SERUM COMPONENTS AND RISK OF CANCER - VII

Serum Soluble Fas Levels and Superoxide Dismutase Activity and the Risk of Death from Pancreatic Cancer: a Nested Case-control Study within the JACC Study

Yingsong Lin[1]*, Shogo Kikuchi[1], Kiyoko Yagyu[1], Teruo Ishibashi[1], Michiko Kurosawa[2], Yoshinori Ito[3], Yoshiyuki Watanabe[4], Yutaka Inaba[5], Kazuo Tajima[6], Kei Nakachi[7], Akiko Tamakoshi[1]; for the JACC Study Group

Abstract

In a search for novel circulating biomarkers for pancreatic cancer, we examined the association between serum soluble Fas (sFas) levels and superoxide dismutase (SOD) activity and the risk of death from pancreatic cancer in a nested case-control study within the Japanese Collaborative Cohort Study. Case subjects were 68 persons who were free of morbidity, had provided a blood sample at baseline (1988-1990), and subsequently died from pancreatic cancer before December 31, 1997. Control subjects were 199 matched persons who were selected from the remaining participants in the cohort. Conditional logistic regression models were used to estimate age-adjusted and multivariate-adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). No statistically significant differences were noted in mean sFas levels (p=0.11) and SOD activity (p=0.42) between cases and controls. Overall, neither serum sFas levels nor SOD activity were associated with the risk of pancreatic cancer deaths, after adjustment for area, BMI, cigarette smoking, and history of diabetes. Furthermore, no significant risk trends were noted. Our results do not support the hypothesis that serum sFas levels and SOD activity are associated with pancreatic cancer risk.

Keywords: JACC Study - nested case-control study - pancreatic cancer - soluble Fas - SOD

Asian Pacific J Cancer Prev, **10**, JACC Serum Component Supplement, 81-85

Introduction

In 2006, 22,650 Japanese men and women died from pancreatic cancer, according to the Vital Statistics published by the Japanese Minister of Health, Labor and Welfare (Vital Statistics 2006). Pancreatic cancer mortality ranks the fifth among the causes of cancer deaths in Japan. Despite extensive research efforts from both epidemiologic and experimental research, the etiology of pancreatic cancer remains largely unknown.

Given the absence of effective screening tools and the difficulty of early detection, one challenge for epidemiologic studies is identifying new circulating biomarkers to predict pancreatic cancer risk in healthy individuals. Although associations between circulating biomarkers such as insulin-like growth factor, transforming growth factor-beta, microphage-inhibitory cytokine-1, and pancreatic cancer risk has been assessed in recent epidemiologic studies, results have been inconsistent (Koopmann et al., 2004; Lin et al., 2004; 2006; Stolzenberg-Solomon et al., 2004).

Since evasion of apoptosis is one of the six hallmarks of cancer (Hanahan. 2000), circulating biomarkers involved in apoptosis may play an important role in pancreatic cancer etiology. Fas is one member of the tumor necrosis factor receptor superfamily that is capable of inducing apoptosis in the presence of Fas ligand (Fas L) (Nagata,1994). Soluble Fas (sFas) is produced by alternative splicing of the Fas messenger RNA to encod a soluble form of the protein that lacks the transmembrane domain. After binding to Fas L, sFas neutralizes Fas L and functionally antagonizes Fas/Fas L-induced apoptosis (Cheng et al., 1994). The significance of serum sFas levels in pancreatic cancer is not clear. A recent nested case-control study showed that higher serum sFas levels were associated with increased risk of total cancer mortality in

*[1]Department of Public Health, Aichi Medical University School of Medicine, [2]Department of Epidemiology and Environmental Health, Juntendo University, School of Medicine, [3]Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, [4]Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Science, [5]Division of Public Health, Department of Food & Health Sciences, Faculty of Human Life Sciences, Jissen Women's University, [6]Aichi Cancer Center Research Institute, [7]Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, Japan *For correspondence: linys@aichi-med-u.ac.jp*

apparently healthy Japanese people (Tamakoshi et al., 2009).

Other important phenomena involved in pancreatic carcinogenesis include chronic pancreatitis, K-ras oncogene mutations, and the subsequent generation of reactive oxygen species (ROS). There is substantial evidence that increase in ROS levels facilitates carcinogenesis by inducing genomic instability (Schumacker, 2006). In order to repair ROS-induced DNA damage, cells activate a large number of intracellular antioxidant enzymes, one of which is SOD. There are three isoforms of SOD (extra-cellular SOD, mitochondria MnSOD, and cytoplasm Cu-Zn SOD,) and they function through dismutating O_2 into H_2O_2. The role of serum SOD activity in pancreatic cancer etiology remains elusive, although previous studies have shown decreased levels of MnSOD and CuZn SOD in human pancreatic ductal carcinoma specimens when compared with normal human pancreas (Teoh et al., 2007).

We thus hypothesize that elevated sFas levels and lower SOD activity may increase the risk for pancreatic cancer. The purpose of this study is to examine the association between serum sFas levels and SOD activity and the risk of death from pancreatic cancer in a nested case-control study within a large, ongoing cohort study of adult Japanese.

Materials and Methods

Study Population

We analyzed data from a nested case-control study within the Japanese Collaborative Cohort Study (the JACC study). The details of the JACC Study have been reported elsewhere (Tamakoshi et al., 2005). Briefly, it is an ongoing, prospective cohort study aimed at evaluating cancer risk associated with lifestyle risk factors in adult Japanese individuals who were recruited from 45 areas throughout Japan. Between 1988 and 1990, 46,465 men and 64,327 women, aged 40-79 years, were enrolled after they responded to a questionnaire. The baseline 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 at baseline. 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 through December 31, 1997 were collected on all cohort participants. During the 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 from pancreatic cancer were coded 'C25' 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.

In the present study, case subjects were defined as those who were free of morbidity at baseline, had provided a blood sample, and subsequently died from pancreatic cancer until December 31, 1997. Control subjects were selected from the remaining participants in the cohort. Controls were matched to the cases on sex, age ±2 years and study area at a ratio of 3:1. Subjects who had a cancer diagnosis before the start of follow-up were excluded from the analyses. A total of 68 cases and 199 control subjects are eligible for the present analysis.

Biochemical assay of sera

All serum samples were assayed at SRL laboratory, Hachioji, Japan. Serum samples were assayed in the same batch to minimize interassay variability, and quality control samples were inserted randomly. Laboratory personnel were unable to distinguish among case, control, and quality control samples. Serum sFas levels were

Table 1. Characteristics of the Case and Control Subjects at the Baseline in the JACC Study

Characteristics	Cases (n=68)	Controls (n=199)	P value
Men, %	45.6	44.2	
Age, years*	65.7 ± 7.5	65.4 ± 7.1	
BMI, kg/m^2*	23.2 ± 2.7	22.8 ± 3.1	0.35
Current Smokers, %	29.4	21.6	<0.01
History of diabetes, %	10.3	5.5	0.35
Serum sFAS, ng/mL	2.2 (1.7-2.6)	2.1 (1.7-2.5)	0.11
Serum SOD, U/mL	2.6 (2.2-3.1)	2.5 (2.3-2.9)	0.42

BMI, body mass index; *mean ± standard deviation values; sFas levels and SOD activity are expressed as medians with 25 and 75 percentiles

Table 2. Associations between Serum sFas Levels and Risk of Death from Pancreatic Cancer

Serum sFas levels	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Cases/Controls	14/43	14/56	16/40	23/59	
Age-adjusted OR (95%CI)	1.00	0.80 (0.35-1.85)	1.31 (0.54-3.17)	1.25 (0.54-2.90)	0.42
Multivariable OR (95%CI)	1.00	0.80 (0.33-1.94)	1.28 (0.48-3.37)	1.10 (0.45-2.69)	0.67

Quartile cutoff points were 1.7, 2.1, and 2.5; OR, odds ratio; CI, confidence interval; Multivariable OR, adjusted for age, area, body mass index, cigarette smoking and history of diabetes

Table 2. The association between Serum SOD activity and Risk of Death from Pancreatic Cancer

Serum sFas levels	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Cases/Controls	18/49	13/39	14/57	23/54	
Age-adjusted OR (95%CI)	1.00	0.89 (0.37-2.10)	0.64 (0.28-1.45)	1.16 (0.84-2.47)	0.91
Multivariable OR (95%CI)	1.00	0.92 (0.38-2.27)	0.75 (0.31-1.77)	1.50 (0.67-3.39)	0.67

Quartile cutoff points were 2.3, 2.5, and 2.9; OR, odds ratio; CI, confidence interval; Multivariable OR, adjusted for age, area, body mass index, cigarette smoking and history of diabetes

assayed by enzyme-linked immunoassay (ELISA), using commercially available kit (MBL Co., Ltd., Nagoya). The intra- and inter-assay coefficient of variation for quality control samples were 2.2-5.6%, and 8.2%-12.3% (Ito et al., 2005). Serum SOD activity was estimated from the decreasing rate of nitrite produced by hydroxylamine and superoxide anions, based on improve nitrite method (Oyanagui, 1984). The intra- and inter-assay coefficient of variation for quality control samples were 4.0-6.8%, and 2.8-5.8%. (Ito et al., 2005)

Statistical analysis

The study subjects were categorized into four groups on the basis of the quartile distribution of serum sFas levels or SOD activity among the control subjects who were selected for matching pancreatic cancer deaths. The baseline characteristics between cases and control subjects were compared using general linear models for continuous variables and chi-square tests for categorical variables. Conditional logistic regression models were used to estimate age-adjusted and multivariate-adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). The lowest quartile of serum sFas levels and SOD activity served as the reference group. The variables included in the multivariate analyses were area, body mass index, cigarette smoking, and history of diabetes. Linear tests for trend were performed using the ordinal variables.

All statistical tests were two-tailed, and a P value<0.05 was considered statistically significant. All analyses were done using SAS Release 9.1 (SAS Institute Inc, Cary, NC).

Results

Table l shows the baseline characteristics of the study subjects. There were more current smokers in the case group than in the control group (p<0.01). No statistically significant difference was noted in mean sFas levels (P=0.11) and SOD activity (p=0.42) between case and control subjects.

Table 2 shows the ORs and 95% CIs for the risk of death from pancreatic cancer associated with the other 3 quartiles compared with the lowest quartile. Overall, serum levels of sFas were not associated with the risk of pancreatic cancer deaths, after adjustment for area, body mass index, cigarette smoking, and history of diabetes. No significant trend in risk was noted with increasing serum sFas levels.

The multivariable OR was 1.50 (95%CI: 0.67-3.39) for individuals in the highest quartile, whereas those in the second and third quartile had decreased risk with ORs being 0.92 (95%CI: 0.38-2.27) and 0.75 (95%CI: 0.31-1.77), respectively. (Table 3)

Discussion

In this nested case-control study, we examined two novel serum biomarkers and their relationship with pancreatic cancer risk. Our results suggest that neither serum sFas levels nor SOD activity was associated with the risk of death from pancreatic cancer. One reason for the null finding on both serum biomarkers may be the small number of study subjects. Barring a strong association, a relatively small number of pancreatic cancer deaths included in the present study made it difficult to detect statistically significant associations.

The role of circulating sFas in malignant diseases has not been elucidated. One important function of sFas is to inhibit Fas-mediated apoptosis by neutralizing FasL or anti-Fas antibody. sFas production has been shown to be present in normal individuals, and elevated circulating sFas levels have been observed in various malignant diseases including bladder cancer and hepatocellular carcinoma (Owen-Schaub, 2001; Ueno et al., 1999; Akhmedkhanov et al., 2003; Sheen-Chen et al., 2003; Midis et al., 1996). Because sFas can functionally antagonize FasL to effectively inactivate cell-surface Fas function, it is plausible that elevated Fas production may promote tumorigenesis and disease progression. Our findings, however, failed to show increased risk of pancreatic cancer with elevated serum sFas levels. One possible reason is that circulating sFas levels may be under genetic control. A recent study has shown that sFas levels in subjects carrying FAS-A/A genotype were significantly higher than that of those carrying the G/G genotype (Mahfoudh et al., 2007). Further studies are warranted to examine both FAS polymorphisms and circulating sFas levels, and their relationship with pancreatic cancer risk.

To date, few prospective studies have examined the association between serum SOD activity and pancreatic cancer risk. We found no significant risk reduction associated with increased serum SOD activity in apparently healthy individuals. However, one notable finding from our study is that individuals in the highest quartile had an increased risk, while those in the second and third quartile had decreased risk with ORs being less than 1.0. Although these associations were statistically insignificant, it is likely that variations in SOD activity according to the amount of inflammation-induced ROS may in part contribute to the above-mentioned results. We consider that the overall null findings might be due to the small number of study subjects. Another possible explanation is that serum SOD activity does not differentiate between the different SOD isoforms. In extracellular fluids including serum, extracellular SOD is the major SOD isoform. The activity of SOD in serum has been shown to closely parallel the concentration of extracelluar SOD (Adachi et al., 1992). The association of serum Mn-SOD and CuZn-SOD with pancreatic cancer risk is unknown and merits further investigation.

Our study has limitations. First, serum sFas levels and SOD activity could not be measured in all deaths from pancreatic cancer during the follow-up period, which may introduce selection bias if people who had provided sera differed systematically from those who did not. However, donation of blood samples depended solely on the subject's intention when they were healthy at baseline. Thus, we believe that any bias due to blood donation would not seriously affect our results. Second, the risk estimates were unstable because of the small number of pancreatic cancer deaths included in the present study. Third, the stability of serum SOD activity after long-term storage may be a concern. However, using the sera from the JACC

Study participants, Ito et al. showed that serum SOD activity was stable during the long-term storage at -80˚C (Ito et al., 2005). Similarly, no significant changes in serum SOD activity with respect to storage time or temperature have also been reported in other studies (Casado et al., 1995).

In conclusion, our results did not support the hypothesis that serum sFas levels and SOD activity are associated with the risk of death from pancreatic cancer in healthy individuals.

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 & 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, 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; and Dr. Hideo Shio, Moriyama Municipal Hospital.

Acknowledgements

The JACC Study has been supported by Grants-in-Aid for Scientific Research (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022, 18014011, 20014026) from MEXT, Japan.

The authors express their sincere appreciation to Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and a former chairperson of the JACC Study, to Dr. Haruo Sugano, the former Director of the Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study, and to Dr. Yoshiyuki Ohno, Professor Emeritus, Nagoya University School of Medicine, who was also a former chairperson of the study. The authors also wish to thank Dr. Tomoyuki Kitagawa, President Emeritus of the Cancer Institute of the Japanese Foundation for Cancer Research and a former chairperson of a Grant-in-Aid for Scientific Research on Priority Area 'Cancer', for his valuable support of this study.

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