SERUM COMPONENTS AND RISK OF CANCER - VI

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

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Abstract

The expression of superoxide dismutases (SODs) has been shown to differ between lung tumor and tumor-free tissues. In the present study, we investigated the association between serum SOD activity and the risk of lung cancer mortality, based on a nested case-control design study within the Japan Collaborative Cohort Study, with a sample of 193 lung cancer patients and 573 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 lung cancer mortality associated with serum SOD quartile levels. The adjusted ORs and 95% CIs for the second, third; and fourth SOD quartiles were 0.80 (95%CI: 0.49-1.29), 1.32 (0.78-2.25), and 1.07 (0.60-1.89), respectively. In analyses stratified by observation period, the adjusted ORs of the respective quartiles were 0.56 (95%CI: 0.30-1.07), 1.16 (0.57-2.37), and 1.11 (0.52-2.35) for the period from the baseline to 1994; and the adjusted ORs of 1.36 (95%CI: 0.65-2.85), 1.71 (0.75-3.87), and 1.06 (0.44-2.53) for the period after 1994. To conclude, we found no significant association between serum SOD level and the risk of deaths from lung cancer in the present study.

Keywords: superoxide dismutase; SOD; lung cancer; nested case-control study; cohort study; JACC Study

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Introduction

Lung cancer is the most common cancer in the world, including Japan, and it is one of the leading causes of cancer deaths (Parkin et al., 2005; Youlden et al., 2008). Tobacco smoking is considered as the most important risk factor for this disease, but viral infection, ionizing radiation, and some kinds of gas exposure may also contribute to the disease. All these factors may cause damage to bronchial and alveolar epithelial cells that may initiate the pathogenesis of lung cancer (Bruske-Hohlfeld, 2009).

Lung injury occurs when there is an imbalance between reactive oxygen species (ROS) and the bodies antioxidant system. In response to this oxidative stress, the body utilizes antioxidant enzymes to remain oxidative-antioxidant balance in the body. The superoxide dismutases (SODs) are a family of antioxidant enzymes responsible for the detoxification of superoxide free radicals. They are thought to be one of the first lines of antioxidant defense. These SODs protect cell and tissues against oxidative stress by catalyzing 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 (Oberley & Buettner, 1979; Fridovich, 1997).

It is known that the activity and expression of SOD in cancer cells differs from that in normal cells (Oberley &

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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 some tumor tissues, including stomach cancer (Izutani et al., 1998), colon cancer (Janssen et al., 1999). With regard to lung cancer, Yamanaka and Deamer reported that the SOD activity in lung fibroblast cells at levels somewhat higher than in human embryonic lung tissues (Yamanaka & Deamer, 1974). Some studies have assessed the SOD activity in sera of cancer patients and their controls, including stomach cancer (Lin et al., 2002), or liver cancer (Clemente et al., 2007). To our knowledge, no study has examined the potential association with lung cancer risk. In the present investigation, we therefore evaluated links between lung cancer and serum SOD activity using a nested case-control design 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 & 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 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 the 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. Lung cancer in the present analysis was defined as code C34 of the ICD-10.

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 193 lung 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 193 cancer deaths and 573 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

Table 1. Selected Baseline Characteristics of Lung Cancer Case and Control Groups

	Cases	Controls	p-value[a]
Number of subjects	193	573	
Age in years (SD)[b]	65.4 (7.8)	65.1 (7.5)	
Male (%)	146 (75.7)	433 (75.5)	
Mean BMI[c] kg/m^2 (SD)[b]	22.1 (2.6)	22.6 (2.7)	0.06
Tobacco smoking status			
Never smoker (%)	39 (20.2)	214 (37.4)	<0.01
Former smoker (%)	37 (19.2)	127 (22.1)	
Current smoker (%)	105 (54.4)	200 (34.9)	
Missing (%)	12 (6.2)	32 (5.6)	
Current drinkers (%)	62 (32.1)	173 (30.2)	0.62
Mean (U/ml) SOD (SD)[b]	3.1 (2.1)	3.2 (2.3)	0.84

[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

Table 2. Crude and Adjusted Odd Ratios (OR)[a] for Serum Levels of SOD with Risk of Lung Cancer Mortality

	Deaths	Controls	Crude OR (95% CI)	Adjusted OR 95% CI
Total				
Quartile 1	50	146	1.00	1.00
Quartile 2	51	179	0.83 (0.53-1.31)	0.80 (0.49-1.29)
Quartile 3	49	119	1.20 (0.73-1.99)	1.32 (0.78-2.25)
Quartile 4	43	129	1.01 (0.59-1.70)	1.07 (0.60-1.89)
p for trend			0.74	0.57
Before 1994				
Quartile 1	28	71	1.00	1.00
Quartile 2	25	114	0.57 (0.31-1.06)	0.56 (0.30-1.07)
Quartile 3	27	65	1.14 (0.58-2.25)	1.16 (0.57-2.37)
Quartile 4	25	63	1.11 (0.55-2.23)	1.11 (0.52-2.35)
p for trend			0.45	0.48
After 1994				
Quartile 1	22	75	1.00	1.00
Quartile 2	26	65	1.37 (0.69-2.72)	1.36 (0.65-2.85)
Quartile 3	22	54	1.30 (0.61-2.77)	1.71 (0.75-3.87)
Quartile 4	18	66	0.84 (0.39-2.01)	1.06 (0.44-2.53)
p for trend			0.79	0.75

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

coefficients of variation were 4.0-6.8% and 2.8-5.8%, respectively.

Statistical analysis

Proportions and mean values of baseline characteristics between lung cancer deaths and their matched controls were compared using the Mantel-Haenszel chi-square test 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 lung cancer death associated with serum SOD levels were estimated using a conditional logistic model (Kleinbaum & Klein, 2002), adjusted for body mass index (computed as weight in kilograms divided by the square of the height in meters), tobacco smoking status, alcohol consumption.

We also conducted analyses stratified by the observation period of lung 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 shows the baseline characteristics of the 193 lung cancer patients and their 573 matched controls. Mean age was 65.4 and 65.1 years, respectively. There was no difference in body mass index between the case and control groups, with p=0.06. The proportion of current smokers in the case group was 54.4%, higher than that of controls (34.9%), with p<0.01. But no difference was seen for current drinkers between the two groups (p=0.62).

Table 2 shows crude and adjusted ORs for risk of lung cancer death according to serum SOD level quartile. The adjusted ORs and 95% CIs for the second; third; and fourth SOD quartiles were 0.80 (95%CI: 0.49-1.29), 1.32 (95%CI: 0.78-2.25), and 1.07 (95%CI: 0.60-1.89), respectively. In analyses stratified by observation period, the adjusted ORs of the respective quartiles were 0.56 (95%CI: 0.30-1.07), 1.16 (95%CI: 0.57-2.37), and 1.11 (95%CI: 0.52-2.35) for the period from the baseline to 1994; and the adjusted ORs were 1.36 (95%CI: 0.65-2.85), 1.71 (95%CI: 0.75-3.87), and 1.06 (95%CI: 0.44-2.53) for the period after 1994.

Discussion

In the present nested case-control study, we examined the potential association between serum SOD levels and the risk of lung cancer deaths among general Japanese population. The results did not show any significant association overall or when stratifying by observation period of lung cancer death from the baseline to 1994, and thereafter.

Most living cells, including lung cells, generate reactive oxygen species (ROS) under normal condition as a natural by-product of the normal metabolism of oxygen. The first ROS is a superoxide anion which participates in the generation of other oxidative metabolites. 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. In response to the oxidative stress, body has antioxidant enzymes, among which SOD is key antioxidant enzyme. The often examined forms of SOD with cancers are the manganese-containing (Mn-SOD) and the copper-zinc-containing (Cu,Zn-SOD) (Hassan & Fridovich, 1981). Different forms of SOD do not only differ in their metal binding ability, which are Mn-SOD and Cu,Zn-SOD, but they may also vary in their distribution in cell compartment, as well as in their sensitivity to various exogenous reagents (Oberley & Buettner, 1979).

For the lung, this organ is directly exposed to ambient air. Local oxygen partial pressure at the alveolar level is high. Thus, lung cells experience directly oxidant stress, such as environmental pollutants, or tobacco smoke (Kinnula & Crapo, 2003). The lung may therefore be exposed to oxidative stress stronger than in the other vital organs. When the balance between oxidative and anti-oxidative is interrupted, the lung may be one of the first affected organs; it may then initiate the carcinogenesis process.

Previous studies have shown that a number of cancer cell lines contain different levels of SOD than normal cell lines. High levels of SOD expression were found in the ovarian cancer tissues compared with normal tissues (Hu et al., 2005). For example, SOD activity was higher in malignant liver tissues than in benign liver tumors, liver cirrhosis, or normal liver tissues (Skrzycki et al., 2008). Pancreatic cancer cell lines had decreased levels of Mn-SOD compared with normal human pancreas (Cullen et al., 2003). A study estimated SOD activity in three breast cell lines, a non-malignant 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).

Studies assessing the role of SOD in tissues of lung related diseases, including lung cancers have been conducted (Chung-man Ho et al., 2001; Kinnula & Crapo, 2003; Harju et al., 2004; Svensk et al., 2004). A study in Finland assessed SOD expression in lung cancer specimens, and distant healthy-looking tissue as controls. The results showed that both Mn-SOD and Cu,Zn-SOD were detected at higher levels in lung adenocarcinoma and squamous than non-magninant lung tissues (Svensk et al., 2004). Another study in Finland evaluated SOD expression at various airway sites from nonsmokers, smokers with and without chronic obstructive pulmonary disease, the results showed an elevation of Mn-SOD expression in smokers compared to nonsmokers (Harju et al., 2004). A study in the US evaluated SOD expression in 16 tumorous and 21 tumor-free lungs, and found the former to have significantly higher levels (Chung-man Ho et al., 2001). Based on the results from these studies, SOD activity may be upregulated, increasing in response to oxidant stress in lung cancer tissues. In the present study, we examined the potential association between serum SOD levels and the risk from lung cancer mortality. However, the results did not reveal any significant relationship. It seems that SOD levels in sera may not always reflect SOD expression in tissues.

In conclusion, we found no significant association between serum SOD level and the risk of deaths from lung cancer in the present study.

Member List of the JACC Study Group

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