RESEARCH COMMUNICATION

Lack of Association between Serum Transforming Growth Factor-beta 1 and Cancer Mortality Risk in a Nested Case-control Study in Japan

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Abstract

We examined the potential role of serum TGF-beta 1 levels to predict cancer mortality risk in a nested case-control study within a large prospective cohort of middle-aged and elderly Japanese subjects. The cases were 893 persons who provided blood samples at baseline and subsequently died of cancer from all sites during the follow-up period. A total of 2,824 subjects were selected from the main study as controls, matched with the cases for sex, age and study area. Serum TGF-beta 1 levels were measured using a quantitative sandwich enzyme immunoassay. The odds ratios and 95% confidence intervals for each quartile were calculated using a conditional logistic regression model. Mean serum TGF-beta 1 levels were approximately 36 ng/ml in both cases and controls, with no significant difference . Overall, serum TGF- beta 1 levels were not associated with total cancer mortality after adjustment for potential confounding factors like age, body mass index or cigarette smoking. Serum TGF-beta 1 levels may thus not be associated with cancer mortality risk in apparently health individuals.

Key Words: TGF-beta1 - cancer mortality - nested case-control study - no association

Asian Pacific J Cancer Prev, **10**, 273-278

Introduction

Cancer is a complex, heterogeneous disease and since 1981 has been the leading cause of mortality in Japan (Yamaguchi, 2000). Despite recent improvements in cancer diagnosis and therapy, early detection of high-risk individuals and prevention remain the major means of easing the health burden associated with cancer.

The promise of new, molecular biomarkers for early detection of cancer and risk prediction has generated considerable scientific interest (Sidransky, 2002). Numerous biomarkers have been suggested as early markers of cancer, with the feasibility and performance of some of these biomarkers having been examined in relatively small case-control studies (Etzioni et al., 2002). One such biomarker that has been studied extensively is transforming growth factor-beta (TGF-β). TGF-β exerts a wide range of biological effects on various cell types, which include regulation of cell growth, cell differentiation, matrix production, apoptosis and angiogenesis (Blobe et al., 2000). There is evidence that mutations in genes coding for TGF-β, its receptors and intracellular signaling are important mechanisms in the development of cancer (Markowitz et al., 1995; Hahn et al., 1996; Bierie and Moses, 2006).

Although TGF-β is a growth inhibitor of normal epithelial cells, in general, cancer cells secrete larger amounts than their normal counterparts. It has been suggested that increased cell growth due to decreased TGF-b growth inhibition may contribute to cancer development (Siegel and Massagué, 2003). TGF-β has three isoforms, of which TGF-β1 is the predominant isoform in humans and most frequently up-regulated in tumor cells (Derynck et al., 2001). Change in TGF-b1 levels can be detected in plasma or serum with elevated levels having been reported in patients with invasive prostate, breast or colorectal cancer (Ivanovic et al., 1995; Sheen-Chen et al., 2001; Shim et al., 1999). It also has been shown that circulating TGF-β1 levels correlate with tumor stage at several cancer sites (Shim et al.,1999; Ivanovi_ et al., 1995), making it a potential predictor of cancer prognosis. However, it remains unclear whether serum TGF-β1 predicts cancer risk in apparently health individuals.

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Given the well-documented role of TGF-β 1 in carcinogenesis, we carried out a nested case-control study to investigate the potential of serum TGF-β1 levels to predict cancer mortality risk. The study population was obtained from a large prospective cohort study of middle-aged and elderly Japanese subjects.

Materials and Methods

Study population

We conducted a nested case-control study within the Japanese Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study). The JACC study is an ongoing prospective cohort study of risk factors for cancer in participants recruited from 45 areas throughout Japan, the details of which have been reported elsewhere (Tamakoshi et al., 2005). Briefly, between 1988 and 1990, 46,465 men and 64,327 women, aged 40-79 years, were enrolled following their response to a questionnaire, which also included consent to participate in the study. The questionnaire included questions on demographic characteristics, medical history and lifestyle factors. In addition, 39,242 people (35% of the participants in the cohort) provided a blood sample for analysis. No significant differences were noted in characteristics such as age, body mass index (BMI), education level and medical history between those who donated the blood sample and those who did not. Sera were separated from the blood samples as soon as possible after blood withdrawal and then stored at -80°C until analysis.

Data on all-cause mortality to December 31, 1999 were collected on all participants in the cohort. During this follow-up period, vital statistics such as the cause and date of death were obtained by reviewing death certificates in each area. The underlying causes of death were coded according to the International Classification of Disease, 10th Revision. Participants who had moved out of their study areas were also identified by reviewing population-register sheets. The Ethics Committee of Nagoya University School of Medicine approved the study.

Case subjects in the present study were defined as those in the JACC Study who were free of morbidity at baseline, had provided a blood sample and subsequently died of cancer at any site during the follow-up period. Control subjects were selected from the remaining participants in the cohort who remained disease-free at the time the cases had died. Controls were matched to the cases for sex, age and study area at a ratio of 3:1 or 4:1. Subjects who had a cancer diagnosis before the start of follow-up were excluded from the analyses.

Of the 12,192 deaths from all causes documented during follow-up until December 31, 1999, 4,538 were from cancer. We selected 893 of these cancer death subjects as the case group and 2,824 subjects as the control group, on the basis of criteria detailed above.

Biochemical assay of sera

Serum TGF-b1 levels were measured by a quantitative sandwich enzyme immunoassay technique using a Quantikine human TGF-β1 kit, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). All samples were assayed at a single laboratory (SRL Inc., Hachioji) with the laboratory technician being blinded to the case and control status of the subjects. The intra-assay coefficient of variation for quality control samples ranged from 2.67 to 6.79% (Ito et al.,2005).

Statistical Analysis

Since the distribution of serum TGF- β1 levels approximated a normal distribution, we used the original measured values in all the analyses. TGF-b1 levels were grouped into quartiles according to the distribution of the control data. We compared baseline characteristics between cases and controls using general linear models for continuous variables and chi-square tests for categorical variables. A conditional logistic regression model was used to calculate age-adjusted, multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for cancer death in each serum TGF-b1 quartile, using the lowest category as the reference group. The multivariate analyses were adjusted for age, month of blood collection, body mass index (BMI), cigarette smoking and alcohol consumption. Linear tests for trend were performed using the median TGF-β1 value within each quartile as an ordinal variable.

Stratified analyses were carried out to determine whether the association between serum TGF-β1 levels and the risk of cancer mortality was modified by factors such as age, BMI and smoking. To examine the influence of undiagnosed cancer at baseline on the association between serum TGF-β1 levels and cancer risk, we conducted unconditional regression model analyses excluding subjects who died during the first 3 years of follow-up. All the statistical tests were two-tailed, and a P value <0.05 was considered statistically significant. All analyses were performed using SAS Release 9.1 (SAS Institute Inc, Cary, NC).

Results

The average duration between blood collection and cancer death was 5.2±2.4 years. The baseline characteristics of the study subjects are presented in Table 1. There were more current smokers and current drinkers in the control group than in the case group (p<0.01). Serum TGF-β1 levels ranged from 7.07-69.1 ng/ml in the cases, and from 5.90-73.8 ng/ml in the controls, with no statistically significant difference (p=0.90).

Table 1. Baseline Characteristics of Cases and Controls

Characteristics	Cases	Controls	P
Age	64.6 ± 8.2	64.5 ± 7.9	MF
Body mass index, kg/m2	22.7 ± 3.1	22.7 ± 3.0	0.52
Smoking status (%)			<0.01
Never	41.3	49.1	
Past	16.9	18.1	
Current	35.5	26.4	
Unknown	6.3	6.4	
Current drinkers (%)	44.6	47.8	<0.01
Serum TGF-b1 (ng/ml)	35.7 ± 8.6	36.0 ± 8.4	0.90

Values are mean±standard deviation; MF, Matching factor

Table 2. Association between Serum TGF-β1 Levels and Risk of Death from All Cancers

	Quartile1 (<30.3)	Quartile2 (30.3-35.7)	Quartile3 (35.8-41.3)	Quartile4 (>41.3)	P for trend
Cases TGF-β1 Concentrations	229	220	214	220	
Controls TGF-β1 Concentrations	692	703	708	703	
Age-adjusted OR (95%CI)	1.00	0.89 (0.71-1.11)	0.84 (0.66-1.05)	0.88 (0.69-1.11)	0.23
Multivariable OR (95%CI)*	1.00	0.89 (0.70-1.12)	0.82 (0.65-1.04)	0.86 (0.68-1.10)	0.20

OR: odds ratio ; CI: confidence interval; *adjusted for age, month of blood collection, body mass index, cigarette smoking and alcohol drinking

Table 2 shows the ORs and 95% CIs for cancer risk in each quartile of serum TGF-β 1 levels. Overall, serum levels of TGF-β1 were not associated with total cancer mortality after adjustment for age, BMI and other potential confounding factors. We found no significant trend in risk with increasing TGF-βb1 levels.

The results of subgroup analyses stratified by age, BMI and smoking status are shown in Table 3. Overall, we found no significant association between serum TGF-β 1 concentrations and cancer risk in all the subgroups analyzed. We also found no association between serum TGF-β1 levels in the analysis that excluded all deaths from cancer during the first 3 years of follow-up (data not shown).

Discussion

In this nested case-control study, we observed no significant association between serum TGF-β1 levels and risk of death from cancer at all sites. As blood samples in our study were collected, on average, 5 years before cancer diagnosis or death, serum TGF-β1 may not have been a good predictor of cancer mortality in apparently healthy individuals.

The value of circulating TGF-β1 level as a prognostic marker for cancer remains controversial, despite higher levels being reported in cancer patients than in healthy individuals, and elevated levels correlating significantly with prognosis in several cancer sites such as the breast, colon and rectum (Ivanovic et al., 1995; Shim et al.,1999). Elevated TGF-β1 levels may have been a result of cancer development in the case-control studies that showed a positive association between serum TGF-β1 levels and cancer risk. This limitation, which is inherent in retrospective case-control studies, may lead to inverse causation and hamper interpretation of the study results. Moreover, the contradictory findings may be due to the considerable variation in measuring plasma or serum TGF-β1 levels. Batch variation and storage and freeze-thawing effects on the biological samples are three important factors that may differ between case-control studies (Rundle et al.,2006).

The prospective design of our study allowed us to explore the potential effect of serum TGF-β1 levels on the risk of subsequent cancer death in apparently healthy individuals. Our results indicated that serum TGF- β1 levels may not predict the risk of cancer death in these individuals, with the association also not being modified

Table 3. Association between Serum TGF-β1 Levels and Risk of Death from All Cancers in Subgroups

	Quartile1 (<30.3)	Quartile2 (30.3-35.7)	Quartile3 (35.8-41.3)	Quartile4 (>41.3)	P for trend
Age 40-59 years					
Cases/Controls	41/133	52/154	59/193	65/207	
Age-adjusted OR (95%CI)	1.00	1.09 (0.68-1.75)	0.99 (0.63-1.56)	1.01 (0.64-1.58)	0.90
Multivariable OR (95%CI)	1.00	0.97 (0.56-1.69)	1.03 (0.61-1.74)	0.97 (0.57-1.66)	0.96
Age 60-79 years					
Cases/Controls	188/559	168/599	155/515	155/496	
Age-adjusted OR (95%CI)	1.00	0.92 (0.72-1.16)	0.90 (0.71-1.15)	0.94 (0.73-1.20)	0.54
Multivariable OR (95%CI)	1.00	0.84 (0.64-1.09)	0.87 (0.66-1.14)	0.89 (0.68-1.17)	0.41
BMI <25					
Cases/Controls	176/551	173/551	163/536	172/514	
Age-adjusted OR (95%CI)	1.00	0.99 (0.77-1.26)	0.96 (0.75-1.22)	1.05 (0.83-1.34)	0.78
Multivariable OR (95%CI)	1.00	0.86 (0.66-1.13)	0.88 (0.67-1.15)	0.98 (0.75-1.28)	0.81
BMI ≥25					
Cases/Controls	43/104	41/122	47/142	40/170	
Age-adjusted OR (95%CI)	1.00	0.81 (0.49-1.34)	0.79 (0.49-1.29)	0.56 (0.34-0.92)	0.03
Multivariable OR (95%CI)	1.00	0.99 (0.56-1.75)	1.04 (0.60-1.80)	0.64 (0.36-1.14)	0.17
Smokers					
Cases/Controls	95/388	99/347	90/340	83/308	
Age-adjusted OR (95%CI)	1.00	1.17 (0.85-1.60)	1.08 (0.78-1.50)	1.09 (0.78-1.52)	0.67
Multivariable OR (95%CI)	1.00	1.06 (0.74-1.81)	1.12 (0.79-1.60)	1.08 (0.74-1.55)	0.62
Nonsmokers					
Cases/Controls	74/143	68/159	79/195	91/237	
Age-adjusted OR (95%CI)	1.00	0.82 (0.55-1.23)	0.78 (0.53-1.14)	0.73 (0.50-1.07)	0.11
Multivariable OR (95%CI)	1.00	0.76 (0.48-1.19)	0.79 (0.51-1.22)	0.71 (0.46-1.10)	0.16

OR: odds ratio ; CI: confidence interval; *adjusted for age, month of blood collection, body mass index, cigarette smoking and alcohol drinking; Unconditional logistic models were used in all the analyses.

by age, BMI or cigarette smoking. As cancer mortality reflects both incidence and survival, analysis including all cancer deaths provides a general assessment of cancer risk attributable to serum TGF-β1 levels. A further analysis by site-specific cancer mortality showed no overall associations between serum TGF-β1 levels and cancer mortality at major sites such as gastric cancer, lung cancer and colon cancer.

There may be several explanations for the lack of association between serum TGF- β1 levels and total cancer risk in our study. First, as shown in the study, as well as in other reports (Shim et al., 1999), serum TGF- β1 levels varied considerably between subjects. Variation over time was also not measured and remains unknown. Given the dual role of TGF- β1 in carcinogenesis, a single measurement of serum TGF- β1 levels at baseline may not be able to capture the critical period involved in multi-stage carcinogenesis. Second, evidence suggests that circulating TGF- β1 levels are under genetic control as mutations at two polymorphic sites of the TGF- β1 gene have been shown to influence plasma levels (Yokota et al.,2000; Saltzman et al.,2008; Grainger et al., 1999). Accordingly, variations in circulating TGF-β 1 levels and the association with cancer risk may be expected in ethnically diverse populations with background genetic variability. Third, selection bias may have been a concern in our study as we included only subjects who had provided sera at baseline. However, the likelihood of selection bias due to differential response would be expected to be small between subjects who donated blood samples and those who did not, given that no significant differences were observed in the characteristics such as age, BMI, education level and medical history between the two groups.

Two limitations of this study warrant consideration. As mentioned earlier, the single measurement of serum TGF-β1 levels at baseline was a limitation. Another limitation was that we were not able to control for the level of platelet activation. As platelets are a rich source of TGF-β1 (Coupes et al.,2001), platelet activation during blood collection may be a factor contributing to variations in circulating TGF-β1 levels.

In conclusion, the results of this nested case-control study indicate that there is no association between serum TGF-β1 levels and overall cancer mortality risk in apparently healthy individuals. However, further work is needed to improve measurement precision of circulating TGF-β1 level and to address its precise role in the prediction of cancer risk.

Acknowledgement

The JACC Study has been supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho), and Grants-in-Aid for Scientific Research on Priority Areas of Cancer, as well as Grant-in-Aid for Scientific Research on Priority Areas of Cancer Epidemiology from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Monbu-Kagaku-sho) (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022 and 18014011).

The authors thank Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and the former chairperson of the JACC Study, and Dr. Haruo Sugano, the former Director of the Cancer Institute, Tokyo, for their contribution in the initiation of the JACC Study.

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RESEARCH COMMUNICATION

Cancer Deaths in a Cohort of Japanese Barbers in Aichi Prefecture

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Abstract

Barbers have frequent occasion to come in contact with hair and beauty products that contain many chemical substances, which could have harmful effects on health. Subjects were barbers belonging to the Barbers' Union of Aichi Prefecture who responded to a questionnaire in 1976. Deaths from all sites of cancers in the subjects were observed over 27 years. Mortalities of several cancers in the subjects were compared with individuals in the Japanese population, calculating standardized mortality ratios (SMRs) using the general Japanese population as a standard. Subjects included 8,360 people (4,674 men). There were a total of 551 deaths (469 men) during the follow-up period, and 277 deaths (211 men) from all cancers. The male and female SMRs (95%CI) were 0.62 (0.58-0.66) and 0.25 (0.16-0.34) for all deaths, 0.46 (0.39-0.53) and 0.41 (0.35-0.53) for all cancers combined, 0.49 (0.35-0.63) and 0.40 (0.12-0.68) for stomach, 0.40 (0.24-0.56) and 0.30 (0.10-0.70) for lung, 0.56 (0.39-0.73) and 0.26 (0.02-0.76) for liver, 0.38 (0.16-0.60) and 0.30 (0.07-0.67) for colon, and 0.48 (0.08-0.88) and 0.22 (0.04-0.79) for blood cancers, respectively, with significantly fewer deaths than in the general population. The female SMRs were 0.90 (0.74-1.06) for breast and 0.55 (0.06-1.04) for ovarian cancer, lacking significance. Thus, no excess mortality of any cancer sites was observed compared with the general population in both Japan overall and in Aichi Prefecture.

Key Words: Barbers - cancer - mortality rate - cohort study

Asian Pacific J Cancer Prev, **10**, 307-310

Introduction

Barbers and hairdressers are often exposed to hairdressing products that contain many chemical substances. Many epidemiological studies have been conducted so far based on the possibility that these chemical products may have somewhat harmful effects on health (Kono et al., 1983; Shibata et al., 1989; Schumacher et al 1989; Kato et al., 1990; Skov et al., 1990; 1994; Silverman et al 199l; Pukkala et al., 1992; Boffetta et al., 1994; Miligi et al., 1999; Teschke et al 1997; Sugiura et al 2000; Czene et al 2003; Ji et al 2005). Positive associations with non-Hodgkin's lymphoma (Boffetta et al 1994) and urinary bladder (Schumacher et al 1989) cancer, but not other sites, have been reported.

In Japan, there have been few such studies on the health of barbers and/or hairdressers (Kono et al 1983; Shibata et al 1989; Kato et al 1990; Sugiura et al 2000). We have conducted earlier studies on hematopoietic diseases in the cohort of the current study, but found that the mortality was lower than in the general Japanese population (Shibata et al 1989; Sugiura et al 2000). In the current study we presented the mortality experience of a cohort of barbers in Aichi Prefecture over 27 years and examined for any excess in mortality from specific cancer sites, including stomach, lung, liver, colon, blood, urinary bladder, breast and ovary, compared with the general population in Japan.

Materials and Methods

Study Population

Subjects were members of the Barbers' Union of Aichi Prefecture who responded to a self-administered questionnaire sent and sent back by mail in October 1976. The questionnaire included items on living habits, medical history, and use of hair dye. Those who could not be accurately identified by name, address, or other information were not included in the study. The subjects were followed for all causes of deaths from October 1976 to December 2002 as a cohort. During the follow-up period, information on deaths from cancers was obtained using deaths' certificates from Barbers' Union of Aichi Prefecture received. The subjects who seceded from the Barbers' Union of Aichi Prefecture or moved out of Aichi Prefecture during the follow-up period were treated as censored cases (473 men, 363 women). We believe the information on vital status for all barbers of the Barber's Union to be reliable, because there were no material cases

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whose death information demonstrated differences from that registered at Aichi Cancer Registry.

This study was approved by the ethics committee of Aichi Medical University School of Medicine, and permission to use cancer registration records was received from the Aichi Cancer Registry at the Aichi Cancer Center.

Types of Cancer Analyzed

The mortality rates were calculated with all sites of, stomach, lung, liver, colon, blood, urinary bladder, breast, and ovarian cancers, based on the ICD-10 classifications. As the standard population, the whole Japanese population was used, and the data were from the Ministry of Health, Labor, and Welfare.

The number of expected deaths (E) in the cohort was calculated stratified by gender and age (5-year age-classes) using the standard population and its mortality rates of cancers. The numbers of people in the cohort of each gender and age-classes were calculated separately and multiplied by the mortality of the standard population each year, and totaled for the 27-year period. Then, Standard mortality ratios (SMRs), the ratio of observed (O) to expected (E) number of deaths, were calculated for all cancers combined, site-specific cancers. Statistical significance of the SMRs was examined using chi-square tests.

Results

Background Factors of Subjects

Table 1 shows the 1976 baseline gender and age distribution of the 8,360 people (4,674 men, 3,686 women) in this study. Table 2 shows demographic characteristics and lifestyle factor of subjects at baseline.

Table 1. Baseline Sex and Age Distribution

Age group (year)	Male (%)	Female (%)	Total (%)
≤19	51 (1.1)	50 (1.4)	101 (1.2)
20-29	963 (20.6)	994 (27.0)	1,957 (23.4)
30-39	1,923 (41.1)	1,548 (42.0)	3,471 (41.5)
40-49	884 (18.9)	600 (16.3)	1,484 (17.8)
50-59	441 (9.4)	371 (10.1)	812 (9.7)
60-69	377 (8.1)	114 (3.1)	491 (5.9)
70-79	31 (0.7)	9 (0.2)	40 (0.5)
≥80	4 (0.1)	0 (0.0)	4 (0.0)
Total	4,674 (100)	3,686 (100)	8,360 (100)

Table 2. Demographic Characteristics of the Study Cohort at Baseline

Characteristics		Male	Female
Barber start age		17.0± 4.3	18.6±5.6
Working years		21.2±11.4	17.0±8.8
Occupational hair dye use	yes	88.9	75.5
(%)	no	11.1	24.5
Personal hair dye use (%)	yes	37.4	58.5
	no	62.6	41.5
Smoking habit (%)	yes	84.4	15.4
	no	15.6	84.6
Drinking habit (%)	yes	78.8	45.9
	no	21.2	54.1

Cancer Mortality Rate in Subjects and General Cohort

Table 3 presents observed number of deaths, expected number of deaths, and SMRs during the follow-up period from 1976 to 2002. A total of 551 deaths due to all causes were observed in this cohort (469 men, 82 women), with 277 (211 men, 66 women) deaths from all cancers. Among both men and women, the observed numbers of cancer deaths were lower than the expected number of cancer deaths based on the referent Japanese population. Among

Table 3. Observed and Expected Numbers of Deaths from All Causes and Cancers, with SMRs by Sex in a Cohort of Barbers, 1976-2002

	Men					Women				
Cause of death	Observed	Expected	SMR	95%CI	P value	Observed	Expected	SMR	95%CI	P value
All deaths	469	752.96	0.62	0.58-0.66	p<0.01	82	329.16	0.25	0.16-0.34	p<0.01
All cancer deaths	211	461.90	0.46	0.39-0.53	p<0.01	66	159.36	0.41	0.35-0.53	p<0.01
Stomach	51	103.23	0.49	0.35-0.63	p<0.01	12	29.85	0.40	0.12-0.68	p<0.01
Lung	37	91.81	0.40	0.24-0.56	p<0.01	5	16.85	0.30	0.10-0.70	p<0.01
Liver	32	57.65	0.56	0.39-0.73	p<0.01	3	11.38	0.26	0.02-0.76	p<0.05
Colon	18	47.67	0.38	0.16-0.60	p<0.01	6	19.99	0.30	0.07-0.67	p<0.01
Blood	11	22.70	0.48	0.08-0.88	p<0.05	2	9.07	0.22	0.04-0.79	p<0.05
Bladder	5	7.84	0.64	0.22-1.06	n.s.	0	1.33	0.00		n.s.
Breast						14	15.59	0.90	0.74-1.06	n.s.
Ovary						4	7.29	0.55	0.06-1.04	n.s.

SMR: standardized mortality ratio; CI: confidence interval

Table 4. Observed and Expected Numbers of Deaths by Time period, with SMRs by Sex in a Cohort of Barbers, 1976-2002

	Men					Women				
Time period	Observed	Expected	SMR	95%CI	P value	Observed	Expected	SMR	95%CI	P value
1976-84	31	73.52	0.42	0.25-0.59	p<0.01	8	26.83	0.30	0.02-0.62	p<0.01
1985-93	73	139.95	0.52	0.41-0.63	p<0.01	24	47.25	0.51	0.31-0.71	p<0.01
1994-2002	107	248.43	0.43	0.34-0.52	p<0.01	34	85.28	0.40	0.24-0.56	p<0.01
All periods	211	461.90	0.46	0.39-0.53	p<0.01	66	159.36	0.41	0.29-0.53	p<0.01

SMR: standardized mortality ratio; CI: confidence interval

men, all SMRs were lower than unity for the major sites of cancer, such as stomach, lung, liver, colon and blood. No excess in bladder cancer mortality was noted in this cohort. Among women, all SMRs were again lower than unity for the stomach, lung, liver and colon. The SMR for breast cancer was near unity. No excess in ovary cancer mortality was noted in this cohort. SMR for bladder cancer could not be calculated because no deaths were observed.

Table 4 shows observed number of cancer deaths, expected number of cancer deaths, and SMRs, stratified by 3 follow-up periods. In all three 9-year periods with 9 years follow-up, the number of deaths was significantly lower than in the general cohort for both men and women.

Discussion

In the present study, mortality from all cancers, and from specific sites such as stomach, lung, liver, colon and blood cancer deaths in this cohort were significantly lower than in the general population for both men and women. Moreover, mortality from urinary bladder cancer in men, and from breast and ovarian cancers in women showed no remarkable elevation. These results do not support the IARC report that hairdressing is a profession that may be related to increased cancer risk (IARC, 1993).

We speculate that low cancer mortality in Aichi Prefecture, where this cohort study was conducted, could may be the main reason for the lower cancer mortality observed in this cohort. Kikuchi et al calculated that SMRs for 1994 to 2002 in Aichi prefecture were lower than 1.0 for male all cancers combined (0.94), male stomach (0.97), and male (0.88) and female (0.90) liver cancers, and higher than 1.0 for female stomach cancer (1.07). SMRs for male and female lung and breast cancer were around 1.0 and less than ones for Aichi Prefecture (unpublished data). There also seems to be a remarkable difference in cancer mortality between the barbers and the general population in Aichi Prefecture. Smoking increases risks of both lung (Wakai et al., 2006) and stomach cancers (Kikuchi et al., 2002). In spite of high rates of smokers in the subjects of the current study, mortality of stomach and lung cancers were low compared with general population.

The reason why the mortality of the barbers was low compared with general population in Aichi Prefecture and in Japan is unknown. One possible explanation is that some cases were not registered on vital status for all barbers, but this seems unlikely because no cases of deaths from cancer in this barbers' cohort were recorded only in Aichi Cancer Registry. The healthy worker effect, which is inherent in occupational cohort studies, may also be responsible for a relatively lower mortality, but the effect seems limited. The subjects of this study were barbers belonging to the Barber's Union in Aichi Prefecture who responded the questionnaire at baseline. They were actually working as barbers and consequently relatively healthy compared with the general population. Another reason may be that most subjects were self-employed persons working indoors and they were free from occupational stress. Stress weakens immune response including activity of natural T-cells (Arranz et al., 2007), which increases risk of cancer (Imai et al., 2000).

Although many epidemiological studies on hair dye and various types of cancer have been conducted to date, no causal relationship has been established (Hennekens et al., 1979; Thun et al 1994; Grodstein et al 1994; La Vecchia et al 1995; Altekruse et al 1999; Gago-Dominguez et al 2001; Negri et al 2001; Zhang et al., 2004). Our results are not consistent with a study by Kono et al (1983), who investigated causes of death from cancer and other diseases in a cohort of female beauticians in comparison with all citizens of Fukuoka Prefecture from 1953 to 1977. They reported that only death from stomach cancer was significantly higher, and that there were no special trends for other cancers.

Epidemiologic studies have suggested a positive association between hematopoietic diseases and occupational exposure of hairdressers (Boffetta et al., 1994; Skov et al., 1994; Miligi et al.,1999), but the results are controversial. Shibata et al (1989) surveyed hematopoietic diseases in the 11 years from 1976 to 1987 in the subjects of the current study, and reported that up to 1987 mortality from leukemia and malignant lymphoma were lower than in the general population. Our previous study followed-up the same subjects until October 1995 and compared the mortality rates of hematopoietic diseases including blood cancer with the general population. We found that all deaths and all cancer deaths were significantly less frequent than in the general cohort, and leukemia and malignant lymphoma were somewhat less frequent.

An increased risk of breast cancer in hairdressers has also been reported in Aichi (Kato et al., 1990). In a Japanese cohort study, Lin (Lin et al., 2005) reported an elevated risk of breast cancer in women who drank alcohol regularly compared with those who did not. In the current study, breast cancer showed a relatively higher SMR than other cancers, although it was still not higher than in the whole Japanese population or in Aichi prefecture. As shown in Table 2, the high rate of alcohol drinkers among female subjects might also explain the relatively high mortality compared with other sites of cancers.

Previous studies have suggested that risk of cancer mortality may differ according to the length of follow-up period. In Finland, Pukkala et al (1992) studied the development of cancer in male and female hairdressers from 1970 to 1987, by dividing the follow-up period into 3 periods. They found that the risk of cancer was elevated in the first period only, but not in the subsequent periods, and stated that the change in risk may have been associated with changes in working conditions in hair salons. Furthermore, Boffetta et al (1994) investigated the incidence rate of ovarian cancer and non-Hodgkin's lymphoma in female hairdressers in Denmark, Sweden, Norway, and Finland. They reported that the increase in risk differed by country, and indicated that the risk of work-related cancer in female hairdressers differed according to time and geographical factors. In the current study, we examined the changes in mortality rate from all cancer deaths over three 9-year periods, but found no appreciable differences in SMRs in each period.

Our study has limitations. Approximately 10% of the study subjects were lost to follow-up during the follow-

up period, which might bias the results if those who were lost differed significantly from those who remained in the cohort. Ten percent during the 27 years means only 0.37 percent per year. Furthermore, no cases of deaths from cancer in this barbers' cohort were recorded on Aichi Cancer Registry but not in the information from the Barber's Union, which means that the association between drop out and death of cancer was limited. The strengths of our study included a long period of follow-up and complete employment record.

Acknowledgement

The authors express their sincere appreciation to the members of the Barbers' Union of Aichi Prefecture for their cooperation in this study.

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Preventive Medicine 48 (2009) 486–492

Contents lists available at ScienceDirect

Preventive Medicine

journal homepage: www.elsevier.com/locate/ypmed

Healthy lifestyle and preventable death: Findings from the Japan Collaborative Cohort (JACC) Study

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ARTICLE INFO

Available online 27 February 2009

Keywords:
Lifestyle
Cohort study
Hazard ratio
Population-attributable fraction

ABSTRACT

Objective. To evaluate the effect of baseline combination of 6 lifestyle factors on all-cause mortality.

Methods. A total of 62,106 Japanese men and women aged 40–79 years were followed for 12.5 years on average. Hazard ratios and 95% confidence intervals (CIs) of all-cause mortality in relation to healthy lifestyle factors (not currently smoking, not heavily drinking, walking 1 h or more per day, sleeping 6.5 to 7.4 h per day, eating green-leafy vegetables almost daily and BMI between 18.5 and 24.9) were calculated from proportional-hazards regression models. We also estimated population-attributable fractions of death to address the impact of potential lifestyle modifications on mortality.

Results. Until 2003, 8497 deaths were observed. Age-adjusted HR of all-cause mortality for the group with 6 healthy lifestyle factors was 0.42 (95% CI: 0.32–0.56) among men and 0.49 (0.39–0.60) among women, respectively, compared with the group with 0–2 healthy lifestyle factors. Even at ages 60–79 years, a healthy lifestyle has a major impact on mortality. Had the subjects achieved even a 1-point increment in their lifestyle scores, death rates of 24.7% among men and 18.5% among women could have been reduced.

Conclusion. We found an inverse association between baseline combination of 6 healthy lifestyle factors and all-cause mortality as well as its impact on preventable fraction of death. Our results also demonstrated that healthy lifestyle behaviors are important even in old age.

Introduction

A large body of existing evidence suggests that behavioral risk factors are leading causes of mortality. Among modifiable lifestyle factors, smoking (Doll et al., 2004; Noale et al., 2005), excessive drinking (Dawson, 2000; Lin et al., 2005), obesity (Hozawa et al., 2008; Tsugane et al., 2002; WHO, 2006), and physical activity (Fujita et al., 2004; Hamer and Chida, 2008) are widely accepted behaviors that have been associated with an increased risk of chronic diseases including cancer and cardiovascular diseases. Because of the complex nature of sleep (Kripke et al., 2002; Tamakoshi et al., 2004) and dietary habits (Genkinger et al., 2004; Takachi et al., 2008), their relationship with mortality is not well-defined, with issues such as the objective assessment remaining to be resolved. Despite the established relationship between these individual lifestyle risk factors and mortality, it remains a difficult task to reduce the total number of deaths from these causes. From a public health perspective, a simple lifestyle assessment is more feasible and can be readily applied to motivate the public to make lifestyle modifications.

Since some lifestyle factors are mutually related to one another (Haenle et al., 2006; Ma et al., 2000), it is important to investigate their combined effects on overall health. Some studies have attempted to clarify the combined effects of lifestyle variables on all-cause mortality (Breslow and Enstrom, 1980; Haveman-Nies et al., 2002; Khaw et al., 2008; Knoops et al., 2004; Spencer et al., 2005; Tsubono et al., 1993; Tsubono et al., 2004). However, the number of subjects in those studies was relatively small, and differences in impact between those in middle age and the elderly were not investigated except in one recent report (Khaw et al., 2008). In addition, assessment of diet and/or physical activity in some studies were complex (Haveman-Nies et al., 2002; Knoops et al., 2004) or required clinical testing (Khaw et al., 2008).

We have previously reported that individual lifestyle factors such as smoking (Ozasa, 2007), heavy drinking (Lin et al., 2005), obesity (Cui et al., 2005), too long or too short sleep (Tamakoshi et al., 2004), daily walking less than 1 h per day (Noda et al., 2005), and low intake of green-leafy vegetables (Iso and Kubota, 2007) were associated with increased risk of mortality in the Japanese Collaborative Cohort Study (JACC Study), a large-scale cohort study of middle-aged and elderly Japanese. In the present study, we sought to examine the risk of all-cause mortality in relation to baseline combination of 6 healthy lifestyle factors (not currently smoking, not heavily drinking, walking 1 h or more per day, sleeping 6.5 to 7.4 h per day, eating green-leafy

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doi:10.1016/j.ypmed.2009.02.017

vegetables almost daily and BMI between 18.5 and 24.9) in the same cohort. We also evaluated the magnitude of the impact on public health, i.e., population-attributable fractions.

Methods

Study subjects and data collection

The study design and methods of the JACC Study have been previously described elsewhere (Ohno et al., 2001; Tamakoshi et al., 2005). Briefly, from 1988 to 1990, healthy subjects in 45 areas throughout Japan replied to a self-administered questionnaire. The cohort consisted of 110,792 subjects (46,465 men and 64,327 women) aged 40–79 years old at baseline. The response rate was 83%. The Ethical Board of the Nagoya University School of Medicine, where the central office of the JACC Study was located, approved our complete study design.

Follow-up

The cause and date of death were identified among the study subjects by reviewing all death certificates in each area by each area investigator with the permission of the Director-General of the Prime Minister's Office (Ministry of Internal Affairs and Communications). Those who moved out of a study area were treated as censored. Follow-ups were done until the end of 2003, except in the 4 areas where follow-ups were discontinued at the end of 1999.

Lifestyle variables

The questionnaires probed smoking status, alcohol consumption, walking duration, sleep duration, consumption of green-leafy vegetables, height, weight, and other health-related variables. Unfortunately, a questionnaire distributed in some areas did not include a question on the total amount of alcohol consumed, walking duration and consumption of green-leafy vegetables. As a result, 20,725 subjects could not be included in the analyses. Smoking status at the time of baseline survey was divided into 3 groups: "currently smoking," "smoked before, but quit," and "never smoked." Alcohol consumption was first divided into "currently drinking," "drank before" and "never drank" categories based on previous year status. Then, current drinkers were asked the total amount consumed per drinking occasion by the Japanese traditional unit of alcohol beverage, i.e., the gou (1 gou = 180 ml of sake (traditional Japanese rice wine containing 22.8 g ethanol)). Walking duration was covered by the question, "How much time per day on average did you walk over the previous year?". Average sleep duration on weekdays one year prior to the survey, and average frequency of green-leafy vegetables in usual life were also included in the questionnaire. Body mass index (BMI; weight in kilograms/[height in meters]^2) was calculated based on the height and weight self-reported at baseline. Each variable was then coded with binary levels, and 1 point was credited for each of the following potentially healthy factors according to previous studies: not currently smoking (includes quitting) (Doll et al., 2004; Ozasa et al., 2008); drinking no more than 1 gou per occasion or not currently drinking (includes quitting) (Lin et al., 2005); walking 1 h or more per day (Fujita et al., 2004; Hamer and Chida, 2008); sleeping 6.5 to 7.4 h per day (Kripke et al., 2002; Tamakoshi et al., 2004); eating green-leafy vegetables almost daily (Iso and Kubota, 2007; Takachi et al., 2008); and desirable weight for height (BMI between 18.5 and 24.9) (Hozawa et al., 2008; WHO, 2006). Points were totaled up to yield an overall lifestyle score ranging from 0 to 6 points, with a higher score indicating a more favorable health-promoting lifestyle. Analysis in this paper was limited to those subjects whose total lifestyle score could be calculated, leaving 62,106 eligible subjects (27,582 men and 34,524 women; 69% of subjects who provided the appropriate questionnaire).

Analysis

To compare proportions of subject's characteristics across lifestyle scores we used Mantel–Haenszel test adjusting for age categories. Number of all-cause mortality and age-standardized mortality rates, standardized to the age distribution of total population separated by gender, were presented according to lifestyle factors, potential confounding factors and lifestyle scores. Hazard ratios (HRs) were calculated according to Cox's proportional hazard model separately by gender. The deceased were treated as uncensored cases when the event occurred. Survivors at the end of 2003 or those who relocated were treated as censored cases. All subjects combined as well as those stratified by age group (younger than 60 years or 60 years and older) were examined. In addition to age-adjusted HRs, we calculated HRs adjusted for potential confounding factors queried in the questionnaire; education (attended school up to 15 years old, 18 years old, older than 18 years or unknown), taking breakfast (almost daily, not daily or unknown), perceived stress (yes, no or unknown), marital status (married, single or unknown), and past history of stroke, myocardial infarction or cancer (yes, no or unknown). Moreover, additional examinations were conducted to exclude subjects whose events occurred within 2 years after baseline to avoid reverse-causality bias.

The population-attributable fraction all (PAF_{all}) was calculated by the standard method (Miettinen, 1974) as follows:

$$PAF_{all}(\%) = \sum P_i(HR_i - HR_6) / \left(1*HR_6 + \sum P_i(HR_i - HR_6)\right)*100,$$

where P_i is the prevalence of the population for each score i at baseline, and HR_i is the age-adjusted relative risk of an exposed group for each score i compared to a group with score 6. This PAF_{all} denotes an estimation of the proportion of deaths attributable to unfavorable behavior in the population, i.e., preventable fraction when all subjects would have altered all their 6 lifestyles to healthy categories. In addition, the population-attributable fraction $+1$ (PAF_{+1}) was calculated as

$$PAF_{+1}(\%) = \sum P_i(HR_i - HR_{i+1}) / \left(1*HR_6 + \sum P_i(HR_i - HR_{i+1})\right)*100,$$

where HR_i and HR_{i+1} are the age-adjusted relative risks of an exposed group for score i and score $i+1$, respectively, compared with a score-6 group. This PAF_{+1} estimates the preventable fraction when all but the subjects with score 6 would have achieved a 1-point increment in their lifestyle scores. We used the SAS program Ver. 9.1 (SAS Institute Inc., Cary, NC) for analyses at the Aichi Medical University Computation Center.

Results

A total of 8497 (mortality rate: 136.8 per 1000) deaths occurred until 2003 (5285 men and 3212 women). Those who moved away totaled 2592 (4.2%). Total cancer deaths accounted for 40.0% in men and 35.5% in women, and the circulatory system for 27.9% and 32.7%, correspondingly. The average follow-up period was 12.5 years.

Compared to those with high total lifestyle scores, those with low scores were younger, less educated, suffered more from high mental stress, less likely to have a spouse, and less likely to eat breakfast everyday (Table 1). The proportion of those with a history of stroke, myocardial infarction or cancer was likely to decrease with increasing total lifestyle scores in women, whereas it tended to increase in men. Age-standardized mortality was higher among those with a past medical history in both men and women. Each of six healthy lifestyle factors showed a lower age-adjusted mortality rate compared with the total population. Also, mortality decreased according to increasing total lifestyle scores.

Table 2 showed the hazard ratios on mortality by total lifestyle scores. Age-adjusted HR of all-cause mortality for the highest group

Table 1
Distribution of some demographic factors according to total lifestyle score and age-standardized mortality rates (Japan Collaborative Cohort Study 1988–2003)

		Men								Women							
		Lifestyle factors						All-cause death		Lifestyle factors						All-cause death	
		0–2	3	4	5	6	Total number	*N*	Age-standardized mortality per 1000	0–2	3	4	5	6	Total number	*N*	Age-standardized mortality per 1000
Number		10,072	8878	6021	2232	379	27,582	5285	191.6[a]	2804	9087	12,869	8014	1750	34,524	3212	93.0[a]
Age category																	
40–59		6446	5122	3264	1103	190	16,125***	1321	81.9[a]	1550	5210	7751	4864	1083	20,458***	689	33.7[a]
	%	64.0	57.7	54.2	49.4	50.1				55.3	57.3	60.2	60.7	61.9			
60–79		3626	3756	2757	1129	189	11,457	3964	346.0[a]	1254	3877	5118	3150	667	14,066	2523	179.4[a]
	%	36.0	42.3	45.8	50.6	49.9				44.7	42.7	39.8	39.3	38.1			
College or higher education		1581	1424	1036	397	79	4517	791	174.8	231	781	1263	869	207	3351***	208	76.7
	%	15.7	16.0	17.2	17.8	20.8				8.2	8.6	9.8	10.8	11.8			
High mental stress		2202	1768	1152	385	66	5573***	725	189.5	541	1598	2243	1387	291	6060**	426	94.2
	%	21.9	19.9	19.1	17.2	17.4				19.3	17.6	17.4	17.3	16.6			
Having a spouse		8849	7769	5333	1994	338	24,283**	4309	184.0	2055	7122	10,315	6557	1429	27,478***	2136	94.2
	%	87.9	87.5	88.6	89.3	89.2				73.3	78.4	80.2	81.8	81.7			
Taking breakfast almost everyday		9385	8421	5797	2149	362	26,114***	5053	191.4	2584	8611	12,281	7742	1705	32,923***	3096	93.4
	%	93.2	94.9	96.3	96.3	95.5				92.2	94.8	95.4	96.6	97.4			
Past medical history																	
Stroke		145	174	102	48	5	474*	224	341.3	53	100	91	49	3	296***	117	210.6
	%	1.4	2.0	1.7	2.2	1.3				1.9	1.1	0.7	0.6	0.2			
Myocardial infarction		243	242	189	80	8	762**	277	245.0	105	219	307	170	32	833***	138	97.9
	%	2.4	2.7	3.1	3.6	2.1				3.7	2.4	2.4	2.1	1.8			
Cancer		77	97	62	30	6	272**	124	301.7	57	170	208	129	31	595	122	169.9
	%	0.8	1.1	1.0	1.3	1.6				2.0	1.9	1.6	1.6	1.8			
Lifestyle factors																	
Not current smoker		2184	4167	4258	1931	379	12,919	2343	161.3	2075	8487	12,547	7965	1750	32,824	2999	91.1
	%	21.7	46.9	70.7	86.5	100.0				74.0	93.4	97.5	99.4	100.0			
Drinking within 1 gou or not current drinker		2962	5475	4758	2036	379	15,610	3293	185.1	2505	8900	12,761	8003	1750	33,919	3169	92.8
	%	29.4	61.7	79.0	91.2	100.0				89.3	97.9	99.2	99.9	100.0			
Walking 1<= hours/day		2725	4558	4047	1907	379	13,616	2564	186.5	219	2218	6935	6560	1750	17,682	1487	86.0
	%	27.1	51.3	67.2	85.4	100.0				7.8	24.4	53.9	81.9	100.0			
Sleep 6.5–7.4 h per night		1568	2840	2898	1555	379	9240	1357	172.5	104	1484	4957	5219	1750	13,514	861	79.3
	%	15.6	32.0	48.1	69.7	100.0				3.7	16.3	38.5	65.1	100.0			
Eating green-leafy vegetables almost everyday		1001	2457	2722	1586	379	8145	1727	188.0	76	1192	4088	4917	1750	12,023	1165	88.2
	%	9.9	27.7	45.2	71.1	100.0				2.7	13.1	31.8	61.4	100.0			
BMI 18.5–24.9		6044	7137	5401	2145	379	21,106	3988	188.6	374	4980	10,188	7406	1750	24,698	2095	87.2
	%	60.0	80.4	89.7	96.1	100.0				13.3	54.8	79.2	92.4	100.0			
Death																	
All-cause death (*N*)		1985	1699	1164	388	49	5285			401	1005	1139	561	106	3212		
Age-standardized mortality per 1000		222.21	188.93	175.0	149.21	110.54				127.32	104.33	90.1	75.127	67.741			
Cancer		761	684	495	153	20	2113			111	348	402	231	48	1140		
	%[b]	38.3	40.3	42.5	39.4	40.8	40.0			27.7	34.6	35.3	41.2	45.3	35.5		
Circulatory system		562	478	325	102	7	1474			154	313	384	175	23	1049		
	%[b]	28.3	28.1	27.9	26.3	14.3	27.9			38.4	31.1	33.7	31.2	21.7	32.7		

College or higher education; attended at school older than 18 years old.
High mental stress; questionnaire reply indicating perceived stress.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$ performed by Mantel–Haenszel test adjusted for age.
[a] Crude mortality rate.
[b] Percentage of deaths per all causes.

Table 2
Hazard ratios and 95% CI of all-cause mortality according to total lifestyle score (Japan Collaborative Cohort Study 1988–2003)

	Men									Women									
	N	Person-years	Cases	HR	95% CI		HR2	95% CI		PAF_{all}(%) PAF_{+1}(%)	*N*	Person-years	Cases	HR	95% CI		HR2	95% CI		PAF_{all}(%) PAF_{+1}(%)
0–2	10,072	123,894	1985	1.00			1.00				2804	34,129	401	1.00			1.00			
3	8878	109,520	1699	0.81	0.76	0.87	0.80	0.75	0.86		9087	114,175	1005	0.79	0.71	0.89	0.82	0.73	0.92	
4	6021	74,423	1164	0.72	0.67	0.78	0.72	0.67	0.77		12,869	163,831	1005	0.67	0.60	0.75	0.70	0.63	0.79	
5	2232	27,912	388	0.59	0.53	0.66	0.58	0.52	0.65	49.4	8014	103,509	561	0.54	0.48	0.62	0.58	0.50	0.66	29.8
6	379	4821	49	0.42	0.32	0.56	0.43	0.32	0.58	24.7	1750	22,795	106	0.49	0.39	0.60	0.53	0.43	0.66	18.5
Trend *p*				<0.0001			<0.0001							<0.0001			<0.0001			
Stratified by age at baseline																				
40<=age<60																				
0–2	6446	84,150	581	1.00			1.00				1550	19,822	80	1.00			1.00			
3	5122	67,481	409	0.84	0.74	0.96	0.84	0.74	0.95		5210	68,538	208	0.73	0.57	0.95	0.75	0.58	0.98	
4	3264	43,052	242	0.75	0.65	0.87	0.75	0.65	0.88		7751	102,288	232	0.55	0.43	0.71	0.58	0.44	0.75	
5	1103	14,779	78	0.69	0.54	0.87	0.70	0.55	0.89	36.3	4864	64,795	138	0.51	0.38	0.67	0.53	0.40	0.70	19.2
6	190	2514	11	0.56	0.31	1.01	0.58	0.32	1.05	17.0	1083	14,390	31	0.50	0.33	0.76	0.53	0.35	0.81	14.6
Trend *p*				<0.0001			<0.0001							<0.0001			<0.0001			
60<=age<80																				
0–2	3626	39,744	1404	1.00			1.00				1254	14,308	321	1.00			1.00			
3	3756	42,040	1290	0.80	0.74	0.86	0.79	0.73	0.85		3877	45,637	797	0.81	0.71	0.92	0.83	0.73	0.95	
4	2757	31,371	922	0.71	0.66	0.77	0.71	0.65	0.77		5118	61,543	907	0.71	0.62	0.80	0.74	0.65	0.84	
5	1129	13,133	310	0.57	0.50	0.64	0.56	0.49	0.63	51.5	3150	38,714	423	0.55	0.47	0.64	0.59	0.51	0.68	33.8
6	189	2307	38	0.39	0.29	0.54	0.40	0.28	0.55	26.7	667	8406	75	0.47	0.37	0.61	0.52	0.40	0.67	20.2
Trend *p*				<0.0001			<0.0001							<0.0001			<0.0001			
Excluded events within 2 years from baseline																				
0–2	9828	123,648	1838	1.00			1.00				2738	34,068	366	1.00			1.00			
3	8660	109,293	1558	0.80	0.75	0.86	0.80	0.74	0.85		8949	114,033	936	0.81	0.72	0.91	0.83	0.74	0.94	
4	5875	74,268	1057	0.71	0.65	0.76	0.70	0.65	0.76		12,687	163,647	1071	0.69	0.61	0.78	0.72	0.64	0.81	
5	2187	27,863	360	0.59	0.53	0.66	0.58	0.52	0.65	49.9	7925	103,404	531	0.56	0.49	0.64	0.59	0.52	0.68	31.6
6	371	4813	45	0.42	0.31	0.56	0.42	0.31	0.57	25.5	1732	22,772	97	0.48	0.39	0.60	0.53	0.42	0.66	19.0
Trend *p*				<0.0001			<0.0001							<0.0001			<0.0001			

HR; adjusted for age categories.
HR2; adjusted for age categories, education, stress, marital status, consumption of green-leafy vegetables, past history of stroke, MI, cancer.

(score of 6) was 0.42 (95% CI: 0.32–0.56) among men and 0.49 (0.39–0.60) among women, respectively, compared with the lowest group (score of 0–2). Statistically, the trends were highly significant. Adjusting for other potential confounders did not alter the results. Dividing subjects according to their age, or excluding events occurring within 2 years also did not change the effects of lifestyle scores. Moreover, the results were not altered when those who suffered from stroke, myocardial infarction or cancer at the baseline were excluded (data not shown). PAF_{all} was 49.4% among men and 18.5% among women, PAF_{+1} was 24.7% and 18.5%, respectively. The values were almost the same when excluding events occurring within 2 years, and also for those aged 60 years and more. However, among both younger men and women the fractions were smaller than among the elderly.

Discussion

Using data from a large population-based cohort study of middle and older subjects followed for 12.5 years on average, we found an inverse association between a baseline combination of 6 healthy lifestyle factors and the risk of all-cause mortality. The risk for the group with a total lifestyle score of 6 was 0.42 among men and 0.49 among women, compared with the group with a lifestyle score of 0–2. To avoid reverse-causality bias that lifestyle factors had changed in response to subclinical but fatal disease, we excluded events that occurred within 2 years from baseline, and almost the same risk reductions were observed. Even in the 60–79 year group, healthy lifestyles were associated with a significantly decreased risk of mortality. Moreover, if the subjects achieved even a 1-point increment in their lifestyle scores, 24.7% deaths among men and 18.5% deaths among women were estimated to be preventable. Such knowledge should prove useful to anyone who considers improving his or her lifestyle as well as to health promoters who plan population-based strategy in health improvement campaigns.

Seven previous studies examined the relative risks of lifestyle variables to all-cause mortality (Breslow and Breslow, 1993; Haveman-Nies et al., 2002; Khaw et al., 2008; Knoops et al., 2004; Spencer et al., 2005; Tsubono et al., 1993; Tsubono et al., 2004). Subjects included in each study were relatively few compared with ours. Lifestyle variables chosen and the criteria dividing healthy from unhealthy varied widely. The number of selected lifestyles were distributed from 3 (Haveman-Nies et al., 2002) to 8 (Spencer et al., 2005). Breslow et al. used 7 healthy variables; never smoking, regular physical activity, moderate or no use of alcohol, sleeping 7–8 h, proper weight, eating breakfast, and not eating between meals (Breslow and Breslow, 1993), and evaluated the association with all-cause mortality. According to their study, the relative risk of those with a health practice score of 6–7 was 0.45 (0.35–0.57), compared with a group of score 0–3. The other studies mentioned above also reported similar combined effects of healthy lifestyle factors.

To evaluate the combined effect of healthy behaviors on all-cause mortality, we constructed a lifestyle score that consisted of 6 lifestyle factors (smoking, drinking, walking, sleeping, green-leafy vegetable intake and weight status) for which information was available from the baseline questionnaire. The selection of these lifestyle factors were based on the results from our previous cohort study and an extensive review of other epidemiologic studies that had reported on the relationship between lifestyle factors and mortality. Another important issue to consider is that the content of lifestyle score should be easily understood by the public and readily applicable to improve lifestyle. Smoking and physical activity were included in lifestyle scores in all previous related studies (Breslow and Breslow, 1993; Haveman-Nies et al., 2002; Khaw et al., 2008; Knoops et al., 2004; Spencer et al., 2005; Tsubono et al., 1993; Tsubono et al., 2004). Excessive drinking is also a well-established risk factor for premature mortality (Lin et al., 2005). These three factors were therefore included in our lifestyle score. Sleeping was added to the score because it is increasingly recognized as an important determinant of health (Kripke et al., 2002), and because our previous cohort study showed the lowest risk of all-cause mortality among people who slept for 6.5–7.5 h (Tamakoshi et al., 2004). Walking duration was used to reflect an individual's usual physical activity in the score because detailed information on physical activity was not available from the baseline questionnaire. For dietary habits, numerous studies have suggested that daily intake of green-leafy vegetable was associated with lower risk of all-cause mortality (Genkinger et al., 2004; Takachi et al., 2008) and was accordingly included in the lifestyle score. Body mass index is a widely accepted risk factor for all-cause mortality (Hozawa et al., 2008; Tsugane et al., 2002). In fact, lifestyle factors selected in this study are key elements of a healthy lifestyle included in "Health Japan 21", a recent health promotion initiated by the Government of Japan.

In the present study, dichotomous criteria were improved from the standpoint of modifiability, i.e., a person in an unhealthy group could change his or her behavior to a healthy group if so motivated; thus, quitting smoking or drinking was categorized as healthy status. Since the mortality risk of former smokers was known to be higher than that of non-smokers (Ozasa et al., 2008), and the risk of one who had quit alcohol was also higher than that of a non-drinker (Lin et al., 2005), this management diminished the differences in mortality risk between high and low total lifestyle scores. As for other variables, our dichotomous categorization is based on previous studies or recommendations for health (Breslow and Breslow, 1993; Davis et al., 1994; Fujita et al., 2004; Hamer and Chida, 2008; Hozawa et al., 2008; Iso and Kubota, 2007; Kaplan et al., 1987; Takachi et al., 2008; Tamakoshi et al., 2004; Tsubono et al., 2004; WHO, 2006).

Our results demonstrated that healthy lifestyle behaviors are important even at older ages. Haveman-Nies et al. reported the increased mortality risks even at 70–75 years of age, as a 3.5- and 3.9-fold risk among men and women, respectively, with the unhealthy lifestyles compared to those with the healthy (high physical activity, no smoking and a high-quality diet) (Haveman-Nies et al., 2002). Almost a 3.5-fold risk was also reported by Khaw et al. among those aged 65 years and older with the 0 health behaviors compared to those with the 4 health behaviors (Khaw et al., 2008). Moreover, Ferrucci et al. found that men at 65 years who never smoked and were in the high physical activity group were expected to survive 7.2 years longer than those who had ever smoked and had a low level of physical activity (Ferrucci et al., 1999). These results strongly underscore the importance of a healthy lifestyle even in the elderly.

Some health behaviors correlated with one another. For example, smokers were more likely to drink alcohol among subjects involved with this cohort. Likewise, the lifestyle variables might even have been associated with other healthy behaviors not chosen here. Allowing people to select whichever lifestyle variable to improve may eventually facilitate a decision or way to begin to adopt a healthier lifestyle. From a public health perspective, knowledge of how complex changes in health behaviors may affect mortality may help health promoters who propose their health plans. Our study estimated that about half and one third of deaths among men and women, respectively, could be avoided if all the people lived with 6 healthy lifestyles, and one fourth and one fifth of deaths, respectively, could be avoided if people achieved even a 1-point increment in their lifestyle scores. Besides, the impact was greater for the elderly than for the middle-aged. However, lifestyle variables other than those chosen in the present study may have some greater impact on mortality. Further studies are thus warranted to promote evidence-based health plans with lifestyle factors.

The strong points of our study were: 1) a large-scale cohort with subjects from all over Japan including more than 8000 deceased; 2) long follow-up period of about 12.5 years; 3) multiple lifestyle variables collected at baseline; and 4) adjusting for potential confounders as much as possible. These advantages allowed us to

estimate healthy lifestyle impacts on all-cause mortality among middle- and older-age groups separately, while adjusting for various factors. The total lifestyle score we adopted was simple, understandable, easy to calculate without any sort of clinical test, and corresponding to lifestyle improvement. Thus, it may serve to motivate both individual and health promoters.

Our study has some limitations. First, we cannot rule out the possibility of a confounding effect by other unknown factors. For example, seat belt use (Cummins et al., 2008; Kerwin et al., 2006) and fat intake (Meng et al., 1999; Stampfer et al., 2000) have also been shown to be associated with all-cause mortality. Unfortunately, we do not have any information on traffic safety behaviors, and nutrition assessment requires complex calculation. Second, measurement errors may be inevitable in the assessment of lifestyle variables because all data were self-reported. However, given the prospective design of our study, misclassification of health status was more likely to occur at random and the estimated HRs might approach null. Although the dichotomous categorization of lifestyle factors was crude and might underestimate the true effect of the various risk factors, our simple lifestyle score can be easily applied to population for lifestyle changes. Third, the data collection was done at baseline only, so behavior changes could not be taken into account. However, Kawado et al. (2005) examined baseline and interim data about 5 years later among some of our participants, and found decreases in smoking and drinking habits. If such behavioral changes occurred not only on smoking and drinking habits but on other lifestyle factors, each lifestyle change in a healthier direction might diminish the differences between healthy and unhealthy groups at baseline, and observed HRs might be diminished according to these changes. A large-scale cohort study with repeated measurements of lifestyle factors will be required to investigate the real relationship between healthy lifestyle factors and mortality. Fourth, about 30% of the subjects excluded from our analyses due to missing data were older than those included, and more likely to be women because of missing data on smoking or drinking. However, even if subjects were decreased at baseline due to missing answers, internal comparison did not alter and results may not be influenced, as no biases occurred on the subjects followed. Fifth, because the study is an observational one and lifestyle behaviors might be interrelated, some lifestyles treated here may not have been direct causes of mortality but rather markers of separate health-related factors. Thus, we must bear in mind that giving some lifestyle behaviors a healthier orientation did not directly translate into a reduction in the mortality risk of each subject and/or a decline in the number of all-cause deaths in society.

Conclusions

Our large-scale cohort study on 62,106 Japanese subjects aged 40–79 years indicated that baseline healthy lifestyle combination (not currently smoking, not heavily drinking, walking 1 h or more per day, sleeping 6.5 to 7.4 h per day, eating green-leafy vegetables almost daily and BMI between 18.5 and 24.9) was associated with a linear decrease in the risk of all-cause mortality among both men and women, as well as among both the middle-aged and elderly. Moreover, if subjects manage to improve their lifestyle by even just one variable, 24.7% of deaths among men and 18.5% among women can be prevented.

Member list of the JACC Study group

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Funding

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho), and Grants-in-Aid for Scientific Research on Priority Areas of Cancer, as well as Grants-in-Aid for Scientific Research on Priority Areas of Cancer Epidemiology from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Monbu-Kagaku-sho) (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022 and 18014011).

Conflict of interest statement
The authors declare that there are no conflicts of interest.

Acknowledgments

The authors wish to express their sincere appreciation to Dr. Kunio Aoki, Professor Emeritus of the Nagoya University School of Medicine and former chairman of the JACC Study, to Dr. Haruo Sugano, former Director of the Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study, and to Dr. Yoshiyuki Ohno, Professor Emeritus of the Nagoya University School of Medicine, who was the ex-chairman of the study. We are also greatly indebted to Dr. Tomoyuki Kitagawa of the Cancer Institute of the Japanese Foundation for Cancer Research and former chairman of the Grant-in-Aid for Scientific Research on Priority Area 'Cancer' and to Dr. Kazao Tajima, Aichi Cancer Center and previous chairman of the Grant-in Aid for Scientific Research on Priority Area of Cancer Epidemiology for their warm encouragement and support of this study.

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Mutation Research 679 (2009) 79–83

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Short communication

Cigarette smoking and serum soluble Fas levels: Findings from the JACC study

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ARTICLE INFO

Article history:
Received 5 November 2008
Received in revised form 18 July 2009
Accepted 5 August 2009
Available online 12 August 2009

Keywords:
Soluble Fas
Inflammation
Cigarette smoking
Male

ABSTRACT

Cigarette smoking enhances low-grade systemic inflammation in the lung and other organs. Activated immune cells play an important role at early and late stages of inflammation, and in recent years, soluble Fas (sFas), an isoform of death molecule Fas, was found to interfere with the apoptotic pathways of these activated immune cells. The aim of this study was to confirm the association between cigarette smoking and sFas levels in healthy male subjects. We measured serum sFas levels of 4415 male subjects selected as controls for a nested case-control study within the large-scale cohort study conducted in Japan, called the JACC Study. Smoking status at baseline was evaluated by a self-administered questionnaire. Least square means of sFas according to smoking status and numbers of cigarettes smoked per day among smokers were calculated and adjusted for possible confounding factors. Mean sFas levels showed an increasing trend across never smokers, past smokers and current smokers, as 2.21 (95% CI: 2.14–2.27) ng/ml, 2.29 (2.22–2.36) ng/ml, and 2.36 (2.30–2.43) ng/ml, respectively. However, no dose-response relationship was observed between the number of cigarettes smoked per day and sFas levels among smokers.

1. Introduction

Cigarette smoking is known to affect many diseases such as autoimmune diseases [1], chronic obstructive pulmonary disease (COPD) [2], and cardiovascular diseases [3]. One plausible underlying mechanism is that cigarette smoking enhances inflammation status in the lung and other organs. In 2007, Yanbaeva and colleagues reviewed systemic effects of long-term smoking from the standpoint of inflammation [4]. They concluded that a low-grade systemic inflammatory response is evident in smokers as confirmed by elevated levels of important markers of inflammation and auto-immunity such as C-reactive protein (CRP), fibrinogen, and interleukin-6, as well as an increased white blood cell count. In recent studies, soluble Fas (sFas), an isoform of death molecule Fas, was found to be a marker of inflammation in hyperthyroidism [5], systemic lupus erythematosus [6], atherosclerosis in end-stage renal disease [7], or dialysis patients [8]. An inflammatory response comprises several steps beginning with molecular clues for tissue penetration by microbes or tissue injury and terminating with healing of tissue damage. If, at any step, progress to the next step is blocked, the inflammatory process may detour into a holding pattern-persistent inflammation [9]. Activated immune cells play an important role in the early and late stages of inflammation, and sFas may interfere with the apoptotic pathways of these cells [10]. Thus, sFas *per se* is thought to play an important role in dysregulation of inflammatory responses that often result in persistent inflammation, possibly by altering apoptotic pathways in activated immune cells as well as remodeling of damaged tissues [11,12]. Therefore, serum sFas may serve as a novel non-specific biomarker for persistent inflammation and disturbed recovery from tissue damage.

To our knowledge, the relationship between smoking and serum sFas levels has not been studied. The aim of the present study was to evaluate the association of smoking with the serum sFas levels in healthy subjects.

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doi:10.1016/j.mrgentox.2009.08.002

2. Materials and methods

2.1. Study subjects

The investigation of the relationship between smoking status and serum sFas levels was conducted in the controls of a nested case-control study within the Japan Collaborative Cohort (JACC) Study, an ongoing large-scale cohort study in Japan initiated between 1988 and 1990. The details of the JACC Study have been described elsewhere [13,14]. In brief, it consisted of 110,792 subjects, aged 40–79 years at baseline living in 45 municipalities all over Japan. At baseline, information on lifestyle factors was collected using self-administered questionnaires. At the same time, blood samples were donated from a part of the cohort members living in 37 areas. Serum samples were stored in deep freezers at −80 °C until analyses. Informed consent to serum donation and its research use was obtained from each participant in 32 areas, though consent was given only by the leader of the area in five areas. The whole study design and use of serum were approved by the Ethical Board of Nagoya University School of Medicine, where the central office of the JACC Study is located.

For the nested case-control study, which was planned to clarify some serum components and mortality, we found 2142 deaths from all-causes up to 1997 and 764 cancer incident cases up to 1994 among subjects whose sera were available at baseline [15]. For each case, we randomly selected 3–4 controls from all members who were still alive at the occurrence of a case event (death or cancer onset), matching them for gender, age (as nearly as possible) and residential area. In this study, the subjects used were the controls selected for the nested case-control study.

The subjects were divided into three groups: (1) smokers, (2) past smokers and (3) non-smokers, according to the answers to the self-administered questionnaire at baseline. The number of cigarettes smoked per day by each smoker was recorded. Since the number of female smokers was few in our cohort (2.9% current smokers and 1.8% past smokers among the selected controls), they were excluded from the analysis.

2.2. Detection of serum sFas

The serum sFas levels were measured by enzyme-linked immunosorbent assay (ELISA), using commercially available kits (MBL Co., Ltd., Nagoya), as described in detail elsewhere [16]. All samples were detected in the same laboratory. The range of the assay for serum sFas levels was 1.0–10 ng/ml; the intra- and inter-assay precisions were 2.1–5.5% and 8.2–12.3%, respectively. Since sFas levels were systematically low in one area, all sera from that area were excluded from the analysis. Those who experienced cancer, stroke or/and myocardial infarction or who showed sFas levels greater than 10 ng/ml were also excluded because of the possibility of undetectable diseases, such as cancer [17], rheumatic [18] or thyroid [5] diseases which were known to be associated with rising sFas level. After the above exclusion, 4415 males were eligible for the present analysis with information on smoking status.

2.3. Analytical method

Baseline characteristics were compared according to smoking status using Cochran-Mantel-Haenszel statistics, adjusting for study area and age category. Since serum sFas levels had logarithmic distributions, all tests and estimations were conducted using log-transformed levels. Least-squares means of sFas according to smoking status were calculated while controlling for possible confounding factors. Among smokers, we estimated simple correlation coefficients between the number of cigarettes smoked per day and serum sFas levels as well as least-squares means of sFas according to the number of cigarettes smoked. Variables adjusted for in multivariate analysis when testing associations between smoking and sFas levels were age at baseline, area, body mass index (BMI;<18.5, 18.5–24.9, ≥25.0, and unknown), alcohol consumption (current drinkers, quitters, non-drinkers, and unknown), walking (walking equal to or more than 1 h per day, walking less than 1 h per day, and unknown), education (≤15 years old, 15–18 years old, >18 old, and unknown), marital status (married, not married, and unknown) and consumption of green leaf vegetables (within 1–2 times per week, 3–4 times per week, almost daily, and unknown). All p values were two-sided, and all statistical analyses were performed using the Statistical Analysis System (SAS 9.1, Cary, NC).

3. Results

Table 1 showed distribution of subjects' baseline characteristics according to smoking status. Smokers were younger, thinner, more frequent drinkers, walking less, eating less green leaf vegetables, and the proportion of the smokers with spouse was low, as compared with never smokers.

Distribution of subjects according to sFas levels and smoking status was shown in Table 2. The highest quartiles of sFas levels were most often observed among smokers, secondary past smokers, then never smokers. Mean sFas levels adjusted for possible confounding factors showed a trend toward increasing across never smokers, past smokers, and current smokers, as 2.21 (95% CI: 2.14–2.27) ng/ml, 2.29 (2.22–2.36) ng/ml, and 2.36 (2.30–2.43) ng/ml, respectively. These mean levels of smokers and past smokers were statistically higher than those of never smokers. No dose-relationship was observed between the number of cigarettes smoked per day and sFas levels among smokers (correlation coefficient = −0.03, $p = 0.17$). However, the adjusted mean levels of sFas increased as the number of cigarettes rose to 20 cigarettes smoked per day and then seemed to peak (Table 3).

4. Discussion

Using data of 4415 apparently healthy men, we found that sFas levels were statistically significantly elevated among current smokers compared with never smokers. This is, to our knowledge, the first report of a positive association between cigarette smoking and serum sFas levels in apparently healthy individuals.

Cigarette smoking triggers various inflammatory responses in the lung and other tissues. The injury induced by direct chemical exposure in the lung, which is the primary target of inhaled smoke, the activation and release of inflammatory cells into the circulation, and an increase in circulating inflammatory mediators characterize the systemic inflammation in smokers [4]. In the late phase of normal inflammation, the clearance of immune complexes and cellular debris occurs to repair injured tissues [9]. sFas may interfere with this apoptotic pathway of activated immune cells and contribute to dysregulated inflammation [11]. Although the present study was not designed to determine the mechanisms underlying the relationship between smoking and sFas levels, its levels could be a novel non-specific biomarker of inflammation among smokers.

To our knowledge, so far the relationship between serum sFas levels and smoking habits among healthy subjects has not been reported. Only Imirzalioglu and colleagues reported that the mean sFas levels in unstimulated saliva of smokers ($N = 13$) was not significantly different from that of non-smokers ($N = 14$) [19]. This was in contrast to our result, but their study sample was small, and measurement of inflammatory markers with saliva is thought to reflect the local inflammatory status of the buccal cavity, not the systemic status [20].

In the present study, no dose-response relationship was found between the number of cigarettes smoked per day and sFas levels among current smokers, though the mean sFas seemed to rise with the number of cigarettes smoked and peaked at 20 cigarettes per day. The reason for this lack of defined dose-response relationship is unclear. However, one plausible interpretation is that, although the production of reactive oxygen species metabolites clearly depends on daily consumption of cigarettes [21], the severity of persistent inflammation may not be directly related to cigarette amounts, due to several immunological steps leading up to the detoured inflammation process [22].

Our data showed that smoking cessation was associated with a decrease in serum sFas levels, but which were still high compared with non-smokers. Although no studies have confirmed this result directly, Wannamethee and colleagues examined the association between years since quitting smoking and inflammatory markers among 2920 British men aged 60–79, and found that most markers improved within 5 years of smoking cessation but took over 20 years to revert to levels of never smokers [23].

The limitations of our study must be discussed when interpreting the results. First, since not all the cohort participants provided blood samples, there was the possibility of a selection bias. However, donation depended solely on the subject's intention, and control selection was only based on matching information to the deceased or cancer cases; age, area and gender. Thus, any bias due to

Table 1
Characteristics of subjects by smoking status[a].

Variables		Never smoker		Current smoker		Past smoker		p value
		N	%	N	%	N	%	
Age distribution								<0.0001[b]
40–49	(%)	65	6.5	161	7.4	49	3.9	
50–59	(%)	259	26.0	527	24.2	218	17.4	
60–69	(%)	445	44.6	1178	54.2	692	55.3	
70–79	(%)	229	22.9	309	14.2	292	23.3	
Mean ± SD		62.7 ± 8.4		61.7 ± 7.6		64.0 ± 7.2		
BMI (kg/m^2)								<0.0001[c]
<18.5	(%)	45	4.5	141	6.5	57	4.6	
≥18.5, <25	(%)	704	70.5	1705	78.4	948	75.8	
≥25	(%)	201	20.1	269	12.4	223	17.8	
Mean ± SD		22.9 ± 2.9		22.1 ± 2.6		22.7 ± 2.7		
Drinking status								<0.0001[c]
Current drinker	(%)	672	67.3	1623	74.6	913	73.0	
Quitter	(%)	31	3.1	80	3.7	83	6.6	
Walking								<0.05[c]
≥1 h/day	(%)	408	40.9	868	39.9	497	39.7	
Education (attained age)								0.1[c]
≤15	(%)	490	49.1	1082	49.7	573	45.8	
15–18	(%)	335	33.6	746	34.3	425	34.0	
>18	(%)	111	11.1	247	11.4	202	16.1	
Mean ± SD		16.1 ± 3.0		16.0 ± 2.4		16.4 ± 2.4		
Marital status								<0.05[c]
Spouse	(%)	756	75.8	1487	68.4	935	74.7	
No spouse	(%)	31	3.1	85	3.9	53	4.2	
Consumption of green leaf vegetables								<0.01[c]
Within 1–2 times/week	(%)	236	23.6	616	28.3	331	26.5	
3–4 times/week	(%)	239	23.9	423	19.4	283	22.6	
Almost daily	(%)	275	27.6	516	23.7	370	29.6	
All		998	100.0	2175	100.0	1251	100.0	

[a] Subtotals were not 100% because of missing values.
[b] Performed by Cochran-Mantel-Haenszel statistics adjusted for area.
[c] Performed by Cochran-Mantel-Haenszel statistics adjusted for area and age category.

blood donation or selection would not seriously affect our results. Second, serum samples were stored for approximately 10 years at −80 °C. The stability of sFas in our cohort samples could not be determined because their levels were not measured at baseline. However, Ito et al. compared newly collected sera and frozen specimens stored for 9 years gathered from a variety of different individuals, and found no statistically significant difference in the distributions of serum sFas levels [16], indicating that the serum sFas levels remained stable after long-term storage at −80 °C. Third, a causal relationship between cigarette smoking and serum sFas levels cannot be proved with our cross-sectional analysis. It is possible that smoking may exert a variety of effects on inflammation status through heterogeneous mechanisms.

In conclusion, although there was no clear dose-response relationship between the number of cigarettes smoked per day and sFas levels among current smokers, sFas levels were statistically significantly elevated among current smokers compared with never smokers. Inflammation caused by smoking may be one of the possible explanations for the elevated serum sFas levels.

5. Member list of the JACC study group

The present members of the JACC Study who co-authored this paper together with their affiliations are as follows: Dr. Akiko Tamakoshi (present chairperson of the study group), Aichi Medical University School of Medicine; Drs. Mitsuru Mori and Fumio Sakauchi, Sapporo Medical University School of Medicine; Dr. Yutaka Motohashi, Akita University School of Medicine; Dr. Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr. Yosikazu Nakamura, Jichi Medical School; Dr. Hiroyasu Iso, Osaka Univer-

Table 2
Smoking status and sFas level.

	Never smoker		Current smoker		Past smoker	
	N	%	N	%	N	%
sFas levels (ng/ml)						
<1.8	298	29.9	454	20.9	288	23.0
1.8–2.2	268	26.9	612	28.1	343	27.4
2.3–2.6	220	22.0	499	22.9	296	23.7
≥2.7	212	21.2	610	28.0	324	25.9
Total	998	100.0	2175	100.0	1251	100.0
Cochran-Mantel-Haenszel p value[a]						p < 0.0001
Least square means[a] (ng/ml)	2.21		2.36		2.29	
95% CI	(2.14–2.27)		(2.30–2.43)		(2.22–2.36)	
p value[b]			<.0001		0.0009	

[a] Adjusted for area, age category, BMI, drinking status, walking, education, marital status, consumption of green leaf vegetables.
[b] Compared with never smoker.