack-years was a 50th percentile of pack-years value for a Japanese population.[4)] From the median of 500 daily smoking numbers × years in this study, we adapted one-pack for 20 years, namely 400, as a cut-off value for subgrouping dependent on historical smoking amount in this study. Therefore the groups were divided into four groups, namely non-smokers, smokers with a single smoking unit of ≤ 400, double units of ≤ 800, and more (> 800) in the present analysis (Table 2).

Dose-dependent effects of cigarette smoking were observed on increased white blood counts in men and women and red blood cell in women (Table 2). The lowest smoking index groups of ≤ 400 gave significant increases in white blood counts in men and women and red blood cell in women as well as triglycerides concentrations in

Table 1. Effects of Cigarette Smoking on the Blood Levels of Cells, Hemoglobin, Lipids, and Glucose from Healthy Volunteers (n = 3015) in Tokyo

Marker	Non-smoker			Smoker		
	Total	Male	Female	Total	Male	Female
	n = 2297	n = 360	n = 1937	n = 718	n = 435	n = 283
White blood cell (μl^{-1})	5324 ± 1457	5708 ± 2244	5253 ± 1271	6496 ± 1688	6697 ± 1701	6187 ± 1622
	(100)	(100)	(100)	(122)**	(117)**	(118)**
Red blood cell (μl^{-1})	425 ± 40	448 ± 57	421 ± 34	445 ± 41	453 ± 45	433 ± 43
	(100)	(100)	(100)	(105)**	(101)	(103)**
Hemoglobin (g/dl)	13.0 ± 1.3	14.0 ± 1.7	12.8 ± 1.1	14.1 ± 1.4	14.5 ± 1.3	13.4 ± 1.7
	(100)	(100)	(100)	(109)**	(104)**	(105)**
Hematocrit (%)	40.0 ± 3.6	42.5 ± 4.7	39.5 ± 3.2	43.0 ± 4.7	44.1 ± 3.7	41.2 ± 3.3
	(100)	(100)	(100)	(107)**	(104)**	(104)**
Triglycerides (mg/dl)	112 ± 64	113 ± 65	111 ± 64	142 ± 93	156 ± 105	120 ± 65
	(100)	(100)	(100)	(127)**	(138)**	(108)
Cholesterol (mg/dl)	211 ± 34	196 ± 31	214 ± 33	207 ± 37	200 ± 36	219 ± 35
	(100)	(100)	(100)	(98)	(102)	(102)
HDL cholesterol (mg/dl)	62 ± 15	58 ± 16	63 ± 15	57 ± 17	52 ± 14	65 ± 18
	(100)	(100)	(100)	(91)**	(90)**	(103)
LDL cholesterol (mg/dl)	126 ± 31	116 ± 28	128 ± 31	122 ± 34	117 ± 34	130 ± 34
	(100)	(100)	(100)	(96)*	(101)	(101)
Glucose (mg/dl)	98 ± 20	106 ± 30	97 ± 17	101 ± 37	106 ± 33	94 ± 14
	(100)	(100)	(100)	(103)	(100)	(97)

Biochemical data in plasma from Japanese in Tokyo were investigated who underwent an annual health check-up from 2003 to 2007 with amount and duration of cigarette smoking. Data are mean and S.D. Numbers in parentheses indicate % of control (non-smokers). $^{*}p < 0.05$ and $^{**}p < 0.01$, significantly different from the control group.

men. Significantly higher levels of hemoglobin and hematocrit in females were seen in the group with ≤ 400 of Brinkman smoking index, but these increases were also observed in male smokers with ≤ 800 of Brinkman index. Heavy smoking also decreased HDL cholesterol levels in men. Decreased cholesterol and LDL cholesterol levels and enhanced glucose levels in blood were seen in heavy smokers in a pooled total group.

Increased ratios for the white blood cell counts and triglyceride concentrations by cigarette smoking were high among the markers tested in this study. The recovery of these parameters by cigarette cessation was investigated in the former smokers. The white cell counts among these parameters were clearly reduced time-dependently after quitting cigarette smoking for 1–3 years as evident with similar blood tests from 38 other male and female subjects who were former smokers (Fig. 1A). This decrease in white blood cells dependent on the observed years was significant by one-way ANOVA analysis ($p < 0.05$). Dunnet's multiple comparison tests revealed decreases after 2 years showed lower values compared with the control values (when they had been smokers). On the other hand, triglyceride levels did not change significantly in the preset conditions (Fig. 1B). The other makers mentioned above did not change under the present condition (results not shown).

DISCUSSION

Cigarette smoking has become an important public health problem in the world. We investigated the data concerning circulating blood cell counts and several biochemical data in plasma from approximately 3000 Japanese subjects who underwent an annual health check-up with cigarette smoking outcome collected in a structured interview concerning the amount and the duration of smoking (Table 1). To clarify the link between changes in blood biomarkers and cigarette smoking, dose-dependent effects of cigarette smoking were analyzed using Brinkman smoking index (daily number of cigarettes × years). White blood cell counts were the best biomarker among the parameters tested because both men and women showed significant in-

Table 2. Dose-dependent Effects of Cigarette Smoking on the Blood Levels of Cells, Hemoglobin, Lipids, and Glucose from Healthy Volunteers

Marker	Group	Brinkman smoking index (daily number of cigarettes x year)							
		0		≤ 400		≤ 800		> 800	
Sample number	Total	2297		288		276		154	
	Male	360		118		196		121	
	Female	1937		170		80		33	
White blood cell (μl^{-1})	Total	5324	(100)	6043	(113)**	6636	(125)**	7092	(133)**
	Male	5708	(100)	6117	(114)**	6789	(119)**	7114	(133)**
	Female	5253	(100)	5992	(107)**	6260	(119)**	7012	(125)**
Red blood cell (μl^{-1})	Total	424	(100)	438	(103)**	450	(106)**	450	(106)**
	Male	448	(100)	448	(100)	457	(102)	451	(101)
	Female	421	(100)	432	(103)**	432	(103)*	445	(106)**
Hemoglobin (g/dl)	Total	13.0	(100)	13.7	(105)**	14.3	(110)**	14.5	(111)**
	Male	14.0	(100)	14.3	(102)	14.7	(105)**	14.6	(104)**
	Female	12.8	(100)	13.3	(104)**	13.4	(104)**	14.0	(109)**
Hematocrit (%)	Total	40.0	(100)	41.9	(105)**	43.6	(109)**	43.9	(110)**
	Male	42.5	(100)	43.4	(102)	44.6	(105)**	44.2	(104)**
	Female	39.5	(100)	40.9	(103)**	41.2	(104)**	42.8	(108)**
Triglycerides (mg/dl)	Total	112	(100)	133	(109)**	153	(137)**	140	(125)**
	Male	113	(100)	155	(137)**	166	(147)**	141	(125)**
	Female	111	(100)	117	(105)	120	(107)	138	(124)*
Cholesterol (mg/dl)	Total	211	(100)	214	(101)	207	(98)	195	(92)**
	Male	196	(100)	206	(105)	201	(103)	190	(97)
	Female	214	(100)	219	(102)	220	(103)	212	(99)
HDL cholesterol (mg/dl)	Total	62	(100)	62	(99)	55	(88)**	51	(82)**
	Male	58	(100)	56	(97)	51	(87)	50	(86)**
	Female	63	(100)	66	(104)	66	(104)	58	(92)
LDL cholesterol (mg/dl)	Total	126	(100)	126	(100)	122	(96)	115	(91)**
	Male	116	(100)	119	(103)	118	(102)	112	(97)
	Female	128	(100)	130	(101)	131	(102)	127	(99)
Glucose (mg/dl)	Total	98	(100)	100	(102)	99	(101)	107	(109)**
	Male	106	(100)	108	(102)	102	(96)	110	(104)
	Female	97	(100)	95	(98)	92	(95)	98	(101)

Biochemical data in plasma from Japanese in Tokyo were investigated from 2003 to 2007. Numbers in parentheses indicate % of control (non-smokers). * $p < 0.05$ and ** $p < 0.01$, one-way ANOVA followed by Dunnet's multiple comparison tests.

creased numbers from the low exposure (≤ 400 of Brinkman index, Table 1). For male smokers, plasma triglycerides determination was also one of the sensitive and detectable biomarkers to cigarette smoking. On the other hand, women were sensitive to change of red blood cell levels caused by cigarette smoking, although the rate of increase was small. The reason for observed sex difference in the responses of blood markers are not known at present. However, for the individual consulting for cigarette cessation, some of these parameters may be used as suitable markers for exposure of cigarette smoking.

The white cell counts among these parameters were clearly recovered time-dependently after quitting cigarette smoking for 1–3 years as evident with the similar blood tests from 38 other subjects who were former smokers. In the present limited numbers of the former smokers, the decreases in triglycerides were not shown by quitting smoking (Fig. 1). Circulating white cell count has been proposed as an indicator of inflammation derived from cigarette smoking.[15] In our preliminary experiments, nicotine in patch formula did not affect the circulating white cell counts. Taken together these findings, levels of reactive oxygen species produced by cigarette smoking or quitting may be a determinant factor for inducing and reducing blood white cell counts. Further mechanistic study is necessary; however, the present results of white cell count as an indicator for smoking cessation in healthy volunteers are of interest.

Recently cigarette smoking has been also re-

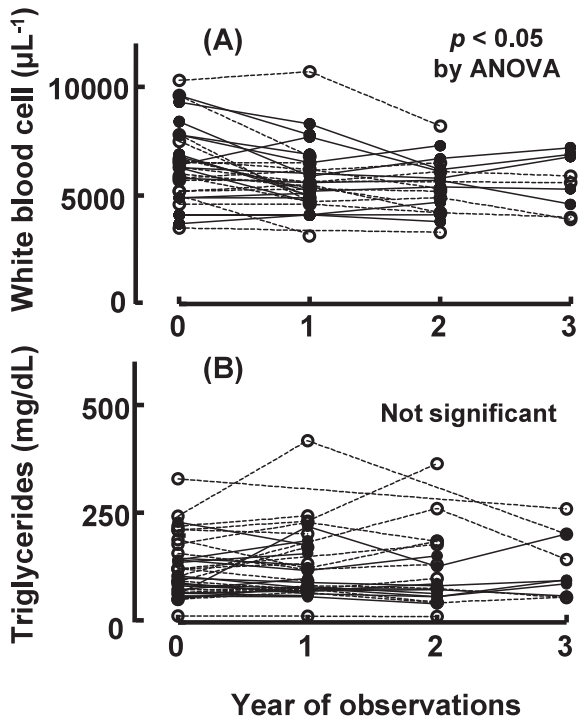

Fig. 1. Changes of White Blood Cell Counts (A) and Concentrations of Triglycerides (B) by Cigarette Cessation in 38 Former Smokers

Male (n = 22) and female (n = 16) subjects shown in solid and dashed lines, respectively, were investigated after cigarette cessation for 1–3 years.

ported as one of the determinant factors for so-called metabolic syndromes.[5, 8, 13] Relationship between blood sugar and smoking has been focused in other disease. There have been inconsistent reports about cholesterol levels in smoking change.[16, 17] In this study, decrease in cholesterol and increase in blood sugar levels were seen in the group with heavy smokers (> 800 Brinkman index) in the combined male and female group, suggesting that this may be late responses to accumulated cigarette smoke exposure. In our follow-up study after quitting smoking, the participants are limited. Larger scale and longer period survey need to be done in future.

In summary, the results suggest that white blood cells would be one of the most sensitive biomarkers for smoking exposures and time-dependent recovery in hearty volunteers. This information would be valuable for understanding the biological effects of smoking and for education of cigarette cessation as useful valid biomarkers for former smokers.

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