SERUM COMPONENTS AND RISK OF CANCER - V

Relationship between Serum Levels of Superoxide Dismutase Activity and Subsequent Risk of Cancer Mortality: Findings from a Nested Case-control Study within the Japan Collaborative Cohort Study

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Abstract

Superoxide dismutases (SODs) are antioxidant enzymes that play a role in the defense system of the body. They may be involved in protection against carcinogenesis processes. In the present study, we investigate the association between serum SOD activity and the risk of deaths due to all cancers combined, based on a nested case-control study within the Japan Collaborative Cohort Study of 914 cancer deaths and 2,739 matched controls. Blood samples were obtained at the baseline and stored at -80ºC until analysis for SOD levels. Serum levels of SODs were divided into quartiles, with the first quartile used as the reference. A conditional logistic model was used to estimate odds ratios (ORs) for total cancer mortality associated with serum SOD quartile levels. The adjusted ORs and 95% confidence intervals (CIs) for the second, third and fourth SOD quartiles were 0.96 (95%CI: 0.77-1.19), 1.18 (0.92-1.51), and 1.32 (1.04-1.69), respectively. In analyses stratified by observation period, the adjusted ORs of the respective quartiles were 0.81 (95%CI: 0.60-1.08), 0.98 (0.70-1.37), and 1.28 (0.92-1.79) for the period from the baseline to 1994; and the adjusted ORs were 1.18 (95%CI: 0.85-1.63), 1.47 (1.04-2.10), and 1.41 (1.00-2.04) for the period after 1994. To conclude, we found a slightly positive association between serum SOD level and the risk of all cancer mortality in the present study. Elevated serum SOD levels might reflect a response to oxidative stress, and then may predict a state of excess reactive oxygen species in the carcinogenesis process. Detailed studies of associations between serum SOD levels and cancers in specific sites should now be performed, with attention to particular tumour types.

Keywords: Cancer mortality - JACC Study - nested case-control study - superoxide dismutase activity

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Introduction

Superoxide dismutases (SODs) are antioxidant enzymes that play a role in the defense system of the body. In humans, two forms of SOD have been identified, the manganese-containing (Mn-SOD) and the copper-zinc-containing (Cu,Zn-SOD) (Hassan and Fridovich, 1981). These SOD enzymes catalyze the dismutation of superoxide anion (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), and the latter is then converted into water by glutathione peroxidase and catalase. In the normal metabolism, reactive oxygen species (ROS) are produced as a natural byproduct. Excessive ROS accumulation under certain conditions will lead to cell injury, such as damage to DNA, and proteins, therefore may be implicated in the process of malignancy (Oberley and Buettner, 1979). As a response to oxidation, the body utilizes scavenging SOD enzymes, which acts to increase the dismutation rate of the superoxide anion. In this way, these enzymes protect cells against oxidative stress (Fridovich, 1997).

It is known that cancer cells exhibit differences in the

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activity and expression of SODs when compared with the normal counterparts (Oberley and Buettner, 1979; Fridovich, 1997). A decrease in manganese-SOD was identified in primary pancreatic cancer cells (Cullen et al., 2003); SOD activity was shown to be increased in tumor tissues, including stomach cancer (Izutani et al., 1998), colon cancer (Janssen et al., 1999). However, a high level of the SOD enzyme was found in the sera of patients with hepatocellular carcinoma compared to healthy subjects (Clemente et al., 2007). Some studies have assessed the possible association between serum SOD activity and the risk of cancer. A case-control study in Japan showed that higher SOD levels were associated with increased risk of stomach cancer incidence (Lin et al., 2002), while other case-control studies did not demonstrate any significance (Lin et al., 2005; Pham et al., 2007).

In the present study, we attempted to investigate the association between serum SOD activity and the risk of deaths due to all cancers combined, based on a nested case-control study within a large-scale cohort study in Japan.

Materials and Methods

Study population

The study was conducted as a nested case-control study within the Japan Collaborative Cohort Study (the JACC Study), a large scale cohort study designed to evaluate the effects of various risks or protective factors on cancer mortality and incidence. Details of the JACC Study have been described elsewhere (Ohno and Tamakoshi, 2001; Tamakoshi et al., 2005). Briefly, a baseline survey was conducted between 1988 and 1990. A total of 110,792 subjects aged 40 to 79 years from 45 areas throughout Japan were enrolled in the study and completed a self-administered questionnaire. At the baseline study, approximately one-third of subjects (39,242 subjects) in study areas where a general health checkup had been performed donated a peripheral blood serum sample. Sera were separated from the blood samples at laboratories in or near the surveyed study areas as soon as possible and kept frozen at -80ºC until analyzed for the presence of biochemical substances.

We followed these 110,792 subjects to identify cancer mortality. The causes and dates of death among the study subjects were determined by reviewing all death certificates in each study area with the permission of the Director-General of the Prime Minister's Office (Ministry of Public Management, Home Affairs, Post and Telecommunications) till 1997. Participants who had moved out from their study areas at baseline were also identified in each area by reviewing the population-register sheets of cohort members. Cause of death was recorded and coded using the International Classification of Diseases and Injuries, 9th Revision (ICD-9), from the baseline survey to the end of 1994, and the 10th Revision (ICD-10) from 1995. All ICD-9 codes were then converted into ICD-10 codes for analysis. Deaths due to cancer involved in the present analysis were coded from C00 to C97 according to the ICD-10 Revision.

The whole study design and use of serum was approved by the Ethical Board at Nagoya University School of Medicine, where the central office of JACC Study was located.

Case identification and control selection

Our study population was initially limited to the 39,242 subjects for whom serum samples were available, followed by exclusion of those with a self-reported history of any type of cancer. We observed 914 cancer deaths through the end of 1997. Each patient was assigned three controls matched for study area, gender, and age using baseline characteristics. All controls were alive, had not migrated, and were free of any cancer at the time of the matched case subject's death. In total, this study enrolled 914 cancer deaths and 2,739 controls.

Laboratory assays

Serum samples were assayed in 1999 and 2000 by trained staff blinded to case/control status. Serum SOD activity was measured from the rate of decrease in nitrite produced by hydroxylamine and superoxide anions, based on an improved nitrite method, and expressed as units of SOD per milliliter (U/mL) of blood (Oyanagi Y, 1984). Assay range was 0.1 – 10.0 U/mL, and intra- and interassay coefficients of variation were 4.0-6.8% and 2.8-5.8%, respectively.

Statistical analysis

Proportions and mean values of baseline characteristics between cases and their matched controls were compared using the Mantel-Haenszel chi-square test

Table 1. Sites of Cancers in Cases of Mortality

Cancer site	ICD-10	Number	%
Esophagus	C15	24	2.6
Stomach	C16	172	18.8
Colon	C18	64	7.0
Rectum	C20	32	3.5
Liver and intrahepatic bile ducts	C22	81	8.9
Gallbladder	C23	32	3.5
Unspecified portion of biliary tract	C24	36	4.0
Pancreas	C25	68	7.5
Bronchus and Lung	C34	193	21.1
Prostate	C61	24	2.6
Lymphoma	C85	21	2.3
Other		167	18.2
All sites		914	100.0

Table 2. Selected Baseline Characteristics of Case and Control Groups

	Cases	Controls	p-value[a]
Number of subjects	914	2,739	
Mean age (SD)[b]	64.6 (8.2)	64.5 (8.0)	
Male (%)	528 (57.8)	1,582 (57.8)	
Mean BMI[c] kg/m^2 (SD)[b]	22.7 (3.1)	22.8 (2.9)	0.31
Current smokers (%)	322 (35.2)	718 (26.2)	<0.01
Current drinkers (%)	228 (25.0)	734 (26.8)	0.27
Mean (U/ml) SOD (SD)[b]	3.4 (2.8)	3.3 (2.5)	0.20

[a]calculated by analysis of variance for continuous variables and Mantel-Haenszel chi-square test for categorical variables; [b]SD, standard deviation; [c]body mass index

and analysis of variance. Serum values were divided into quartiles based on the distribution of serum values in all control subjects, with the first quartile used as reference. SOD quartile values for quartiles 1, 2, 3, and 4 were ≤2.2; 2.3-2.6; 2.7-3.1; and ≥3.2 U/mL, respectively. The Odds ratios (ORs) for total cancer mortality associated with serum SOD levels were estimated using the conditional logistic model (Kleinbaum and Klein, 2002), adjusted for body mass index (computed as weight in kilograms divided by the square of the height in meters), tobacco smoking status, and alcohol consumption. We also conducted analyses stratified by the observation period of cancer death from the baseline to 1994, and thereafter, in order to examine whether subjects in later or earlier stage of cancers at baseline might affect the potential association. The statistical significance of trends across exposure quartiles was assessed by including ordinal terms for each serum level quartile and entering the variable as a continuous term in the model. All p values and 95% confidence intervals (95% CI) presented in the tables were based on two-sided tests. All statistical analyses were performed using Stata software version 9.0 (StataCorp, 2005).

Results

Table 1 describes numbers of cancer deaths by the anatomic sites. Of the 914 patients who died from all cancers, we observed 193 deaths from lung cancer (21.1%), 172 from stomach cancer (18.8%), 81 from liver cancer (8.9%), 68 from pancreatic cancer (7.4%), and 64 from colon cancer (7.0%).

Table 2 shows the baseline characteristics of the 914 cancer patients and their 2,739 matched controls. Mean age was 64.6 and 64.5 years, respectively, with no difference in body mass index between the two groups. The proportion of current smokers was higher in the case group than among controls (p<0.01), but no difference was seen for current drinkers (p=0.27).

Table 3 shows crude and adjusted ORs for risk of total cancer mortality according to serum SOD level quartile. The adjusted ORs and 95% CIs for the second; third; and fourth SOD quartiles were 0.96 (95%CI: 0.77-1.19), 1.18 (95%CI: 0.92-1.51), and 1.32 (95%CI: 1.04-1.69), respectively (p for trend<0.01). In analyses stratified by observation period, the adjusted ORs of the respective quartiles were 0.81 (95%CI: 0.60-1.08), 0.98 (95%CI: 0.70-1.37), and 1.28 (95%CI: 0.92-1.79), (p for trend=0.08) for the period from the baseline to 1994; and the adjusted ORs were 1.18 (95%CI: 0.85-1.63), 1.47 (95%CI: 1.04-2.10), and 1.41 (95%CI: 1.00-2.04) (p for trend=0.05) for the period after 1994.

Table 3. Crude and Adjusted Odd Ratios (OR)[a] for Serum Levels of SOD with Risk of Total Cancer Mortality

	Deaths	Controls	Crude OR (95% CI)	Adjusted OR 95% CI
Total				
Quartile 1	218	685	1.00	1.00
Quartile 2	251	837	0.95 (0.77-1.18)	0.96 (0.77-1.19)
Quartile 3	196	564	1.14 (0.90-1.46)	1.18 (0.92-1.51)
Quartile 4	249	653	1.29 (1.02-1.64)	1.32 (1.04-1.69)
p for trend			p=0.02	p<0.01
Before 1994				
Quartile 1	127	368	1.00	1.00
Quartile 2	136	481	0.81 (0.61-1.08)	0.81 (0.60-1.08)
Quartile 3	94	289	0.96 (0.69-1.34)	0.98 (0.70-1.37)
Quartile 4	130	321	1.25 (0.90-1.73)	1.28 (0.92-1.79)
p for trend			p=0.10	p=0.08
After 1994				
Quartile 1	91	317	1.00	1.00
Quartile 2	115	356	1.16 (0.84-1.60)	1.18 (0.85-1.63)
Quartile 3	102	275	1.40 (0.97-1.99)	1.47 (1.04-2.10)
Quartile 4	119	332	1.38 (0.96-1.98)	1.41 (1.00-2.04)
p for trend			p=0.06	p=0.05

[a]adjusted for body mass index (kg/m^2), smoking habit, and alcohol consumption

Discussion

In the present study, associations of highest quartile levels of serum SOD with all cancer mortality were observed. The positive association seem to be stronger for the period after 1994 than that from the baseline to the end of 1994.

Under normal physiological conditions, ROS are produced during metabolic process in the body. ROS formation and elimination are balanced by the action of antioxidant enzymes. This balance is important for maintaining proper cellular states. A moderate increase in ROS can stimulate cell growth. However, excessive ROS generation will contribute to cellular injury, such as damage to DNA, protein. Therefore, antioxidant defense mechanisms, including SOD, must promptly eliminate harmful ROS in order to protect tissues from oxidant damage. The SOD enzymes are a major cellular defense system against superoxide. Different forms of SOD do not only differ in their metal binding ability, which are Mn-SOD and Cu,Zn-SOD. But they differ also in their distribution in cell compartment, and sensibility to various reagents (Oberley and Buettner, 1979).

Several studies have assessed the role of SOD in various cancers. Previous studies have showed that a number of cancer cell line contain elevated levels of SOD. High levels of SOD expression were found in the ovarian cancer tissues compared normal tissues (Hu et al., 2005). In a study in Poland, SOD activity was higher in malignant liver tissues than in benign liver tumor, liver cirrhosis, or normal liver tissues (Skrzycki et al., 2008). In recent work in the US, various pancreatic cancer cell lines had decreased levels of Mn-SOD compared with normal human pancreas (Cullen et al., 2003). A second US study estimated SOD activity in three breast cell lines, a nonmalignant breast epithelial cell line and two human mammary adenocarcinomas, and found that malignant cells had lower total SOD activity as well as lower Mn-SOD activity than non-malignant cells (Weydert et al., 2006). A case-control study in Japan examined the role of Mn-SOD levels and stomach cancer and revealed a weak increase in risk in the highest quartile of Mn-SOD, but the association was without statistical significance (Lin

et al., 2005), while a second case-control study in Japan showed that high serum levels of Cu/Zn-SOD were significantly elevated in gastric cancer patients (Lin et al., 2002). These results reflected a discrepancy in association between SOD levels and cancers, which might be due to different cancer sites.

In present nested case-control study, cancer deaths were identified and recorded from baseline study through the end of 1997. Each of them was then matched to three controls by using the study area, gender, and age at baseline. We examined the association between serum SOD levels and cancer mortality from all sites combined. As results, we observed an association of the highest quartile level of serum SOD activity with increased risk of deaths from all cancers. We also performed analysis by stratification of observation period of cancer death from the baseline to 1994, and thereafter. The results showed almost the same pattern of increased risk with cancer mortality during period after 1994, while the results did not show any association between high SOD levels and cancer risk for the period from the baseline to 1994. Thus, carcinogenesis may be involved in a long process, and the increased serum SOD level may predict for early carcinogenesis process. Further, the association of serum SOD levels with all cancers combined, since the degree of this association may be different for each cancer site. The more detailed examination of this association by specific sites should be performed, then it may provide more detailed evidence of the relationship between SOD and specific cancers.

The results from the present study showed that elevated serum SOD levels are associated with increased risk of deaths from all cancers combined. The findings from our study were in line with some previous studies. A study in Italy compared serum Mn-SOD levels of 70 healthy subjects with those of liver cirrhotic patients as well as liver cirrhotic patients with hepatocellular carcinoma (HCC) (Clemente et al., 2007). Data showed that Mn-SOD activity is significantly higher in liver cirrhotic patients, and activity is more elevated in cirrhotic patients with HCC. The elevated SOD levels have been also reported in other cancer sites, for instance in ovian cancer (Hu et al., 2005), stomach cancer (Lin et al., 2002), and colon cancer (Janssen et al., 1999). The underlying mechanism of this association might be cellular adaptation to cope with ROS, which might induce the upregulation of SOD, rather than increased serum SOD levels being a potential risk factor for cancer mortality. Increased activity of SOD might occur after responding to ROS exposure. In fact, an excessive ROS state is usually difficult to be identified or immeasurable. Hence, the increase of serum SOD might be a proxy reflecting cellular response to oxidative stress.

The nested case-control design of our study, conducted within a large-scale cohort study, is one of its strengths. Nested investigations are able to establish the temporal nature of an association which traditional case-controls studies may not. Further, our analyses were conducted on a large numbers of subjects, consisting of 914 cancer deaths and 2,739 controls. Sex-, age-, and study area based matching between cases and controls aided in reducing confounding factors.

In conclusion, we found a slightly positive association between serum SOD level and the risk of all cancer mortality in the present study. Elevated serum SOD levels might be regulated in order to response to oxidative stress, and then may predict excessive ROS state in the carcinogenesis. The association between serum SOD levels and cancers from specific sites should be performed, and it may therefore be helpful to clarify more detailed association by specific sites of cancer.

Member List of the JACC Study Group

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