RESEARCH COMMUNICATION

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

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Abstract

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

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

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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.

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

Materials and Methods

Study population

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