表2　国際がん研究機関(IARC)による禁煙とリスク低下に対する評価

がん種	①禁煙した人のリスクが現在喫煙者より低いか	②禁煙継続によるリスクの低下	③非喫煙者のレベルまでのリスクの低下	備　考
口腔	◎	○	○(20年以上)	一般に禁煙期間の増加に伴いリスクも低下。
鼻咽頭・副鼻腔	○	—	—	
食道	◎	○	○(20年以上)	リスク低下は扁平上皮がんで認められる。禁煙後長期間(20年以上)リスクは高い。禁煙後10年で非喫煙者の2倍までリスクが下がる。腺癌に関する研究結果は少なく，関連を判断するには不十分。
胃	◎	○		禁煙期間の増加に伴いリスクも低下し，禁煙した年齢が若いほど大きく低下する。
肝	○	—	—	リスクは低下すると思われるが，地域によって研究結果に違いがある。
膵臓	◎	○	○(15年以上)	研究報告が少ない。
喉頭	◎	◎	○(20年以上)	禁煙後，急速にリスクは低下する。10～15年で約60%低下。20年以上かけて非喫煙者レベルに達する。
肺	◎	○	△	リスクは5～9年で低下を始め，長い期間かかり徐々に低下，禁煙後長期間経っても非喫煙者レベルより高い。
子宮頸部	◎	◎	◎	リスク低下は扁平上皮がんで認められる。禁煙によって非喫煙者のリスクレベルまで急速に下がる。
腎細胞	◎	○	○(20年以上)	研究報告が少ない。
膀胱	◎	○	○(25年以上)	
骨髄性白血病	○	—	—	リスクは低下すると思われるが，研究結果が一致していない。

①禁煙した人のリスクが現在喫煙者より低いか
　◎：禁煙した人のリスクは現在喫煙者より低い
　○：禁煙した人のリスクは現在喫煙者より低いと考えられる
②禁煙継続によるリスクの低下
　◎：禁煙後，急速にリスクが低下する
　○：禁煙期間の増加に伴いリスクが低下する
　—：情報が不十分
③非喫煙者のレベルまでのリスクの低下
　◎：禁煙後，非喫煙者のレベルまでリスクが下がる
　○：禁煙後，非喫煙者のレベルまでリスクが下がるが，長期間かかる。(　)内はかかる年数
　△：禁煙後，非喫煙者のレベルまでリスクが下がらない
　—：情報が不十分
(空白)：記載なし

出典：国際がん研究機関がん予防ハンドブック，たばこ対策，第11巻，2007年[15]

に，カリフォルニア州環境保護庁の報告書(2005年)[17]と，米国公衆衛生総監報告書(2006年)[18]がある。これら二つの報告書においても，受動喫煙は，タバコを吸わない大人と子どもの両方に，さまざまな病気や早期死亡を引き起こすと結論づけている。

表3は，これら三つの報告書の評価結果をまとめたものである。肺がんについては，三つの報告書とも，受動喫煙との因果関係があると判定している。同居者の喫煙による受動喫煙による非喫煙者の肺がんリスクは1.2～1.3倍であり，職場での受動喫煙による非喫煙者の肺がんリスクは1.1～1.2倍である。鼻腔がんおよび乳がんは，カリフォルニア州環境保護庁報告書のみが因果関係ありと判定している。乳がんについては，前述の通り能動喫煙との関連が否定的であるため，米国公衆衛生総監報告書では，「因果関係が示唆される」という評価にとどまった。小児がんおよび全がんについては，いずれの報告書も因果関係があるとは判定していない。

表3　非喫煙者における受動喫煙とがんとの関連についての評価結果

がん種	①IARC モノグラフ 83巻 (2002年)	②カリフォルニア州環境保護庁報告書 (2005年)	③米国公衆衛生総監報告書 (2006年)	各報告書のその他の記述
肺がん	○	○	○	①配偶者の喫煙による受動喫煙で肺がんリスクは女性で約1.2倍，男性で約1.3倍。職場での受動喫煙で肺がんリスクは1.12～1.19倍。 ③同居者の喫煙による受動喫煙で肺がん死亡リスクは1.2～1.3倍。
頭頸部がん	—	関連を示唆する研究はあるが結論は出せない	—	
鼻腔がん	×	○	△	
鼻咽頭がん	×		データが不十分	
乳がん	×	○*	△	①能動喫煙と乳がんとの間の関連が十分でないことから，受動喫煙との関連の可能性は低い。
子宮頸がん	×	受動喫煙が病因の一つである可能性がある	—	
小児がん	関連が示唆されるが他の要因の影響を否定できない	△‡	△†	
全がん	×	△	—	

○：因果関係がある，△：因果関係が示唆される，×：データが一致しない，—：(評価なし)
*：閉経前若年女性について，†：白血病・リンパ腫・脳腫瘍について，‡：リンパ腫と脳腫瘍について

2．日本人を対象とした研究

日本人を対象とした受動喫煙とがんとの関連は，平山によって夫の喫煙と非喫煙女性の肺がんとの関連が最初に報告された[19]。以後，受動喫煙に関する日本人を対象とした疫学研究は，配偶者など同居者の喫煙による受動喫煙を対象としたものが比較的多い。

表4は，主に非喫煙女性について夫の喫煙による受動喫煙の健康影響を調べた疫学研究のまとめである。肺がんについては，平山研究以後の疫学研究で報告された非喫煙女性の夫からの受動喫煙の相対リスクはおおむね1.3～2.0に分布しており，平山研究や国際機関などの報告と大きな相違はない。組織型別では，近年肺腺がんが受動喫煙とより関連が強いとの報告があり，副流煙が主流煙より肺末梢に到達しやすいこと，腺がんとの関連が指摘されている4-(N-ニトロソメチルアミノ)-1-(3-ピリジル)-1-ブタノン(NNK)の関与などが機序として考えられている(Kurahashi N, et al)。

肺がん以外のがんでは，2000年以降，乳がんを対象としたコホート研究が三つある。これらの結

表4　夫の喫煙または家庭での受動喫煙に関する日本の疫学研究のまとめ

文　献	研究手法	調査年	対象者	エンドポイント	曝露レベル把握方法	調整変数・マッチングなど	曝露形態	疾患・組織型など	曝露レベル・層別など	相対リスク[95%信頼区間]*
Hirayama T BMJ 282： 183-185, 1981	コホート	1965	非喫煙女性 91,540 名	死亡	質問票	年齢，職業	夫の喫煙	肺がん 肺気腫・喘息 子宮頸がん 胃がん 虚血性心疾患	現在喫煙 (20 本/日以上)	2.08(p=0.001) 1.49(p=0.474) 1.14(p=0.249) 0.99(p=0.720) 1.03(p=0.393)
Hirayama T Prev Med 13： 680-690, 1984	コホート	1965	非喫煙女性 91,540 名	死亡	質問票	年齢，職業	夫の喫煙	肺がん 胃がん 鼻腔がん 脳腫瘍 全がん	現在喫煙 (20 本/日以上)	1.91 [1.34～2.71] 1.01 [0.86～1.19] 2.55 [1.04～6.27] 4.32 [1.53～12.19] 1.23 [1.12～1.35]
Akiba S Can Res 46： 4804-4807, 1986	コホート内ケースコントロール	1982	原爆生存者男女。 症例 428 名， 対照 957 名	肺がん罹患	インタビュー	出生年，居住地，性，健診対象者か否かをマッチング	夫の喫煙		非喫煙男性 非喫煙女性	1.80 [0.50～5.60] 1.50 [1.00～2.50]
Inoue R & Hirayama T Smoking and Health 283-285, 1987	ケースコントロール (病院対照)	1980～1983， 1973～1981	女性。症例 37 名， 対照(脳血管疾患) 74 名	肺がん死亡	インタビュー	出生年，死亡年，地域をマッチング	夫の喫煙		非喫煙女性	2.25 [0.91～7.10]
Shimizu H, et al Tohoku J Exp Med 154： 389-397, 1988	ケースコントロール (病院対照)	1982～1985	非喫煙女性 (症例 90 例， 対照 163 例)	肺がん罹患	質問票	病院，年齢，入院日をマッチング	夫の喫煙			1.1(NS)
Sobue T Int J Epi 19： S62-S66, 1990	ケースコントロール (病院対照)	1986～1988	非喫煙女性 (症例 144 例， 対照 713 例)	肺がん罹患		年齢階級と教育歴調整	夫の喫煙			1.13 [0.78～1.63]
Nishino Y, et al Can Causes Cont 12： 797-802, 2001	コホート	1984	非喫煙女性 9,675 名	がん罹患	質問票	年齢，地域，飲酒，緑黄色野菜摂取，果物摂取	夫の喫煙	全がん		1.1 [0.91～1.4]
						十肉摂取，肺疾患既往		肺がん		1.8 [0.67～4.6]
						十肉摂取		結腸がん		1.1 [0.54～2.4]
						十肉摂取		直腸がん		1.9 [0.87～4.2]
						十初産年齢，出産回数，初経年齢，BMI		乳がん		0.58 [0.32～1.1]
Hanaoka T, et al Int J Can 114： 317-322, 2005	コホート	1990～1992	非喫煙女性 20,193 名	乳がん罹患	質問票	年齢，地域，就労状況，教育歴，BMI，乳がん家族歴，良性乳腺腫瘍既往，飲酒，初経年齢，出産回数，ベースライン閉経状態，女性ホルモン使用	家庭		ベースライン時閉経前 ベースライン時閉経後 全例	1.6 [0.9～2.7] 0.7 [0.4～1.1] 1.0 [0.7～1.4]
							家庭または，職場 and/or 公共の場所		ベースライン時閉経前 ベースライン時閉経後 全例	2.6 [1.3～5.2] 0.7 [0.4～1.0] 1.1 [0.8～1.6]
Ozasa K Asian Pacif J Can Prev 8： 89-96, 2007	コホート	1988～1990	非喫煙男性 67,997 人年 非喫煙女性 420,201 人年	肺がん死亡	質問票	年齢，地域	家庭		ほぼ毎日 3 時間/日以上 時々，1～4 時間/週 ほぼ毎日 3 時間/日以上 時々，1～4 時間/週	0.45 [0.09～2.22] 5.29 [1.03～27.20] 1.48 [0.57～3.84] 1.06 [0.68～1.64] 1.12 [0.55～2.28] 0.84 [0.49～1.46]
Lin Y, et al J Epi 18： 77-83, 2008	コホート	1988～1990	非喫煙女性 32,023 名	乳がん罹患	質問票	年齢，地域，BMI，乳がん家族歴，飲酒，日常歩行，初経年齢，初産年齢，出産回数，ベースライン閉経状態，女性ホルモン使用	家庭		時々 ほぼ毎日	0.59 [0.33～1.05] 0.71 [0.48～1.05]
Kurahashi N, et al Int J Can 122： 653-657, 2008	コホート	1990，1993	非喫煙女性 28,414 名	肺がん罹患	性・姓・住所・年齢(産 15 歳未満)で夫婦同定，夫の喫煙状況は質問票に基づく	年齢，地域，飲酒，肺がん家族歴，閉経状態	夫の喫煙	肺がん全体 肺腺がん	現在喫煙 現在喫煙	1.34 [0.81～2.21] 2.03 [1.07～3.86]

NS：有意でない，*：Hirayama は 90%信頼区間

果は一致していないが，前述のカリフォルニア州環境保護庁報告書での乳がんの評価には，閉経前女性で受動喫煙との有意な関連がみられた日本の研究が影響している。平山研究では鼻腔がん，脳腫瘍，および全がんでも有意なリスク上昇を観察したが，その後同様の報告はない。

おわりに

喫煙の健康影響については，過去半世紀近くにわたって研究が蓄積し，因果関係が認められる疾患や症状が一貫して増えてきた。今日，能動喫煙はがんだけでなく，循環器疾患，呼吸器疾患など，さまざまな疾患との因果関係が科学的に確立され，最近では大規模なメタアナリシスにより糖尿病との関連も確立されつつある。今後も，受動喫煙を含めて，タバコとの関連が科学的に確立される疾患や症状は増え続けることが予想される。

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RESEARCH COMMUNICATION

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

Yingsong Lin[1]*, Kei Nakachi[2], Yoshinori Ito[3], Shogo Kikuchi[1], Akiko Tamakoshi[1], Kiyoko Yagyu[1], Yoshiyuki Watanabe[4], Yutaka Inaba[5], Kazuo Tajima[6] for the JACC Study Group

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

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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.

*[1]Dept of Public Health, Aichi Medical University School of Medicine, Nagakute-cho, Aichi, [2]Dept of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, Hiroshima, [3]Dept of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, Nagoya, [4]Dept of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, [5]Division of Public Health, Dept of Food and Health Sciences, Faculty of Human Life Sciences, Jissen Women's University, Tokyo, [6]Aichi Cancer Center Research Institute, Nagoya, Japan *For Correspondence: linys@aichi-med-u.ac.jp*

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.

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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 chairman of the study group), Aichi Medical University School of Medicine; Drs. Mitsuru Mori & 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 University School of Medicine; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Michiko Kurosawa, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr. Hiroshi Suzuki, Niigata University School of Medicine; Dr. Koji Tamakoshi, Nagoya University School of Medicine; Dr. Kenji Wakai, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, Nagoya City University Graduate School of Medical Sciences; Dr. Koji Suzuki, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Yasuhiko Wada, Kansai Rosai Hospital; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Drs. Yoshiyuki Watanabe & Kotaro Ozasa, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Tsuneharu Miki, Graduate School of Medical Science, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Nara Women's University; Dr. Kiyomi Sakata, Iwate Medical University; Dr. Yoichi Kurozawa, Tottori University Faculty of Medicine; Dr. Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr. Yoshihisa Fujino, University of Occupational and Environmental Health; Dr. Akira Shibata, Kurume University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; Dr. Hideo Shio, Moriyama Municipal Hospital.

The past investigators of the study group were listed in reference (Tamakoshi et al., 2005) except for the following eight members (affiliations are those at the time they participated in the study): Dr. Takashi Shimamoto, Institute of Community Medicine, University of Tsukuba; Dr. Heizo Tanaka, Medical Research Institute, Tokyo Medical and Dental University; Dr. Shigeru Hisamichi, Tohoku University Graduate School of Medicine; Dr. Masahiro Nakao, Kyoto Prefectural University of Medicine; Dr. Takaichiro Suzuki, Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases; Dr. Tsutomu Hashimoto, Wakayama Medical University; Dr. Teruo Ishibashi, Asama General Hospital; and Dr. Katsuhiro Fukuda, Kurume University School of Medicine.

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RESEARCH COMMUNICATION

Cancer Deaths in a Cohort of Japanese Barbers in Aichi Prefecture

Shuji Sugiura[1], Kiyoko Yagyu[1], Yuki Obata[2], Yingsong Lin[1], Akiko Tamakoshi[1], Hidemi Ito[3], Keitarou Matsuo[3], Kazuo Tajima[3], Kunio Aoki[4], Shogo Kikuchi*[1]

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

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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 1991; 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

[1]Department of Public Health, Aichi Medical University School of Medicine, Nagakute, [2]Department of Pharmacy, Kinjogakuin University, [3]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, [4]Nagoya University, Nagoya, Japan
**For Correspondence: kikuchis@aichi-med-u.ac.jp*

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 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.

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

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.

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

Akiko Tamakoshi [a,*], Koji Tamakoshi [b], Yingsong Lin [a], Kiyoko Yagyu [a], Shogo Kikuchi [a] for the JACC Study Group

[a] Department of Public Health, Aichi Medical University School of Medicine, Aichi, Japan
[b] Department of Nursing, Nagoya University School of Health Sciences, Nagoya, Japan

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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

* Corresponding author. Fax: +81 561 62 5270.
E-mail address: tamaa@aichi-med-u.ac.jp (A. Tamakoshi).

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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	3764	1103	190	16,125***	1321	81.9[a]		1550	5210	7751	4864	1083	20,458***	689	33.7[a]	
%	64.0	57.7	62.5	49.4	50.1		25.0			55.3	57.3	60.2	60.7	61.9		21.5		
60–79	3626	3756	2257	1129	189	11,457	3964	346.0[a]		1254	3877	5118	3150	667	14,066	2523	179.4[a]	
%	36.0	42.3	37.5	50.6	49.9		75.0			44.7	42.7	39.8	39.3	38.1		78.5		
College or higher education	1581	1424	1036	397	79	4517	791	174.8		231	781	1263	859	207	3351***	208	76.7	
%	15.7	16.0	17.2	17.8	20.8		15.0			8.2	8.6	9.8	10.8	11.8		6.5		
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		13.7			19.3	17.6	17.4	17.3	16.6		13.3		
Having a spouse	8849	7769	5333	1994	338	24,283**	4300	184.0		2055	7122	10,315	6557	1429	27,478***	2136	94.2	
%	87.9	87.5	88.6	89.3	89.2		81.4			73.3	78.4	80.2	81.8	81.7		66.5		
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		95.6			92.2	94.8	95.4	96.6	97.4		96.4		
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		4.2			1.9	1.1	0.7	0.6	0.2		3.6		
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		5.2			3.7	2.4	2.4	2.1	1.8		4.3		
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		2.3			2.0	1.9	1.6	1.6	1.8		3.8		
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		44.3			74.0	93.4	97.5	99.4	100.0		93.4		
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		62.3			89.3	97.9	99.2	99.9	100.0		98.7		
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		48.5			7.8	24.4	53.9	81.9	100.0		46.3		
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		25.7			3.7	16.3	38.5	65.1	100.0		26.8		
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		32.7			2.7	13.1	31.8	61.4	100.0		36.3		
BMI 18.5–24.9	6044	7137	5401	2145	379	21,106	3988	183.6		374	4980	10,183	7406	1750	24,698	2095	87.2	
%	60.0	80.4	89.7	96.1	100.0		75.5			13.3	54.8	79.2	92.4	100.0		65.2		
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	22,705	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	76	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	1250	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

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 University School of Medicine; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Michiko Kurosawa, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr. Hiroshi Suzuki, Niigata University School of Medicine; Dr. Koji Tamakoshi, Nagoya University School of Health Sciences; Dr. Kenji Wakai, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, Nagoya City University Graduate School of Medical Sciences; Dr. Koji Suzuki, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Yasuhiko Wada, Kansai Rosai Hospital; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Drs. Yoshiyuki Watanabe and Kotaro Ozasa, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Tsuneharu Miki, Graduate School of Medical Science, Kyoto Prefectural University of Medicine; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Nara Women's University; Dr. Kiyomi Sakata, Iwate Medical University; Dr. Yoichi Kurozawa, Tottori University Faculty of Medicine; Dr. Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr. Yoshihisa Fujino, University of Occupational and Environmental Health; Dr. Akira Shibata, Kurume University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; Dr. Hideo Shio, Moriyama Municipal Hospital.

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

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Short communication

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

Akiko Tamakoshi[a,*], Koji Suzuki[b], Yingsong Lin[a], Yoshinori Ito[c], Kiyoko Yagyu[a], Shogo Kikuchi[a], Yoshiyuki Watanabe[d], Yutaka Inaba[e], Kazuo Tajima[f], Kei Nakachi[g], for the JACC Study Group

[a] Department of Public Health, Aichi Medical University School of Medicine, Aichi, Japan
[b] Department of Public Health, School of Health Sciences, Fujita Health University, Aichi, Japan
[c] Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, Nagoya, Japan
[d] Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, Japan
[e] Division of Public Health, Department of Food & Health Sciences, Faculty of Human Life Sciences, Jissen Women's University, Hino, Japan
[f] Aichi Cancer Center Research Institute, Nagoya, Japan
[g] Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, Hiroshima, Japan

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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.

* Corresponding author. Tel.: +81 561 62 3311; fax: +81 561 62 5270.
E-mail address: tamaa@aichi-med-u.ac.jp (A. Tamakoshi).

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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/m²)								<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.

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.

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 University School of Medicine; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Michiko Kurosawa, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr. Naohito Tanabe, Niigata University School of Medicine; Dr. Koji Tamakoshi, Nagoya University Graduate School of Health Science; Dr. Kenji Wakai, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, National Institute of Health and Nutrition; Dr. Koji Suzuki, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Yasuhiko Wada, Kansai Rosai Hospital; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Dr. Yoshiyuki Watanabe, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Kotaro Ozasa, Radiation Effects Research Foundation; Dr. Tsuneharu Miki, Kyoto Prefectural University of Medicine, Graduate School of Medical Science; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Nara Women's University; Dr. Kiyomi Sakata, Iwate Medical University; Dr. Yoichi Kurozawa, Tottori University Faculty of Medicine; Dr. Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr. Yoshihisa Fujino, University of Occupational and Environmental Health; Dr. Akira Shibata, Kurume University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; and Dr. Hideo Shio, Moriyama Municipal Hospital.

Table 3
sFas distribution among smokers according to cigarettes smoked per day.

	Number of cigarettes smoked per day																			
	1–5		6–10		11–15		16–20		21–25		26–30		30–							
	N	%	N	%	N	%	N	%	N	%	N	%	N	%						
sFas levels (ng/ml)																				
<1.8	12	23.1	60	18.8	82	21.4	193	20.1	27	25.0	57	24.4	22	20.6						
1.8–2.2	16	30.8	101	31.6	92	24.0	256	26.6	34	31.5	78	33.3	34	31.8						
2.3–2.6	17	32.7	76	23.8	97	25.3	224	23.3	22	20.4	44	18.8	15	14.0						
≥2.7	7	13.5	83	25.9	113	29.4	288	30.0	25	23.1	55	23.5	36	33.6						
Total	52	100.0	320	100.0	384	100.0	961	100.0	108	100.0	234	100.0	107	100.0						
Cochran-Mantel-Haenszel p value[a]														p = 0.26						
Least square means[a] (ng/ml)	2.28		2.37		2.40		2.42		2.34		2.35		2.41							
95% CI		(2.11–2.47)		(2.26–2.48)		(2.29–2.51)		(2.32–2.51)		(2.20–2.49)		(2.24–2.47)		(2.27–2.56)						
p value[b]				0.33		0.19		0.11		0.54		0.44		0.19						

[a] Adjusted for area, age category, BMI, drinking status, walking, education, marital status, consumption of green leaf vegetables.
[b] Compared with smoker who smoked 1–5 cigarettes per day.

Conflict of interest

The authors declare that there are no conflicts of interest.

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総　　論

H. pylori 感染症の疫学と感染経路の解明

菊地正悟　林　櫻松

Epidemiology of *H. pylori* infection and exploration of its infection route

Shogo Kikuchi, Yngsong Lin
Department of Public Health, Aichi Medical University School of Medicine

Abstract

To build up a preventive strategy for *H. pylori* related diseases including gastric cancer, infection ages and routes are important. Recently, several studies have explored them. In advanced countries, most infections occur under five years of age. Mother to child in some studies and sibling to sibling in others were dominant infection routes. As infection ages and routes depend on countries, studies in Japan are indispensable. Infection in kindergartens, nursery and elementary schools as well as in families should be explored. Based on the findings, a strategy preventing infection to children should be build up to prevention *H. pylori* related diseases.

Key words: intra-familial infection, infection in kindergarten or school, DNA fingerprinting, infection route, infection age

はじめに

H. pylori 感染は胃癌を含めた多くの疾患の原因となることが明らかにされている．*H. pylori* 感染の防止によるこれらの疾患の予防を考えるうえで，*H. pylori* 感染症の疫学，特に感染経路は重要である．胃癌については，一度も感染したことのない者に比べ，感染者では20倍以上胃癌のリスクが高いことが明らかになっており[1]，感染防止ができれば胃癌は稀少がんとなることが予想されている．*H. pylori* 感染症の疫学，感染経路について，最近の報告を中心にreviewするとともに，今後の研究の方向性について検討した．

1. *H. pylori* 感染症の疫学

H. pylori の感染率にはっきりした性差はない．年齢別には，**図1**に示した某町の健診受診者の血清抗体陽性率[2]のように，高齢ほど陽性率が高くなる．*H. pylori* は一度持続感染が成立すると，除菌や胃粘膜の強度萎縮などの環境変化がないかぎり感染が持続するので，感染の累積によって年齢とともに感染率が上昇する．また，後述するように小児期の衛生環境が感染率に大きく影響するので，経済発展による社会基盤の整備がなされると後から生まれる世代ほど感染率は低下する（コホート効果）．感染の累積に加え，コホート効果によっても年齢とともに感染率が上昇する．**図1**では2003年の方が1997年

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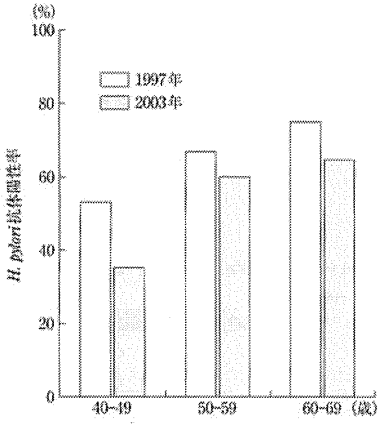

図1　某町の基本健康診査受診者の *H. pylori* 抗体保有率

表1　これまでに明らかにされた *H. pylori* の感染時期，感染経路

主な感染時期は小児期である．
小児期の衛生環境*が悪いと感染率が高い．
保育園，幼稚園の通園期間が長いと感染率が高い．
低い社会階層に属するほど感染率が高い．

*上下水道の整備状況，1人あたりの部屋数などの居住環境など．

より同じ年齢での感染率が低くなっており，この低下は我が国の社会基盤の整備によるコホート効果によると考えられる．

コホート効果による感染率の減少は，欧米の先進国では我が国に先立って起こったと推定される．一方，現在経済発展が著しい諸国でも，コホート効果による若い世代での感染率の低下が観察されている．

2．感染経路に関する既存の知見

H. pylori の感染経路に関しては，この細菌の発見以来これまで多くの研究がなされてきた．その中で，明らかにされてきたことは，**表1**に示すような内容である．

H. pylori の特異的な感染経路については，不完全な消毒の内視鏡による感染以外，いまだ不明である．ヒトが日常接する環境中からはほとんど検出されないので，ヒト→ヒト感染が主な経路と考えられている．胃の粘液が生息場所であるので，経口感染することは間違いないとされているが，口→口感染であるか便→口感染であるかは明らかでない．

3．感染年齢

主な感染時期は小児期であるが，その中でどの年齢での感染が多いかが注目されている．これは，除菌後の再感染の可能性がどのくらいあるかなど，適切な除菌時期を考えるうえで重要だからである．

Rowland らは，ダブリン（アイルランド）での研究で年齢別の新規感染率を観察した結果を報告している[3]．279人の小児を追跡した結果，2–3歳で5人/99人年（以下同じ），3–4歳で9/214，4–5歳で5/241，5–6歳で0/224，6–7歳で1/146，7–8歳で0/46と，主な感染時期は5歳以下であるが，6–7歳でも感染が観察されている．

Konno らは，*H. pylori* 抗体陽性の母親から生まれた44人の便中 *H. pylori* 抗原を用いて5年間追跡調査し，5人の感染児を確認した．感染時期は1歳が4人，4歳が1人であった[4]．

Okuda らは，乳児237人の感染時期を便中抗原検査によって追跡調査した結果，5人の感染を確認し，感染時期は乳児3人，1歳，2歳がそれぞれ1人であった[5]．

これらの追跡調査から先進国における主な感染時期は5歳頃までと推測される．しかし，ダブリンで6歳での感染の報告や，我が国の他の研究でも，6歳や10歳代での感染が報告されるなど，例外も少なくない．

4．感染予防に向けた感染経路の研究

H. pylori の特異的な感染経路の特定が難しいことから，広めの範囲に網をかけて感染を防止することが考えられるようになっている．*H. pylori* については，感染しているヒトが感染源であるが，除菌をすることで感染源でなくすることが可能である．最近の内外の研究には，この視点に立っていると思われるものが散見される．

表2　小児における *H. pylori* 感染経路の分類

1．家族内感染
母　→　子
父　→　子
同胞　→　同胞
2．集団生活での感染（保育園・幼稚園・学校）
児童/生徒　→　児童/生徒
教員/職員　→　児童/生徒
3．その他
家族や集団生活以外の人から
環境中から

る．これらの研究は，**表2**に示すような分類の経路での感染が，それぞれどのくらいの比重であるのかを明らかにすることを目的としている．

Farrell らは，ベルファスト（英国）で52家族（小児は126人）の *H. pylori* 感染を調査し，母の感染，父の感染，同胞（兄弟姉妹）との部屋の共有，同胞とのベッドの共有のどれが最も強く小児の感染に関連するかを分析した．オッズ比（95％信頼区間，以下95％ CI）は母の感染では2.5（1.0–6.1），父の感染では3.9（1.0–8.6），同胞との部屋の共有では3.7（1.3–10.8），同胞とのベッドの共有では4.8（1.5–15.2）であった．同胞とのベッドの共有が最も関連が強く，この経路による感染の頻度が高いとしている[6]．家族内感染のうち，同胞間感染の比重が大きいことを示す結果である．

Weyermann らは，ウルム（ドイツ）で612人の3歳児とその両親の *H. pylori* 感染を調べ，児の感染と両親の感染の関連を分析した．国籍やもう片方の親の感染の有無で補正すると，母親の感染はオッズ比13（95％CI: 3.2–52.5）で有意な関連を示したが，父親の感染は1.4（95％CI: 0.4–4.6）と有意ではなかった．このことから，父子感染に比べ，母子感染優位であると結論している[7]．

またWeyermann らは，メタアナリシスの手法で493例の4歳児の *H. pylori* 感染の有無と，その家族の感染の関係を分析した．両親の民族，配偶者の感染と同胞の感染の有無，同胞の有無で補正したオッズ比（95％CI）は，母の感染の有無13.0（3.0–55.2），父の感染の有無3.0（0.8–11.2），同胞の1人以上が感染あり3.7（0.5–26.2）と，母の感染の有無だけが有意であった[8]．ウルムでの研究と同様に，母から子への感染の頻度が高いことを示す結果である．

Kivi らは，ストックホルム（スウェーデン）で，同一家族内で *H. pylori* 感染陽性の者同士で *H. pylori* の株（strain）が一致するかを分析した．一致率（一致した組数/分析した組数）は，同胞同士81％（29/36），母がいずれとも異なる株に感染している同胞同士82％（14/17），母子56％（10/18），父子0％（0/8），夫婦22％（5/23）であった[9]．親から子への感染よりも同胞間での感染が主であることを示す結果である．

Konno らは，前述した研究での5人の感染児とその母親に感染している *H. pylori* が同一起源であるかを確認するために，Random amplified polymorphic DNA fingerprinting（RAPD）法によるDNAパターンの分析を行った．その結果，5例全例ともその母親とRAPD法によるDNAパターンが一致しており，すべて母子感染であったと結論している[4]．

Konno らは更に，上部消化管内視鏡検査と生検組織で診断された小児の *H. pylori* 胃炎42例を発端児として，家族内で *H. pylori* のDNAパターンが一致するかRAPD法によって分析した．家族に便中抗原検査HpSAを行い，陽性者からは胃液の採取，希望者には内視鏡検査を施行して胃粘膜を採取し，菌株を培養してRAPD法による分析を行った．家族の *H. pylori* 陽性率（陽性数/検査数）は，母が86％（36/42），父が82％（32/39），同胞が43％（18/42）であった．DNAパターンの一致率（一致した組数/分析した組数）は，母子で69％（29/42），父子で37％（7/19）で，父子間に比べ母子間で有意に高かった．発端児と同胞の一致率は全体で80％（8/10），母親と発端児のDNAパターンが一致する家族では88％（7/8）であった（1家族で発端児と他の2人の同胞が一致する例を含む）．また，発端児42例中32例（76％）で，家族のいずれか（祖母と一致した1例を含む）とDNAパターンが一致した[10]という詳細な結果を報告している．

これらの結果は，**表2**の家族内感染の部分に

関するものである．家族内感染が小児における感染の主要な部分を占めていること，父子感染に比べれば母子感染の頻度が高いことも間違いない．しかし，母子感染と同胞間感染に関しては結果が分かれている．

5. *H. pylori* の除菌による胃癌の予防

胃癌予防の方法の一つとして，一定の年齢に達した段階で感染の有無を確認して感染者の除菌を行うという方法が考えられる．除菌の時期は早い方が感染期間が短いので，より効果的である．一方，除菌治療の安全性は小児では確立されていないため，無症状の場合の除菌はできるだけ成人に近い年齢で，あるいは成人になってから行うべきである．更に，再感染の頻度を考慮して除菌の年齢を決める必要がある．

この方法には，感染者は一定期間感染を受けることになるという欠点があるが，対象の選択が容易で実施しやすい方法と考えられる．

6. *H. pylori* の感染防止

もう一つの方法として，小児期の感染自体を防止するものがある．まず，**表2**に示す各感染経路の比重を明らかにする．ほとんどの感染が小児期に起こることから，仮に家族内感染以外に子への感染がないとすると，第1子出産以前の段階で同居家族の感染者を除菌すれば子への感染は防止できることになる．また，家族内感染と集団生活での感染以外に子への感染がなかったとすると，第1子出産以前の段階での同居家族の感染者の除菌と保育園，幼稚園，学校の職員の感染者を除菌することで，子への感染は防止できることになる．

生活習慣や育児方法は，小児での *H. pylori* 感染に影響を与えていると思われるが，国や地域などによって異なる．同胞数も感染経路に影響を与えている可能性が高く，先進国と発展途上国では同胞数が異なる．母子感染が優位であるという報告と同胞間感染が優位であるという報告があることも，これらの要因の国や地域による違いで説明できる．我が国において，小児期の感染防止を考えるうえでは，我が国で感染がどのように起こっているのかを明らかにすることが不可欠である．我が国で家族内感染を詳細に検討したデータはKonnoらの研究[10]だけである．地域を変えて，同様の研究を行うことにより，我が国における家族内感染の実態を十分なデータで明らかにしていく必要がある．

上下水道の整備状況と *H. pylori* の陽性率に負の関連がある[11]ことから，先進国では環境からの感染は少なく，大部分が家族内と集団生活での感染であると推測される．我が国においても家族内と集団生活以外での感染は，少なくなっていることが予測される．家族内感染より対象を広げて，保育園児，幼稚園児，小学生の *H. pylori* 感染者について，その家族と接触のあった教職員を含めてDNAパターンを検査し，どのような一致がみられるかを明らかにすることも，今後の研究の方向性として重要である．家族内と集団生活以外での感染が極めてまれであることが明らかとなれば，前述したような対策で次の世代への感染を0に近づけることが可能となる．

このような感染防止の方法は，感染検査の対象の選択が複雑で，情報の管理に細心の注意を要するなど実施がやや難しい方法である．しかし，感染そのものを防止するので，除菌による方法に比べ胃癌などの疾患の予防効果は確実であり，検討すべき方法であると思われる．

おわりに

H. pylori の感染防止や除菌による，胃癌などの *H. pylori* 関連疾患の予防を考えるうえで，年齢ごとの感染頻度や感染経路を明らかにすることが重要である．これまでの報告で，我が国などの先進国では5歳までが主な感染時期であるが例外もあることが明らかとなっている．感染経路に関しては，母子感染が優位という報告と同胞間感染が優位という報告がある．感染年齢や感染経路は国によって異なる．我が国ではこの分野の研究が少ない．今後，対策を構築するうえでは，根拠となりうる十分なデータを我が国で集める必要がある．

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胆管細胞癌の疫学

Epidemiology of cholangiocarcinoma

田中政宏　津熊秀明

Key words：胆管細胞癌，肝内胆管癌，疫学，罹患率，リスク

はじめに

原発性肝癌は，国際疾病分類(ICD-10)では病理組織像の特性に基づき，肝細胞癌(C22.0)，胆管細胞癌(C22.1)，肝芽腫(C22.2)，肝血管肉腫(C22.3)，その他の肝の肉腫(C22.4)，その他の明示された肝の癌(混合型を含む)(C22.7)，詳細不明の肝の悪性腫瘍(C22.9)の7群に分類される．胆管細胞癌(肝内胆管癌)は，肝内胆管上皮に由来する癌腫で，肝門部に近い比較的太い胆管から末梢の細い胆管に至るまで，どの部位からでも発生しうる．なお左右の肝管および両者の合流部近傍に発生する胆管癌は，肝門部胆管癌とも呼ばれ，肝外胆管癌に分類される．胆管細胞癌は，原発性肝癌の中では肝細胞癌に次ぐ頻度である．

本稿では，はじめに我が国における胆管細胞癌の現況・動向について述べ，次いで胆管細胞癌の発生要因について，これまでの知見を総説的に述べる．

1. 我が国における胆管細胞癌発生の現況・動向

我が国の原発性肝癌の性別頻度分布を**表1**に示した．このうち人口動態死亡統計は厚生労働省大臣官房統計情報部による2000-04年の5年間の成績，罹患統計は，大阪府がん登録データによる2000-04年値，および，日本肝癌研究会による2002-03年の新規登録例(第17回全国原発性肝癌追跡調査報告[1])についての集計値である．

大阪府がん登録は，大阪府在住者に発生したすべてのがんを，府内の医療機関に依頼して届け出てもらい登録する制度であり，診断・治療・病理組織型などに関する一定の情報をデータベース化している．**表1**における同データにおいては，罹患年が2000-04年に診断された肝癌についてまず国際疾病分類腫瘍学形態学コード(ICD-OM第3版)による集計を行い，次にParkinらの国際比較研究のための組織型群の考え[2]に従いグループ化し，最終的にICD-10に基づく7つのサブカテゴリーに再構成した．

胆管細胞癌の占める割合は，死亡統計では男性6.0％，女性10.0％，大阪府がん登録では男性5.0％，女性8.5％，全国肝癌登録では男性3.7％，女性5.5％であった．ただし，それぞれのデータの解釈には留意が必要である．死亡統計では，原死因が'肝癌'とされた場合C22.0とコード化されるため，胆管細胞癌の比率が過小評価される可能性のあること，全国肝癌追跡調査では登録症例の代表性に制約があると推測されること，大阪府がん登録では登録症例の代表性には大きな問題はないが，病理組織型報告例の割合が低いことを保留しておく，ともあれ我が国では胆管細胞癌が原発性肝癌の4-10％程度を占め，その割合は男性より女性で高いと推測される．

Masahiro Tanaka, Hideaki Tsukuma: Department of Cancer Control and Statistics, Osaka Medical Center for Cancer and Cardiovascular Diseases　大阪府立成人病センター　調査部

0047-1852/09/¥40/頁/JCLS

表1　原発性肝癌の組織型分布(臨床診断または組織診断による)

肝および肝内胆管の悪性新生物		人口動態死亡統計 厚生労働省大臣官房統計情報部 2000-04年死亡				地域がん登録資料 大阪府がん登録 2000-04年罹患*				全国臓器別がん登録 日本肝癌研究会** 2002-03年新規登録症例			
		男性	%	女性	%	男性	%	女性	%	男性	%	女性	%
C22		117,810		53,718		13,054		5,784		13,017		5,196	
C22.0	肝細胞癌	109,888	93.9	47,785	89.8	11,070	94.7	4,606	91.1	12,341	94.8	4,818	92.7
C22.1	胆管細胞癌	6,979	6.0	5,301	10.0	583	5.0	431	8.5	485	3.7	285	5.5
C22.2	肝芽腫	37	0.0	29	0.1	10	0.1	6	0.1	8	0.1	4	0.1
C22.3	肝血管肉腫	41	0.0	28	0.1	2	0.0	4	0.1	11	0.1	8	0.2
C22.4	その他の肝の肉腫	47	0.0	28	0.1	13	0.1	6	0.1				
C22.7	その他の明示された肝の癌	50	0.0	22	0.0	8	0.1	3	0.1	172	1.3	81	1.6
総計(C22.9：詳細不明の肝の悪性腫瘍等を除く)		117,042	100.0	53,193	100.0	11,686	100.0	5,056	100.0	13,017	100.0	5,196	100.0

いずれの報告も臨床診断のみによる症例を含む．
*2000-04年の総報告数18,838例から，詳細不明の肝の悪性腫瘍(C22.9)(2,030例)およびParkinらの分類に含まれない組織型のもの(66例)を除く．
**C22.1にはcholangiocellular carcinomaだけでなくcystadenocarcinomaを含めた．C22.7にはmixed carcinoma and othersを計上した．

この図にみられる傾向と同様に，世界的には胆管細胞癌の罹患率および死亡率が近年増加しているとの報告が複数あり[3-5]，この現象の原因としては，肝癌の報告方法の変更または改善，診断技術の向上，そして罹患率の真の増加などが考えられる．そして，この増加が複数の国・地域でみられること，増加割合に性差がみられること，報告例中の早期がんの割合に変化がみられないことなどの理由から罹患率は真に増加しているとの主張もある[6]．この真偽については，胆管細胞癌のリスク要因も考慮した今後の研究が待たれる．

2. 胆管細胞癌のリスク要因

胆管細胞癌のリスクとされている要因には，肝吸虫の寄生，原発性硬化性胆管炎，肝臓結石，胆管枝の奇形，化学物質，ウイルス性肝炎などがある[5]．これらの要因の多くは胆管上皮の慢

表2 年齢階級別にみた肝癌の組織型分布（大阪府がん登録，2000-04年）

年齢階級	肝細胞癌 (C22.0)	胆管細胞癌 (C22.1)	肝芽腫 (C22.2)	肝血管肉腫 (C22.3)	その他の肝の肉腫 (C22.4)	その他の明示された肝の癌 (C22.7)	総 計
0-14	2 (10.0)	0 (0.0)	16 (80.0)	0 (0.0)	2 (10.0)	0 (0.0)	20 (100)
15-34	42 (95.5)	1 (2.3)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	44 (100)
35-44	148 (90.2)	16 (9.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	164 (100)
45-54	1,069 (93.0)	79 (6.9)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	1,150 (100)
55-64	3,272 (93.2)	229 (6.5)	0 (0.0)	4 (0.1)	3 (0.1)	2 (0.1)	3,510 (100)
65-74	7,028 (94.9)	360 (4.9)	0 (0.0)	0 (0.0)	10 (0.1)	5 (0.1)	7,403 (100)
75-	4,110 (92.4)	329 (7.4)	0 (0.0)	2 (0.0)	2 (0.0)	3 (0.1)	4,446 (100)
総計	15,671 (93.6)	1,014 (6.1)	16 (0.1)	6 (0.0)	19 (0.1)	11 (0.1)	16,737 (100)

ICD-10への分類：国際疾病分類腫瘍学形態学コード（ICD-OM第3版）による集計を行った後，Parkinらの国際比較研究のための組織型群の考えに従いグループ化した．

2000-04年の総報告数18,838例から，詳細不明の肝の悪性腫瘍（C22.9）（2,030例），C22.0における年齢不明例（5例）およびParkinらの分類に含まれない組織型のもの（66例）を除いた．

（ ）内の数値は％．

性炎症と関連すると考えられる．

世界的にみると，肝吸虫症は胆管細胞癌の地域集積と密接に関連しており[7]，とりわけ，タイ，ラオス，カンボジアで流行のみられる*Opisthorchis viverrini*と，中国・台湾，韓国，ベトナムで流行のみられる*Clonorchis sinensis*との関連が注目されている．タイ東北部，中国，韓国で認められるように，肝吸虫は淡水魚の生食を通してヒトに感染し，成虫は主として肝内胆管に寄生する．

肝臓結石および原発性硬化性胆管炎は，肝吸虫症と同じく慢性胆管炎の原因となり，胆管の慢性炎症が過形成性変化，異型性変化へと移行する過程で，胆管細胞癌の発生と密接に関与すると考えられている[6]．肝臓結石は胆道の細菌感染と胆汁のうっ滞とが関連して発生し，我が国を含め極東地域の胆管細胞癌の前駆性病変になっている場合が多い[6]．原発性硬化性胆管炎は，とりわけ西欧諸国で胆管細胞癌の前駆性病変として考えられており[8]，原発性硬化性胆管炎の8-40％程度に胆管細胞癌の合併がみられる[6]．また，潰瘍性大腸炎患者に胆管細胞癌が併発する例も知られている．胆管枝の奇形などによる胆管嚢胞性拡張も結果的に慢性胆管炎を引き起こし，胆管細胞癌の危険因子になると考えられている．

胆管細胞癌の原因となりうる化学物質としては，放射性造影剤として使われたトロトラスト，ダイオキシン，ニトロサミン，喫煙に関連する物質，アルコール摂取などが報告されている[6]．

胆管細胞癌のほとんどは非硬変肝から発生するが，ウイルス性，アルコール性などの非胆汁性肝硬変に発生する胆管細胞癌も報告されている[9]．Kobayashiら[9]は，C型肝炎ウイルスによる肝硬変を有する日本人600人を平均7.2年間追跡調査し，11人に胆管細胞癌，3人に肝細胞癌と胆管細胞癌の混合型，206人に肝細胞癌の発生を認め，この群からの胆管細胞癌発生率が日本人一般人口からの発生率と比べ極めて高いと報じた（なおこの報告では，罹患率を全国肝癌追跡調査を基に推計している点で留意が必要である）．また，近年ウイルス肝炎と胆管細胞癌の関係を示唆する報告も複数みられる．大阪，イタリア，米国からはC型肝炎ウイルス感染によって胆管細胞癌罹患リスクが6-9倍程度高くなること[8,10,11]が報告されている．肝細胞と胆管細胞は同じ前駆細胞をもつこと，またC型肝炎ウイルスのRNAが胆管細胞癌組織に確認されたとの報告などもこの因果関係を示唆するが，正確な発癌のメカニズムは今後の課題である．また韓国からは，B型肝炎ウイルス感染により胆管細胞癌の罹患リスクが2倍程度になること

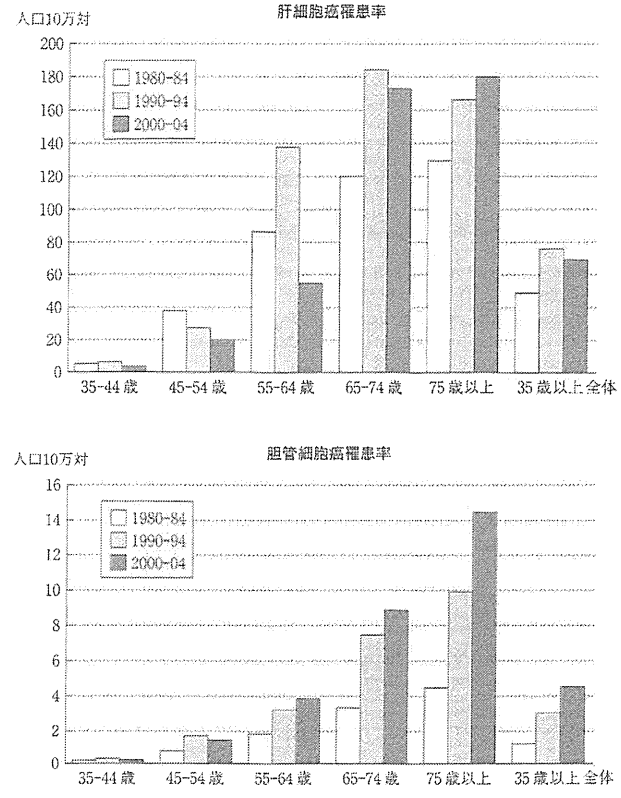

図1 肝細胞癌，胆管細胞癌の年齢別，年代別罹患率の推移（人口10万対）
（大阪府がん登録データより）

それぞれの年齢階級における詳細不明例（C22.9）の報告分は，C22.9を除いた'C22.0-22.4およびC22.7全体'におけるそれぞれの割合で按分して加えることにより，各10歳年齢階級の肝細胞癌と胆管細胞癌の罹患数を推計した．

が報告されている[12]. 1980年代後半から90年代まで, 我が国ではC型肝炎による肝癌が著明に増加した. その大多数は肝細胞癌によるものであるが, このことが大阪がん登録データで示唆された胆管細胞癌罹患率の上昇とどのような関連があるのかにつき, 今後の検討が必要である.

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Induction of SCEs in CHL cells by dichlorobiphenyl derivative water pollutants, 2-phenylbenzotriazole (PBTA) congeners and river water concentrates

Takeshi Ohe[a,*], Aki Suzuki[a], Tetsushi Watanabe[b], Tomohiro Hasei[b], Haruo Nukaya[c], Yukari Totsuka[d], Keiji Wakabayashi[d]

[a] Department of Food and Nutrition, Faculty of Home Economics, Kyoto Women's University, Kyoto 605-8501, Japan
[b] Department of Public Health, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan
[c] School of Pharmaceutical Science, University of Shizuoka, Shizuoka 422-8526, Japan
[d] Cancer Prevention Basic Research Project, National Cancer Center Research Institute, Tokyo 104-0045, Japan

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PBTA-1
PBTA-2
PBTA-6

ABSTRACT

We recently identified dichlorobiphenyl (DCB) derivatives and 2-phenylbenzotriazole (PBTA) congeners as major mutagenic constituents of the waters of the Waka River and the Yodo River system in Japan, respectively. In this study we examined sister chromatid exchange (SCE) induction by two dichlorobiphenyl derivatives, 3,3'-dichlorobenzidine (DCB, 4,4'-diamino-3,3'-dichlorobiphenyl) and 4,4'-diamino-3,3'-dichloro-5-nitrobiphenyl (5-nitro-DCB); three PBTA congeners, 2-[2-(acetylamino)-4-[bis(2-methoxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-1), 2-[2-(acetylamino)-4-[*N*-(2-cyanoethyl)ethylamino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-2), and 2-[2-(acetylamino)amino]-4-[bis(2-hydroxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-6); and water concentrates from the Waka River in Chinese hamster lung (CHL) cells. Concentration-dependent induction of SCE was found for all DCBs and PBTAs examined in the presence of S9 mix, and statistically significant increases of SCEs were detected at 2 μg per ml of medium or higher concentrations. SCE induction of MeIQx was examined to compare genotoxic activities of these water pollutants. According to the results, a ranking of the SCE-inducing potency of these compounds is the following: 5-nitro-DCB ≈ MeIQx > PBTA6 > PBTA-1 ≈ PBTA-2 > DCB.

Water samples collected at a site at the Waka River showed concentration-related increases in SCEs at 6.25-18.75 ml-equivalent of river water per ml of medium with S9 mix. The concentrations of 5-nitro-DCB and DCB in the river water samples were from 2.5 to 19.4 ng/l and from 4100 to 18,900 ng/l, respectively. However, these chemicals showed only small contribution to SCE induction by the Waka River water.

1. Introduction

Genotoxic compounds are detected in many surface waters in the world. These compounds are often released directly from industrial discharges as a result of insufficient treatment of wastewater or unintentional formation in the environment after discharge of effluents [1-5]. In the previous studies, we found two novel chemical classes, dichlorobiphenyl derivatives and 2-phenylbenzotriazole (PBTA) congeners, as major mutagenic constituents in the water of rivers flowing through several industrial areas in Japan [6-20].

Among dichlorobiphenyl derivatives, 3,3'-dichlorobenzidine (DCB, 4,4'-diamino-3,3'-dichlorobiphenyl), 4,4'-diamino-3,3'-dichloro-5-nitrobiphenyl (5-nitro-DCB), and so forth were identified as major mutagens in the water of the Waka River flowing through an industrial area in Wakayama, where a number of large chemical plants are found [6-9]. 5-Nitro-DCB is a novel chemical and is presumed to be formed unintentionally by the process of wastewater treatment of drainage water containing DCB discharged from chemical plants [6]. DCB is a raw material in the manufacture of polymers and dye intermediates, and there are large-scale chemical plants producing DCB in this industrial area. 5-Nitro-DCB is highly mutagenic in the Ames assay using *Salmonella typhimurium* YG1024, which is an *O*-acetyltransferase-overproducing derivative of TA98, with S9 mix, and its activity was ~7 times higher than that of DCB.

5-Nitro-DCB was detected in river water concentrates at the maximum level of 6.9 μg/g of blue rayon. DCB was also detected in the concentrates at 13.2-104 μg/g of blue rayon. The percent contributions of 5-nitro-DCB and DCB to the mutagenicity of the water concentrates in YG1024 with S9 mix were 11% and 28%, respectively,

* Corresponding author. Tel.: +81 75 531 7124; fax: +81 75 531 7170.
E-mail address: ooe@kyoto-wu.ac.jp (T. Ohe).

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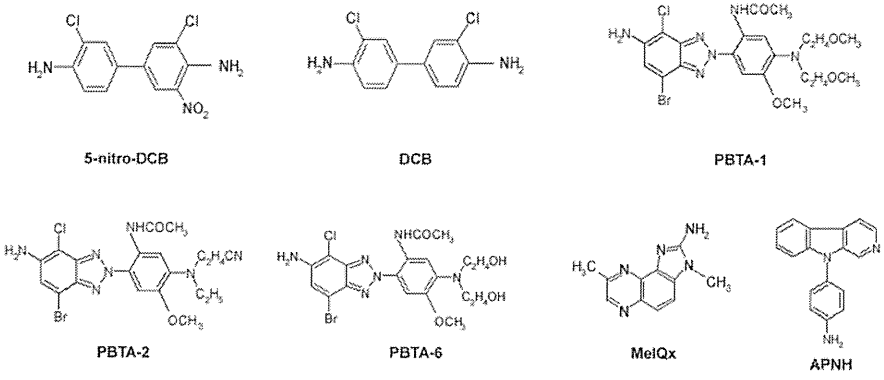

Fig. 1. Chemical structures used in the present study.

on average. 5-Nitro-DCB is a new chemical, and it has no biological activity data except for mutagenicity in the Ames assay.

PBTA congeners were identified as major indirect-acting river water mutagens, and seven kinds of such compounds were detected in highly mutagenic river waters, e.g., the Yodo River system, the Asuwa River, the Nikko River and so on, flowing through areas of textile dyeing industries [10–20]. PBTA congeners were suggested to be formed from corresponding dinitrophenylazo dyes via reduction with sodium hydrosulfide during the industrial dyeing process and following chlorination in the disinfection process at sewage plants. PBTA congeners show potent mutagenicity in *S. typhimurium* YG1024 in the presence of S9 mix. 2-[2-(Acetylamino)-4-[bis(2-methoxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-1), 2-[2-(acetylamino)-4-[*N*-(2-cyanoethyl)ethylamino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-2), and 2-[2-(acetylamino)amino]-4-[bis(2-hydroxyethyl)amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzo-triazole (PBTA-6) were detected in many river water concentrates [10–15]. However, there are few reports on the genotoxicity of PBTA congeners in mammalian cells.

In this study, we investigated the induction of sister chromatid exchanges (SCEs) by DCB, 5-nitro-DCB, PBTA-1, PBTA-2, PBTA-6, and water concentrates from the Waka River in Chinese hamster lung (CHL) cells to evaluate the genotoxic effect of water pollutants and environmental samples contaminated with DCB and 5-nitro-DCBs. DCB and 5-nitro-DCB in the river water concentrates were quantitatively analyzed, the mutagenicity of the water concentrates were evaluated in YG1024, and the contribution of 5-nitro-DCB and DCB to the mutagenicity of the river water concentrates are also estimated.

2. Materials and methods

2.1. Materials

5-Nitro-DCB (CAS 1073239-90-3) was synthesized according to the method reported previously [6]. Dichlorobenzidine dihydrochloride (CAS 612-83-9) was purchased from Sigma–Aldrich Co. Ltd. (MO, USA). PBTA-1 (CAS 194590-84-6), PBTA-2 (CAS 215245-16-2), and PBTA-6 (CAS 392274-07-6) were synthesized as described previously [11,13,16]. 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx, CAS 77500-04-0) was obtained from Wako Pure Chemical Industries (Osaka, Japan). The chemical structures of six compounds used in the present study are shown in Fig. 1. All other chemicals and reagents were of analytical grade.

2.2. Preparation of river water concentrates and analysis of DCBs

Each 10 l water sample was collected at a site where wastewater was discharged from chemical plants and a sewage treatment plant into the Waka River in Wakayama, Japan from September 2006 to March 2007. Collected water samples were passed through Supelpak2 columns (SUPELCO, PA, USA, 20 mm i.d. × 800 mm), and adsorbed materials were then extracted with methanol (300 ml) according to our previous paper [8]. Each extract was used for SCE assay, the Ames assay, and quantification of 5-nitro-DCB and DCB by HPLC. Quantification of 5-nitro-DCB and DCB was performed according to the method reported in the previous paper [6].

2.3. Chemical treatment

All chemicals tested for SCE assay were dissolved in dimethyl sulfoxide (DMSO), and freshly prepared solutions were added to cultures in appropriate final concentrations. The final concentration of DMSO in all cultures was 0.5% (v/v). Aminophenyl-norharman (APNH, Fig. 1) was used as the positive control [22] and was dissolved in DMSO before use.

2.4. SCE assay

CHL cells, obtained from Health Science Research Bank (HSRRB), Japan, were subcultured at a cell density of 1.5×10^5 cells per 60-mm dish and cultivated in 4 ml of Eagle's minimum medium (MEM, Gibco BRL) supplemented with 10% heat-inactivated fetal bovine serum (Trace Scientific Ltd., Melbourne, Australia). In the system with metabolic activation, cells were treated with each chemical for 6 h in the presence of S9 mix. The S9 mix was prepared immediately before use by mixing 1 ml of phenobarbital- and 5,6-benzoflavone-induced rat liver S9 (Oriental Yeast Co. Ltd., Tokyo, Japan) with 0.1 ml of 0.5 M glucose-6-phosphate, 0.4 ml of 0.1 M NADP, 0.2 ml of 1.65 M KCl solution, 0.2 ml of 0.4 M $MgCl_2$ solution, 4 ml of 0.25 M phosphate buffer (pH 7.4), and 4.1 ml of distilled water. The final concentration of S9 used was 1.25% (v/v). After treatment, cells were washed with phosphate buffered saline (PBS) and cultured in fresh medium for a recovery period of 24 h. In an experiment designed to detect SCEs, 5-bromodeoxyuridine (BrdU; final concentration 5 μg/ml) was added to the cultures just after addition of the test chemical.

Colcemid (final concentration 0.2 μg/ml; Gibco) was added to each culture 2 h prior to harvest. Harvested cells were then treated with a hypotonic solution of 75 mM KCl and fixed with cold methanol:acetic acid (3:1, v/v). Solvent-treated cells served as the negative control. Air-dried chromosome preparations prepared for SCEs were stained with the fluorescence plus Giemsa method described by Perry and Wolff [23]. SCEs were scored in 25 well-spread metaphases for each treatment. The results were expressed as the frequency of SCEs per metaphase. The significance between mean SCE in treated versus control groups were determined using the Student's *t*-test.

2.5. Ames assay

Ames assay was performed for DMSO solutions of chemicals and water concentrates described above using *S. typhimurium* YG1024 according to the method reported previously [24–26]. *S. typhimurium* YG1024 were kindly provided by Dr. T. Nohmi from the National Institute of Health Sciences, Tokyo. The S9 mix contained

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Table 1
SCEs induced by 5-nitro-DCB, DCB, PBTA-1, PBTA-2 and PBTA-6 in CHL cells in the presence of S9 mix.

Sample	Dose (μg/ml)	SCEs per metaphase		
		MI (%)[c]	Mean ± S.D.	Range
5-Nitro-DCB[a]	1.25	2.0	13.32 ± 6.27**	4–29
	2.5	2.5	15.92 ± 7.43**	6–31
	5	2.1	18.58 ± 6.01**	7–29
	10	2.2	25.04 ± 9.50**	11–48
DCB[a]	1	2.9	11.16 ± 4.14*	4–20
	2	2.2	14.76 ± 4.35**	7–23
	10	2.9	16.96 ± 5.95**	8–35
	20	2.5	19.16 ± 7.69**	7–37
PBTA-1[b]	1.25	2.6	17.88 ± 5.21**	11–29
	2.5	2.6	18.60 ± 8.29**	9–34
	5	2.0	19.06 ± 6.27**	7–29
	10	1.7	20.04 ± 7.37**	10–43
PBTA-2[b]	1.25	2.8	14.28 ± 3.60*	8–23
	2.5	2.9	14.56 ± 6.58**	6–38
	5	2.7	17.84 ± 5.74**	8–28
	10	2.0	20.96 ± 7.81**	9–34
PBTA-6[b]	1.25	2.9	15.08 ± 4.95	8–26
	2.5	2.7	16.28 ± 6.13**	8–26
	5	2.9	19.40 ± 5.22**	12–31
	10	1.8	22.76 ± 7.04**	10–36
MeIQx[b]	1.25	3.5	15.97 ± 8.20**	6–39
	2.5	3.9	18.20 ± 5.12**	9–35
	5	3.7	23.96 ± 9.20**	9–43
	10	2.7	26.68 ± 9.02**	13–53

SCE frequency for positive control (APNH, 0.005 μg/ml) was 21.44 ± 5.04 (mean ± S.D.).
[a] SCE frequency for Control (DMSO) was 8.68 ± 4.28 (mean ± S.D.).
[b] SCE frequency for Control (DMSO) was 10.16 ± 2.65 (mean ± S.D.).
[c] MI; mitotic index. MI (%) was calculated by counting the number of mitotic cells among 1000 round nuclei.
* Significantly different from control, $p < 0.05$.
** Significantly different from control, $p < 0.01$.

25 μl of S9 (25 mg of protein/ml) at a total volume of 500 μl. Mutagenic activities of test samples were calculated from the linear portions of the dose-response curves obtained with four doses with duplicate plates in two independent experiments, and the results were the average of two independent experiments. The positive controls were 2-aminoanthracene (0.1 μg/plate) and Trp-P-1 (0.01 μg/plate) with S9 mix. The mutagenic potencies were expressed as revertants/l of river water.

3. Results

3.1. SCE induction by DCBs and PBTAs

We evaluated the genotoxic effect of 5-nitro-DCB, DCB, three PBTA congeners, and MeIQx using SCEs in cultured CHL cells in the presence of S9 mix. These chemicals are indirect-acting mutagens for bacteria and show potent mutagenicity with S9 mix [6,7,10,11,13,27]. As shown in Table 1, statistically significant increases in SCEs were found for DCBs and PBTAs at doses used in this study (up to 10 or 20 μg/ml). Among DCBs and PBTAs, the highest SCE frequency, 25.04 ± 9.50 (mean ± S.D.), was detected for 5-nitro-DCB at the dose of 10 μg/ml. MeIQx is a well-known mutagenic and carcinogenic heterocyclic amine [28], and it shares structural features common to those of DCBs and PBTAs. Dose-related increases in the frequencies of SCEs were found for MeIQx between the concentration of 1.25 and 10 μg/ml. The highest SCE frequency, 26.68 ± 9.02, was detected at 10 μg/ml. Concentrations of DCBs, PBTAs, and MeIQx leading to two-fold increases of SCE frequencies relative to that of control were shown as SCE-inducing activity in Table 2, with mutagenicity data by Ames test. SCE-inducing activities of DCBs and PBTAs were from 4.5 to 13.9 μg/ml. The SCE-inducing activity of 5-nitro-DCB, 4.5 μg/ml was as high as

Table 2
SCE-inducing activity and mutagenicity data of 5-nitro-DCB, DCB, PBTA-1, PBTA-2, PBTA-6 and MeIQx in the presence of S9 mix.

Sample	SCE-inducing activity (μg/ml)[a]	Mutagenicity (revertants/μg)[b]	
		TA98	YG1024
5-Nitro-DCB	4.5	8,700	24,200
DCB	13.9	100	3,400
PBTA-1	8.4	88,000	3,000,000
PBTA-2	8.4	93,000	3,200,000
PBTA-6	6.9	17,900	485,000
MeIQx	4.6	117,000	1,400,000

[a] Concentration leading to a two-fold increase relative to control level.
[b] Data from reference [6,9,12,17,38].

that of MeIQx, 4.6 μg/ml. APNH, which was used as a positive control, significantly increased SCE induction at a dose of 0.005 μg/ml in the presence of S9 mix, and this result was consistent with that of our previous report [22].

3.2. SCE induction by the water concentrates from the Waka River

The dose-response effects of SCE induction for three water concentrates obtained from the Waka River are shown in Table 3. For all water concentrates, dose-related increases in the frequencies were found between the concentration of 6.25 and 18.75 ml eq/ml of medium in the presence of S9 mix. The highest SCE frequency, 20.84 ± 5.08, was detected for river water concentrate No. 2 at the dose of 18.75 ml/ml, but this concentrate showed toxicity at a higher dose.

3.3. Concentrations of DCBs in the river water and mutagenicity in Salmonella

Table 4 shows amounts of 5-nitro-DCB and DCB in the three water samples from the Waka River and mutagenicity of water concentrates toward *S. typhimurium* YG1024 in the presence of S9 mix. 5-Nitro-DCB and DCB were detected in all the water samples. The concentrations of 5-nitro-DCB and DCB in the river water samples were from 2.5 to 19.4 ng/l and from 4100 to 18,900 ng/l, respectively. The three water concentrates showed potent mutagenicity in YG1024, and the highest activities was detected for the water concentrate No.2. The percent contributions of DCB to the mutagenicity of the river water concentrates were from 8% to 26%, but those of 5-nitro-DCB were less than 1%.

4. Discussion

In the present study, we evaluated the genotoxic effect of the water pollutants 5-nitro-DCB, DCB, PBTA-1, PBTA-2, and PBTA-6, and river water samples, which included 5-nitro-DCB and DCB as constituents, using SCEs in CHL cells. In addition, SCE induction of MeIQx was examined to compare genotoxic activities of these water pollutants. MeIQx was deduced to be possibly carcinogenic to human (Group 2B) by the International Agency for Research on Cancer (IARC) [28], and it has the structural features of an aromatic amine similar to those of DCBs and PBTAs. MeIQx was reported to induce SCEs in human lymphocyte cultures and to show mutagenicity in CHL cells for diphtheria toxin resistance in the presence of S9 mix [29,30]. As shown in Table 1, the dose-response effects of SCE induction were detected for DCBs, PBTAs, and MeIQx at doses from 1.25 to 10 μg/ml or from 1 to 20 μg/ml with S9 mix, and the increases of SCEs were statistically significant at almost all doses tested. All chemicals tested in the present study induced SCE induction in cultured mammalian CHL cells in the presence of S9 mix. Among the composites tested, 5-nitro-DCB was found to have