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Table1. Age distribution and age adjusted hazard ratios and 95% confidence intervals of ovarian cancer death

Age at baseline survey	Category	Subjects (%)	Person-Years	Cases	HR[a]	95% CI[b]	*P* Value
57.8±10.1 years	40–49	15,391 (24.2)	217,332	15	1.00		
(Mean±Standar deviation)	50–59	19,720 (31.0)	272,028	23	1.23	0.64–2.35	0.54
	60–69	19,391 (30.5)	250,328	25	1.45	0.77–2.76	0.25
	70–79	9,039 (14.2)	103,825	14	1.99	0.96–4.12	0.07
	Total	63,541 (100)	843,513	77		Trend *P* = 0.06	
Items	Categories	Subjects (%)	Person-Years	Cases	HR[c]	95% CI	*P* Value
Age at onset of menarche	≦14	24,385 (42.6)	325,980	23	1.00		
	≧15	32,922 (57.4)	435,703	41	1.21	0.71–2.09	0.49
Menopausal state	No	24,424 (38.4)	343,569	27	1.00		
	Yes	39,117 (61.6)	499,944	50	0.89	0.59–1.34	0.58
Number of pregnancy	0	2,001 (3.5)	26,238	6	1.00		
	≧1	54,968 (96.5)	729,606	61	0.38	0.17–0.89	0.03
Number of childbirth	0	2,054 (3.7)	26,756	6	1.00		
	≧1	54,077 (96.3)	716,978	61	0.40	0.17–0.92	0.03
Age at first birth	≦24	26,284 (50.0)	343,096	29	1.00		
	≧25	26,299 (50.0)	352,015	32	1.08	0.65–1.79	0.76
History of sex hormone use	No	45,310 (95.1)	591,603	52	1.00		
	Yes	2,359 (4.9)	30,555	4	1.51	0.54–4.17	0.43
History of cancer in first-degree relatives	No	49,521 (77.9)	663,196	62	1.00		
	Yes	14,020 (22.1)	180,317	15	0.36	0.51–1.58	0.71
Body mass index (BMI)	<18.5	3,728 (6.3)	46,815	6	1.71	0.72–4.06	0.22
	18.5–25.0	42,143 (70.9)	563,614	39	1.00		
	≧25.0	13,545 (22.8)	180,835	21	1.69	1.00–2.87	0.054
Sports activity	Seldom	38,376 (76.1)	502,391	49	1.00		
	≧1–2 hours/week	12,032 (23.9)	156,505	8	0.51	0.24–1.07	0.08
Smoking	No	50,914 (92.7)	679,863	58	1.00		
	Yes	4,013 (7.3)	52,153	3	0.68	0.21–2.18	0.52
Alcohol consumption	No	42,442 (73.8)	561,384	53	1.00		
	Yes	15,044 (26.2)	202,275	12	0.65	0.35–1.23	0.19

a: Hazard ratio (unadjusted), b: Confidence interval
c: HR adjusted for age (category)

Table2. Age adjusted hazard ratios and 95% confidence intervals of ovarian cancer death

Item	Category	Person-Years	Cases(n)	HR	95% CI
Pork	≦1-2 times/month	200,084	13	1.00	
	1-2 times/week	283,399	23	1.27	0.64-2.51
	≧3-4 times/week	137,031	15	1.72	0.81-3.65
					Trend P = 0.16
Beef	Seldom	168,220	13	1.00	
	1-2 times/month	184,023	13	0.92	0.42-1.97
	≧1-2 times/week	220,089	19	1.12	0.55-2.27
					Trend P = 0.73
Chicken	≦1-2 times/month	208,077	15	1.00	
	1-2 times/week	314,075	27	1.21	0.64-2.28
	≧3-4 times/week	147,088	11	1.05	0.48-2.29
					Trend P = 0.84
Ham and sausges	≦1-2 times/month	330,873	27	1.00	
	1-2 times/week	237,364	16	0.84	0.45-1.57
	≧3-4 times/week	118,694	14	1.47	0.77-2.83
					Trend P = 0.38
Egg	≦1-2 times/week	225,785	25	1.00	
	3-4 times/week	229,136	21	0.84	0.47-1.50
	Almost every day	335,309	26	0.71	0.41-1.23
					Trend P = 0.22
Fresh fish	≦1-2 times/week	282,291	29	1.00	
	3-4 times/week	254,766	17	0.66	0.36-1.20
	Almost every day	195,989	18	0.91	0.51-1.65
					Trend P = 0.63
Dried or salted fish	≦1-2 times/month	203,482	10	1.00	
	1-2 times/week	237,687	18	1.55	0.72-3.36
	≧3-4 times/week	180,423	20	2.30	1.08-4.92*
					Trend P = 0.03
Milk	≦1-2 times/month	194,641	16	1.00	
	1-4 times/week	222,220	17	0.95	0.48-1.88
	Almost every day	350,954	37	1.27	0.71-2.29
					Trend P = 0.35
Cheese	Seldom	323,120	24	1.00	
	1-2 times/month	150,601	12	1.04	0.52-2.10
	≧1-2 times/week	125,328	11	1.16	0.56-2.36
					Trend P = 0.70
Butter	Seldom	301,248	24	1.00	
	1-2 times/month	135,928	10	0.91	0.43-1.90
	≧1-2 times/week	156,440	13	1.03	0.52-2.02
					Trend P = 0.98
Yougurt	Seldom	304,286	24	1.00	
	1-2 times/month	112,854	9	1.02	0.47-2.19
	≧1-2 times/week	157,080	14	1.13	0.58-2.18
					Trend P = 0.73
Cabbage and lettuce	≦1-2 times/week	238,356	26	1.00	
	3-4 times/week	201,132	11	0.50	0.25-1.02
	Almost every day	195,495	17	0.80	0.44-1.48
					Trend P = 0.39
Chinese cabbage	≦1-2 times/month	123,436	4	1.00	
	1-2 times/week	199,001	21	3.22	1.10-9.36*
	≧3-4 times/week	257,323	25	2.95	1.03-8.49*
					Trend P = 0.09

*: P<0.05

Table 2 (*Continued*)

Item	Category	Person-Years	Cases(n)	HR	95% CI
Green leafy vegetables	≦1-2 times/week	232,195	22	1.00	
	3-4 times/week	202,710	20	1.04	0.57-1.90
	Almost every day	231,781	14	0.62	0.32-1.22
					Trend P = 0.18
Carrots and squash	≦1-2 times/week	292,561	27	1.00	
	3-4 times/week	202,927	19	1.02	0.57-1.83
	Almost every day	143,668	9	0.67	0.32-1.43
					Trend P = 0.37
Tomatoes	≦1-2 times/month	209,633	23	1.00	
	1-2 times/week	198,692	15	0.69	0.36-1.33
	≧3-4 times/week	252,104	17	0.62	0.33-1.16
					Trend P = 0.13
Potato	≦1-2 times/week	357,587	33	1.00	
	3-4 times/week	260,027	22	0.90	0.52-1.55
	Almost every day	159,165	16	1.04	0.57-1.89
					Trend P = 0.99
Soybean curd (Tofu)	≦1-2 times/week	236,373	28	1.00	
	3-4 times/week	259,623	22	0.72	0.41-1.26
	Almost every day	241,382	14	0.49	0.26-0.93*
					Trend P = 0.03
Oranges	≦1-2 times/week	217,267	20	1.00	
	3-4 times/week	145,425	6	0.45	0.18-1.11
	Almost every day	267,150	28	1.12	0.63-2.00
					Trend P = 0.60
Fruits other than oranges	≦1-2 times/week	187,879	16	1.00	
	3-4 times/week	153,303	5	0.38	0.14-1.05
	Almost every day	263,453	29	1.29	0.70-2.38
					Trend P = 0.27
Fruit juice	Seldom	139,044	13	1.00	
	≦1-2 times/week	229,181	15	0.69	0.33-1.46
	≧3-4 times/week	206,656	16	0.83	0.40-1.72
					Trend P = 0.67

*: P<0.05

Table3. Hazard ratios adjusted for multiple variables and 95% confidence intervals of ovarian cancer death

Item	Category	HR[a]	95% CI[b]	*P* Value
Dried or salted fish	≦1–2 times/month	1.00		
	1–2 times/week	1.29	0.55–3.03	0.55
	≧3–4 times/week	2.12	0.93–4.85	0.08
			Trend *P* = 0.07	
Chinese cabbage	≦1–2 times/month	1.00		
	1–2 times/week	4.46	1.01–19.6	0.048
	≧3–4 times/week	5.47	1.28–23.4	0.02
			Trend *P* =0.02	
Soybean curd (Tofu)	≦1–2 times/week	1.00		
	3–4 times/week	0.73	0.35–1.52	0.40
	Almost every day	0.46	0.20–1.07	0.07
			Trend *P* = 0.07	

a: Hazard ratio adjusted for age, menopausal state, number of pregnancy, history of sex hormone use, BMI, and sports activity (categories in Table1)

b: Confidence interval

LETTER TO THE EDITOR

Helicobacter pylori Infection as an Essential Factor for Stomach Cancer

Asian Pacific J Cancer Prev, 7, 163

To the Editors,

A recent article (Wu et al., 2005) provided state-of-the-art information on the relationship between *Helicobacter pylori* (*H. pylori*) and stomach cancer. It is particularly useful for understanding the biology and mechanisms regarding the virulence of *H. pylori* (Covacci et al.,1999; Hatakeyama and Higashi, 2005) and host genetic polymorphisms (El-Omar et al., 2000; Graham and Graham, 2002) which impact on defence against the bacterium, which may of course play a crucial role in gastric carcinogenesis.

In this context we ned to stress the very low gastric cancer incidence rates observed in Yogyakarta and Semarang which appear to be due to the rarity of appreciable *H. pylori* infection (Tokudome et al., 2005a, b). The bacterium seems to be an egg, without which nothing can happen. This is in direct line with earlier findings suggestive that *H. pylori* is essential and necessary for gastric carcinogenesis, at least, for non-cardia gastric adenocarcinoma (Uemura et al., 2001; Brenner et al., 2004).

From the etiological standpoint of stomach cancer, we therefore need to make a radical paradigm shift away from the general emphasis on lifestyle-related cancer, with risk factors including smoking, consumption of salt, and low intake of vegetables and fruit (World Cancer Research Fund/ American Institute for Cancer Research,1997; IARC, 2004) towards infectious disease. *H. pylori* should be placed at the top of the environmental factors, not parallel with them (Wu et al., 2005).

Thus, for the practical prevention of gastric cancer, the infection route/vehicle of *H. pylori* must be explored and pinpointed for infection control. Development of a vaccine to be applied during early childhood is also urgently required. An effective eradication strategy for infected people must be put in place. Periodic screening using gastroscopy should be launched along with consultation on modulations of the related environmental/behavioral/lifestyle factors for patients for whom eradication treatment proves unsuccessful or who have already developed chronic atrophic gastritis.

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ORIGINAL ARTICLE

Ningen Dock 2006 ; **20** : 35−39

Longitudinal Changes in Medical Examination Data of Ex-Smokers in Comparison with Smokers and Non-Smokers

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Objective This study was aimed to clarify differences in the medical examination data of ex-smokers in reference to those of smokers and non-smokers.

Methods From 19410 males who underwent medical check-ups at Kasugai City Medical Care Center between April 1994 and March 2002, an ex-smoker group (93 subjects), a smoker group (135 subjects) and a non-smoker group (120 subjects) were defined. The following data were recorded for people in each of the three groups in four consecutive annual examinations : body weight, body mass index, body fat percentage, blood pressure, total cholesterol, HDL cholesterol and triglyceride concentration. Changes in these measurements over time were calculated for each group and their differences were compared.

Results The body weight, body mass index, body fat percentage, blood pressure, total cholesterol and HDL cholesterol remained roughly the same or increased slightly for the smoker and non-smoker groups. No remarkable differences were observed in any data categories for the ex-smoker group between the year prior to smoking cessation and the year after cessation. However, significant increases were measured after 1 year in the following : 1.3 kg in body weight, 0.5 kg/m^2 in body mass index, 0.7% in body fat percentage, 1.7 mmHg in both systolic and diastolic blood pressure, 8.6 mg/dl in total cholesterol and 2.9 mg/dl in HDL cholesterol. Increased blood pressure and total cholesterol were correlated strongly with increased body fat.

Conclusion It appears necessary for medical practitioners to advise clients to quit smoking and provide guidance regarding the importance of minimizing gains in body weight and body fat which lead to hypertension or hyperlipidemia after quitting smoking. (*Ningen Dock* 2006 ; **20** : 35−39)

Key Words : longitudinal study, smoking cessation, body fat, body weight

A considerable amount of research has been conducted in Japan and overseas on the harmful effects of smoking. Research articles have revealed that smoking causes serious damage to health[1-3], harming all vital organs and causing various diseases including cancer and ischemic heart disease[4-9]. According to the World Health Organization (WHO), approximately 5 million people die from smoking-related illnesses each year in the world, about half of whom are middle-aged in the prime of their lives[10]. WHO predicts that this number will rise remarkably unless the number of smokers declines[10].

It is estimated that 100000 people die each year in Japan as a result of smoking ; anti-smoking measures have been implemented in various places throughout the country[11,12]. In addition, public concern over smoking has increased with the enactment of the Health Promotion Law in May 2003, which mandates the management of public facilities to take necessary measures to prevent users' exposure to environmental tobacco smoke.

We conducted a longitudinal study in which we collected data from annual medical examinations of local residents and compared ex-smokers' data with those of smokers and non-smokers. We also studied factors that are associated with changes in parameters over time.

Methods

The study was carried out on a total of 19410 males who had undergone a comprehensive medical examination at Kasugai City Medical Care Center between April 1994 and March 2002 who were not receiving treatment for a disease. Three groups were defined by a questionnaire as follows : the ex-smoker group was defined as those who had given up smoking following a comprehensive examination and had undergone three consecutive examinations during the time which they had not smoked ; the smoker group was defined as those who had smoked at least 10 cigarettes for a period of at least 10 years and who had undergone at least four consecutive examinations ; and the non-smoker group was defined as those who

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Table 1. Characteristics of the study subjects at baseline

	Ex-smoker group	Smoker group	Non-smoker group
Number	93	136	120
Age	56.9 ± 8.2	56.9 ± 8.7	57.2 ± 7.4
Body weight (kg)	62.2 ± 8.0	63.2 ± 9.7	61.9 ± 8.7
Body mass index (kg/m^2)	22.7 ± 2.4	22.8 ± 2.6	22.4 ± 2.4
Body fat (%)	21.0 ± 5.0	21.3 ± 4.6	20.3 ± 4.6
Systolic blood pressure (mmHg)	72.0 ± 9.5	70.0 ± 9.4	73.3 ± 7.9
Diastolic blood pressure (mmHg)	118.5 ± 13.4	116.7 ± 13.9	121.0 ± 12.9
Total cholesterol (mg/dl)	198.8 ± 30.3	198.7 ± 33.9	204.5 ± 31.2
HDL cholesterol (mg/dl)	53.5 ± 16.1	51.1 ± 13.2	58.2 ± 14.9
Triglycerides (mg/dl)	149.2 ± 116.0	152.4 ± 194.1	120.9 ± 68.0

Mean ± SD

had never smoked at all and had undergone at least four consecutive examinations. The first year, year 0, was defined as the examination immediately prior to giving up smoking for members of the ex-smoker group, and arbitrarily for the other two groups.

The following data were taken for people in all three groups from examinations in years 0 to 3 (i.e., four consecutive examinations) : body weight, body mass index, body fat percentage, blood pressure, total cholesterol, HDL cholesterol and triglyceride concentration. Changes in these measurements over time were calculated for each group and differences were statistically analyzed. Additionally, for the ex-smoker group, the correlation between measurement differences from year 1 (first examination after stopping smoking) to year 2 was analyzed. Statistical analyses were carried out using the Statistical Analysis System (SAS) (Windows ver. 8.02 ; SAS Institute Inc., NC, USA).

Results

The ex-smoker group comprised 93 people, the smoker group 136 people and the non-smoker group 120 people. The respective groups had similar age distributions : 48–49% between the ages of 60 and 69, 26–28% between 50 and 59, and 24–25% between 40 and 49. Almost half the people in each group were between the ages of 60 and 69. **Table 1** shows that the mean ages and standard deviations (SDs) at the time of the first examination (year 0) were very similar for all three groups. Also, other medical examination data were not significantly different between the groups.

Fig. 1 shows that the body weight, body mass index, body fat percentage, blood pressure, total cholesterol, and HDL cholesterol stayed roughly the same or increased slightly for the smoker group and the non-smoker group. No significant differences were observed in any data categories for the ex-smoker group, with the exception of triglycerides, between year 0 (prior to stopping smoking) and year 1 (after stopping smoking), as had been observed with the other two groups. However, significant increments of 1.3 kg in body weight (95% CI : 0.82–1.80), 0.5 kg/m^2 in body mass index (95% CI : 0.28–0.63), 0.7% in body fat percentage (95% CI : 0.20–1.17), and 1.7 mmHg in diastolic blood pressure (95% CI : 0.10–3.30) were detected between years 1 and 2. The changes of 1.7 mmHg in systolic blood pressure, 8.6 mg/dl in total cholesterol and 2.9 mg/dl in HDL cholesterol were not significant between years 1 and 2. This phenomenon did not occur from years 2 to 3 : measurements either remained unchanged or increased slightly, as had been found between years 0 and 1. No definite trends were observed for triglycerides.

Table 2 shows the correlation between measurement differences for the ex-smoker group. Between years 1 and 2, a significant positive correlation was found in the respective differences between body fat percentage and each of systolic blood pressure, diastolic blood pressure and total cholesterol. Such a correlation was also found between body weight and total cholesterol. Between years 2 and 3, a significant positive correlation was found for differences in body fat percentage and systolic blood pressure and for differences in body weight and systolic blood pressure.

Fig. 2 shows differences in both systolic blood pressure and total cholesterol for ex-smokers between years 1 and 2 for five different subgroups, determined by changes in body fat percentage over the same period. Apparently, changes in systolic blood pressure and total cholesterol were slight for people whose changes in body fat percentage were small.

Discussion

It has been reported that smoking actually lowers the risk of developing certain diseases such as ulcerative colitis[13], Parkinson's disease[14] and Alzheimer's disease[15]. However, the research methods employed in those studies might be deficient[16-18]. Far more numerous studies have indicated that smoking harms all vital organs and causes various diseases[1-9] than have shown that smoking reduces the risk of certain diseases[13-15]. Furthermore, various academic societies are opposed to smoking and have adopted anti-smoking measures. This society has also declared an opposition to smoking and has stepped up initiatives in line with the contents of the WHO Framework Convention on Tobacco Control.

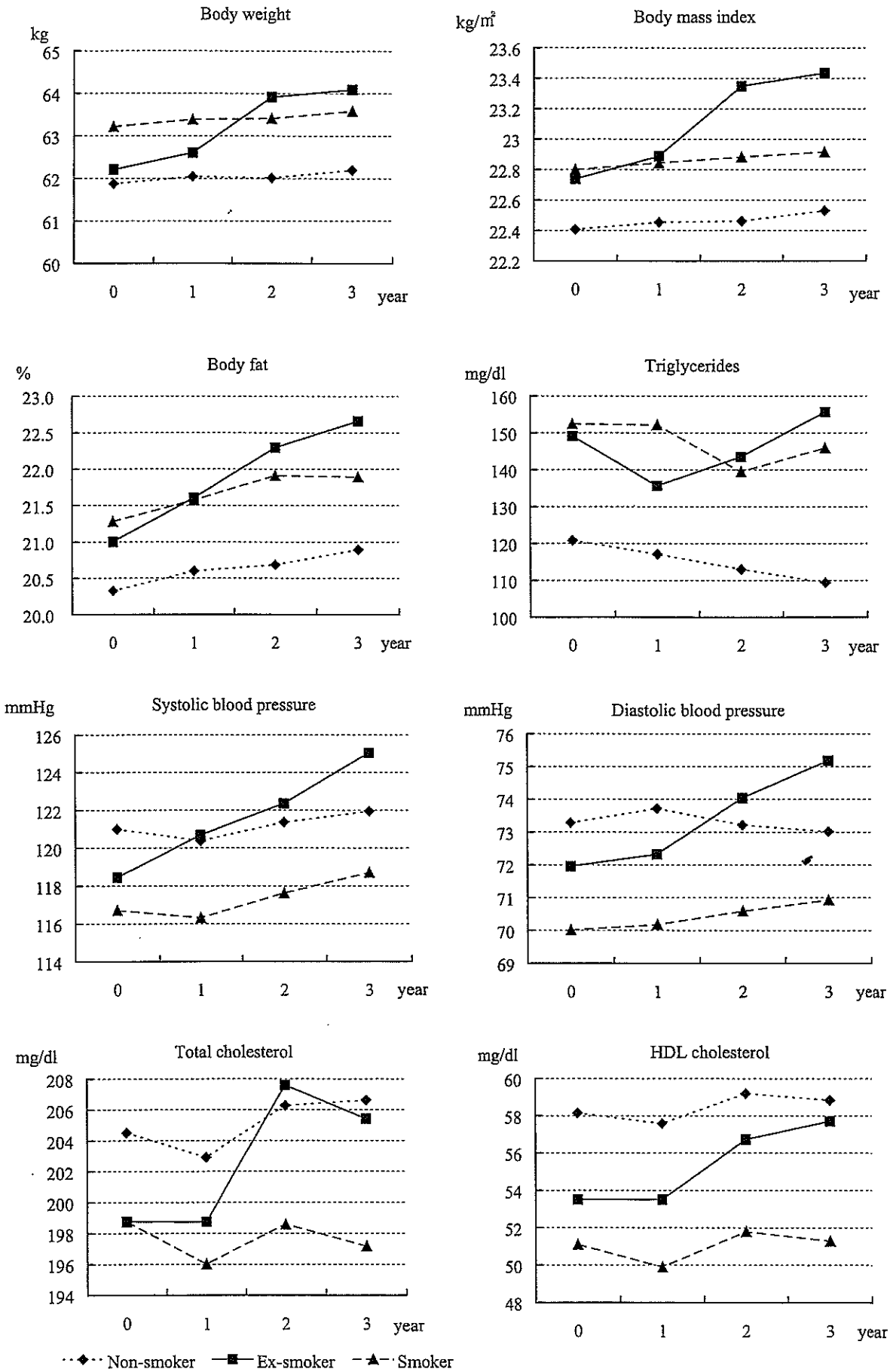

Fig. 1. *Temporal changes of measurements of each examination*

Table 2. Correlation coefficients of body weight and body fat vs. blood pressure and plasma cholesterol among ex-smokers

	Between year 1 and year 2		Between year 2 and year 3	
	Body weight	Body fat	Body weight	Body fat
Systolic blood pressure (mmHg)	0.11567	0.22524*	0.25524*	0.34389**
Diastolic blood pressure (mmHg)	0.06993	0.20906*	0.01840	0.19471
Totel cholesterol (mg/dl)	0.23008*	0.20966*	0.01162	0.17166
HDL cholesterol (mg/dl)	−0.05449	0.07313	0.17434	−0.00380

*$p<0.05$ **$p<0.001$

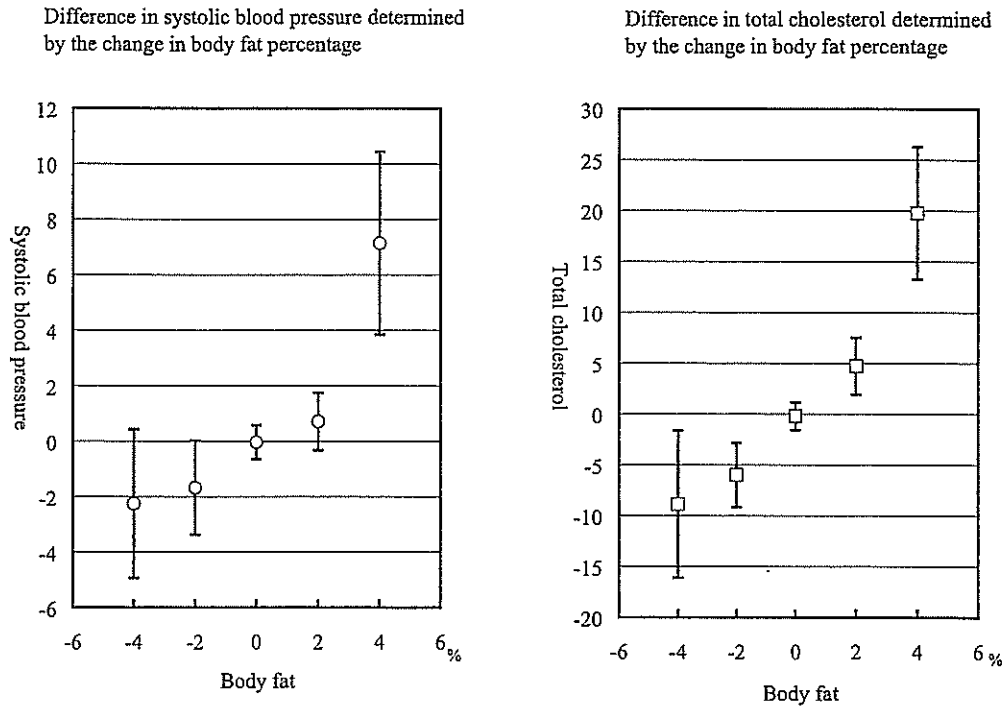

Fig. 2. *The difference in both systolic blood pressure and total cholesterol between years 1 and 2 by change fat percentage for ex-smokers*

Smoking affects not only smokers' health, but also the health of people around them. The effects of passive smoking[19-21] are so serious that prohibiting smoking in public places has become an important social priority.

Our research showed that increases in body weight, body fat percentage, blood pressure and cholesterol levels occur as a result of smoking cessation. A positive correlation was found between changes in the body fat percentage and both changes in blood pressure and total cholesterol for the ex-smoker group, indicating that changes in blood pressure and total cholesterol were slight for people whose changes in body fat percentage were small. These results suggest that increases in blood pressure and total cholesterol are attributable to increases in body fat percentage and body weight and not simply due to stopping smoking.

Sato *et al.*[22] reported significant mean increases of 1.5 kg in body weight, 7.7 mg/dl in total cholesterol, 1.5 mmHg in diastolic blood pressure and 2.0 mmHg in systolic blood pressure after quitting smoking. They also reported that the increases in total cholesterol and blood pressure were higher for people whose body weight had increased significantly. These findings are consistent with the results of our research. Body weight is inferred to increase after smoking cessation because of the heightened appetite of ex-smokers caused by the recovery of taste and smell[23,24]. Moreover, smoking cessation causes the absence of tobacco components that raise metabolic rates and thereby burn body fat[25]. The weight gain that occurs after smoking cessation is perceived as natural and indicative of the body returning to health after breaking away from smoking effects. However, gaining too much weight can damage health.

For the reasons explained above, using comprehensive medical tests to encourage smokers to give up smoking will benefit their health. However, it is insufficient simply to tell people to stop smoking. It is

necessary to monitor changes in measurements such as body fat percentage and provide suitable guidance after cessation to enhance health-related benefits.

Conclusion

Encouraging smokers who are undergoing comprehensive medical examinations to give up smoking is crucial for their health. However, our research suggested that it is also necessary for medical practitioners to provide guidance and instruction about the importance of minimizing increases in body weight and body fat to prevent hypertension or hyperlipidemia after giving up.

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cancer. The authors found an inverse association between circumcision and cervical cancer among women whose husbands had engaged in high-risk sexual behavior. These results are consistent with the HPV link to circumcision and support their conclusion of an inverse association between circumcision and penile HPV infection. It is obvious that circumcision cannot reduce the risk of acquiring or transmitting HPVs in or from the penile shaft or the scrotum. However, by removing the foreskin, the potential sites for HPV entry and/or transmission are reduced. Furthermore, it is likely that the risk of transmission to the cervix of HPVs in the mucosal part of the prepuce, and in the coronal sulcus and glans is higher than that of HPVs detected in the skin of the shaft or the scrotum. For all these reasons, failure to collect samples from the skin does not invalidate the results of a reduced risk of cervical cancer linked to the circumcision status of the husband. What Castellsagué et al.'s results may imply is that HPVs, as detected in the penile shaft and scrotum, are not that relevant to transmission or that they do not increase the risk of cervical cancer in the female partner. If they did, their study would not have detected such a strong protection.

Correct classification of exposure and outcome categories is essential in epidemiologic studies. We concur with the suggestion that clinicians who participate in future studies on sexually transmitted diseases should be specifically trained to classify the lengths of foreskins (1), and we consider that visual aids should be encouraged to standardize the procedure. Similarly, genital sampling schemes in epidemiologic HPV studies in males should aim at being accurate yet efficient. An overzealous evaluation of the genital area may be invasive to the participant, burdensome to the investigator, costly, and it may prove to be unnecessary.

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Marine *n*-3 Fatty Acids and Colorectal Cancer: Is There a Real Link?

To the Editor: Oh et al. (1) investigated the possible inverse association between consumption of fish/*n*-3 highly unsaturated fatty acids (HUFAs, marine omega-3 fatty acids mainly consisting of icosapentaenoic acid and docosahexaenoic acid) and the risk of colorectal cancer in the Nurses' Health Study, but failed to observe any link. However, we have documented elevated apoptosis in normal colonic membranes in Japanese patients, in the Dietary Intervention in Polypectomized Patients Study, who adhered to our regimen of high fish consumption along with fish oil and perilla oil (rich in α-linolenic acid; ref. 2), suggesting a reduced risk of colorectal adenomas/tumors.

n-3 PUFAs (or *n*-3 HUFAs) compete with *n*-6 PUFAs (or arachidonic acid) in various enzymatic processes and the absolute consumption of *n*-3 PUFAs (or *n*-3 HUFAs) may be crucial for colorectal carcinogenesis (3). The median energy percentages from fish/*n*-3 HUFAs in the study group of Oh et al. were distributed from 0.03 to 0.18, according to quintile categorization (1), while our Japanese Dietitians' Epidemiologic Study noted a distribution of 0.26 to 0.53 (4), indicating that the quantity of fish/*n*-3 HUFAs consumed by Americans is only approximately one-tenth of the Japanese level. Indeed, there is no overlap with each other, the amount of the highest quintile for Americans being less than the lowest quintile for Japanese, as also discussed by the authors. Therefore, the findings in Americans, with an intake possibly insufficient to exert pharmacologic influence, may at least not be applicable to Japanese.

Furthermore, the ratio of *n*-6 PUFAs/*n*-3 PUFAs (or *n*-3 HUFAs) may also be critical for colorectal carcinogenesis. The intake of *n*-6 PUFAs by Americans is exceedingly high and their median ratios of *n*-3 HUFAs/*n*-6 PUFAs appear to be distributed from 0.006 to 0.04 (or *n*-6 PUFAs/*n*-3 HUFAs: 25.0-166.7; ref. 1), whereas those for Japanese are 0.05 to 0.11 (or *n*-6 PUFAs/*n*-3 HUFAs: 9.2-21.1; ref. 4), again with no overlap between the two populations. Plasma phospholipids in Americans would be expected to be highly saturated with *n*-6 PUFAs and the concentrations of *n*-3 HUFAs, even in the highest quintile group, might not effectively compete (5). Accordingly, we would like to stress that *n*-3 PUFAs and/or fish/*n*-3 HUFAs may indeed be favorable for the prevention of colorectal adenomas/tumors in populations, including Japanese, who consume appreciable amounts of fish and other marine foods.

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ORIGINAL PAPER

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Elevated risk of colorectal cancer associated with the AA genotype of the *cyclin D1* A870G polymorphism in an Indian population

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Abstract *Purpose*: To investigate whether the common *cyclin D1* (*CCND1*) A870G polymorphism is a risk factor for colorectal cancer (CRC) in an Indian population. *Methods*: In this study, 301 newly diagnosed CRC patients and 291 healthy control subjects were genotyped by the PCR-RFLP method. Genotype frequencies were compared between cases and controls, and the association of genotypes with CRC was studied. *Results*: The *CCND1* 870 A allele was more frequently observed in CRC patients than controls (0.63 vs. 0.56, $P=0.01$), and after adjustment for age, sex, smoking habits, family history, family income and the consumption of meat, fish, vegetables and fruit, an increased risk was observed for the AA genotype compared to the GG+AG genotype (OR = 1.56; 95% CI: 1.10–2.21). The increased risk were also found for colon (OR = 1.96; 95% CI: 1.08–3.57) and rectal cancer (OR = 1.51; 95% CI: 1.04–2.19). No correlation was observed between genotypes and age of diagnosis of CRC (49.9, 48.7 and 49.4 years for the GG, AG and AA genotypes, respectively; $P=0.84$). Multivariate analysis also revealed a stronger positive association with the AA genotype among patients with high meat intake (OR = 2.67; 95% CI: 1.29–5.51), and particularly significant inverse associations with the GG+AG genotypes were also found for those with high vegetable consumption (OR = 0.46; 95% CI: 0.27–0.79 of 2–3 servings/day, and OR = 0.31; 95% CI: 0.18–0.53 for >3 servings/day) and fish intake (OR = 0.48; 95% CI: 0.28–0.82). *Conclusion*: These data support the hypothesis that the *CCND1* A870G polymorphism may increase the risk of CRC in our Indian population.

Keywords Colorectal cancer · Cyclin D1 · A870G polymorphism

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Introduction

Cyclin D1, a protein encoded by the *CCND1* gene located on chromosome 11q13, is a key regulatory protein for the cell cycle transition from G1 phase to S phase (Sherr 1996), whose overexpression disrupts normal cell cycle control, possibly promoting the development and progression of cancers (Zhou et al. 1996; Wang et al. 1994; Donnellan and Chetty 1998). Furthermore, amplification of the *CCND1* gene and/or aberrant induction of cyclin D1 activity in colorectal cancer tissue have been found to be associated with enhanced cell proliferation and malignant progression in CRCs (Arber et al. 1996; McKay et al. 2000; Sutter et al. 1997; Bahnassy et al. 2004). A single nucleotide adenine-to-guanine substitution (A870G) in the splice donor region of exon 4 has been shown to influence the splicing variation coding for two mRNA transcripts. The G allele tends to produce mostly transcript-a, whereas the A allele is associated with the production of an aberrant splicing product termed

transcript-b which lacks an exon five sequence containing the PEST-rich region, which destabilizes the protein (Betticher et al. 1995). Therefore, A allele leads to a prolonged half-life and increases levels of cyclin D1 protein in cells, in turn promoting cell proliferation (Sawa et al. 1998).

A number of studies have linked the *CCND1* 870 A allele to increased cancer risk (Zhang et al. 2003; Buch et al. 2005; Shu et al. 2005; Shi et al. 2003; Wang et al. 2003; Wang et al. 2002), but the evidence is not entirely consistent (Yu et al. 2003; Ceschi et al. 2005; Cortessis et al. 2003; Forsti et al. 2004), and some controversy exists regarding the effects on CRC development (McKay et al. 2000; Kong et al. 2001; Porter et al. 2002; Le Marchand et al. 2003; Grieu et al. 2003). We have therefore evaluated links between the *CCND1* A870G polymorphism and susceptibility to CRC in an Indian population. In addition, we also investigated whether the association differs due to the location of tumors in either the colon or rectum, and whether the association is modified by dietary or environmental factors.

Materials and methods

Subject selection and data collection

The participants and data collection methods for this study have been described previously in detail (Jiang et al. 2005). Briefly, from 1999 to 2001, we recruited 301 colorectal cancer patients and 291 controls from Chennai and the surrounding area in southeastern India. Cases were recruited at the Madras Cancer Institute in Chennai, India, all enrolled patients with a first diagnosis of histologically confirmed colorectal cancer. Control subjects were cancer-free individuals, consisting of randomly selected attendants to patients having cancers other than CRC during the same time period of case collection. They were frequency-matched to case patients by sex and age (within 5 years). Informed consent was obtained from all study subjects. Trained interviewers collected information on socioeconomic status, medical history, alcohol, smoking, and tobacco chewing habits using a standard questionnaire. A 114 food –and- beverage item food-frequency questionnaire (FFQ) specific to this population was used to measure long-term intake of foods/food groups. All subjects were asked for their average frequency of consumption of food items per week over the past 1-year period (for cases, 1 year before the diagnosis of CRC). After the interview, a 7- ml blood sample was collected from each fasting subject. Soon after the blood sampling, blood was separated by centrifugation at 2,500 rpm for 15 min at 4°C and aliquoted into plasma (four tubes), buffy coat (one tube) and red blood cells (one tube), and immediately stored at −80°C. The study was approved by the internal review board of the Madras Cancer Institute in Chennai.

Genotyping

The DNA samples of the subjects were extracted from peripheral blood leukocytes using a GenTLE solution Kit (TaKaRa, Japan), and analyses were essentially carried out as previously described. (Betticher et al. 1995). To assess *CCND1* genotypes, a 167- bp fragment including the A870G polymorphism was amplified using forward and reverse primers (5′-GTG AAG TTC ATT TCC AAT CCG C-3′ and 5′-GGG ACA TCA CCC TCA CTT AC-3′, respectively). The A870G change creates a restriction site for the ScrF1 enzyme (New England Biolabs, Beverly, MA, USA) with the expected products after digestion with ScrF1 being 167 bp for AA, 145, 22 bp for GG, and 167, 145, 22 bp for AG. For quality-control purposes, 30 randomly selected DNA samples (5% of all samples) were determined by sequencing analysis using a BigDye Terminator Cycle Sequencing Kit, v 3.1 (Applied Biosystems, Foster City, CA, USA) with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) (Kong et al. 2000). There were no discrepancies between the two results.

Statistical analysis

Differences in characteristics between cases and controls were assessed with the chi-square test, as well as disparities in genotype and allele frequencies. The Hardy–Weinberg equilibrium was checked with the chi-square test. One-way analysis of variance was employed to assess differences in age at diagnosis between genotypes in case subjects.

Unconditional logistic regression analyses were performed under a codominant model (risk differing across all three genotypes), a dominant model (subjects with one or two A alleles having the same increased risk) or a recessive model (only subjects with two A alleles at increased risk) to calculate odds ratios (ORs) and confidence intervals (95% CIs) for associations between genotypes and risk of CRC. To estimate dominant or recessive effects of the *CCND1* A870G genotype on CRC risk, log-likelihood statistics of nested and codominant models were compared. Adjustments were made for matching variables (age and sex) and for possible confounders. Covariates were identified as potential confounders by examining their distribution by case–control status. The BMI was excluded from covariates to avoid information bias, as it was affected by cancer in cases. The covariates were included in the model if they changed the ORs by more than 20% or significantly changed the likelihood ratio statistic ($P < 0.05$) on univariate analysis.

To examine the combined effects of *CCND1* A870G genotypes and certain risk factors, stratified analyses were conducted. Criteria for assessing effect modifiers were based on biological plausibility, and whether the risk estimation differed substantially across strata. The