

Serum Copper/Zinc Superoxide Dismutase (Cu/Zn SOD) and Gastric Cancer Risk: a Case-Control Study

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We conducted a case-control study to evaluate the association between serum levels of copper/zinc superoxide dismutase (Cu/Zn SOD) and the risk of gastric cancer. Cases were 214 patients who had been diagnosed with gastric cancer and controls were 120 persons who underwent medical checkups. Serum levels of Cu/Zn SOD were determined by enzyme-linked immunosorbent assay (ELISA). Compared with the lowest quartile, the OR (odds ratio) was 4.54 (95% CI (confidence interval), 1.62–12.66) for the third quartile and 15.75 (95% CI, 5.84–42.46) for the highest quartile. With both early and advanced cancers, as well as with the intestinal and diffuse types, a significant increase in risk was observed with increasing levels of serum Cu/Zn SOD. Our case-control study showed that serum levels of Cu/Zn SOD were significantly elevated in gastric cancer patients compared with apparently healthy controls, and higher Cu/Zn SOD levels may be associated with an increased risk of gastric cancer.

Key words: Cu/Zn SOD — Gastric cancer risk — Case-control study

Despite the high prevalence of *Helicobacter pylori* (*H. pylori*) infection in patients with gastric cancer, most apparently healthy people infected with *H. pylori* never develop gastric cancer. It is important, therefore, to identify other factors that may determine risk among those infected with *H. pylori*. Studies have shown that gastric cancer may be caused by reactive oxygen species (ROS), and dietary antioxidants may protect against this disease.¹⁾ Superoxide dismutase (SOD), which is known to be present in at least two forms, cytoplasmic copper/zinc SOD (Cu/Zn SOD) and mitochondrial manganese (Mn SOD), plays a key role in the detoxification of superoxide radicals.²⁾ Several studies have reported that Mn SOD expression is increased in colorectal, ovarian and gastric cancer.^{3–6)} However, few epidemiological studies have addressed the relationship between serum levels of Cu/Zn SOD and gastric cancer risk. Cu/Zn SOD catalyzes the dismutation reaction of the toxic superoxide radical to molecular oxygen and hydrogen peroxide and thus forms a crucial part of the cellular antioxidant defense mechanism.

Using a case-control study design, we aimed to examine the association between serum levels of Cu/Zn SOD and gastric cancer risk.

The details of study design and case/control selection have been described previously.⁷⁾ Briefly, case subjects were patients who were diagnosed as having stomach can-

cer at one of nine hospitals in the Tokyo Metropolitan Area between 1993 and 1995. Patients who had undergone treatment for gastric cancer were excluded at entry. An endoscopy was performed on all eligible cases and the diagnosis was confirmed by an examination of the resection or biopsy specimen. Data on pathological findings, including the type and stage of the cancer, were then recorded. Gastric cancer was subdivided into early or advanced cancer, as well as intestinal or diffuse type, based on the criteria proposed by the Japanese Research Society for Gastric Cancer (JRSGC).⁸⁾ Control subjects were recruited from a group of apparently healthy people who underwent medical checkups at a health promotion center in the same area. The cases and the control subjects were asked to provide sera, and written informed consent was obtained from all subjects. Between 1993 and 1995, we enrolled 800 gastric cancer patients and 1000 apparently healthy controls. From this group, we randomly selected 214 cases and 120 controls, who aged from 40 to 69 years, as the subjects of the present study, based on sex, age, histological type and disease stage.

Serum samples of cases and controls were collected in the same method and stored -80°C until analysis. Serum levels of Cu/Zn SOD were measured by a commercially available enzyme-linked immunosorbent assay (ELISA, Amersham, Pharmacia Biotech, NJ) using the manufacturer's assay procedure. The summary of this procedure has been reported elsewhere.⁹⁾ During measurement, serum samples were analyzed in randomly ordered duplicates in

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Table I. Selected Characteristics of Cases and Controls

	Cases (n=207)		Controls (n=119)		P for difference
	N	%	N	%	
Age distribution					<0.01
40-49	25	12.1	40	33.6	
50-59	92	44.4	40	33.6	
60-69	90	43.5	39	32.8	
Mean age±SD	57.6±7.4		54.3±8.6		<0.01
Cigarette smoking					
Nonsmoker	53	25.7	39	33.6	<0.01
Former smoker	56	27.2	13	11.2	
Current smoker	97	47.1	64	55.2	
Cu/Zn SOD levels ^{a)}					<0.01
40-49	23.3 (12.7 and 50.9)		8.7 (6.3 and 13.4)		
50-59	17.6 (10.9 and 37.5)		8.2 (6.0 and 10.4)		
60-69	15.7 (10.7 and 29.2)		9.1 (7.2 and 12.6)		
<i>H. pylori</i> infection					<0.01
Negative	5	2.4	42	35.3	
Positive	202	97.6	77	64.7	

a) Data are shown as median (25th and 75th percentile).

Table II. Odds Ratios for Gastric Cancer According to the Quartile of Serum Levels of Cu/Zn SOD

	Cases		Controls		OR	95% CI
	N	%	N	%		
Serum Cu/Zn SOD						
0-6.4	9	4.3	30	25.2	1.00	
6.5-8.8	13	6.3	30	25.2	1.37	0.45-4.16
8.9-11.5	40	19.3	30	25.2	4.54	1.62-12.66
11.6-	145	70.1	29	24.4	15.75	5.84-42.46
					P for trend <0.01	

Adjusted for sex, age stratified by 10 years, cigarette smoking and *Helicobacter pylori* infection.

order to reduce systematic and interassay error. All assays were performed by laboratory personnel who were blinded to the case/control status.

The demographic and clinical characteristics of cases and controls were compared by use of the Mann-Whitney *U* test or χ^2 test. An unconditional logistic model was used to calculate the odds ratios (ORs) and 95% confidence interval (CI) for gastric cancer by quartile levels of serum Cu/Zn SOD. Cut points for quartiles were determined according to the controls' serum Cu/Zn SOD distribution, and the ORs were adjusted for sex, age, cigarette smoking and *H. pylori* infection. In the logistic model, trend *P* was calculated by treating categorical variables as continuous variables. All *P*-values were from two-tailed tests and values lower than 0.05 were considered statistically signifi-

cant. All analyses were conducted using SAS release 6.12 (SAS, Inc., Cary, NC).

We excluded seven cases and one control because of hemolysis. The remaining 326 subjects were eligible for the present analysis. Table I shows the selected characteristics of case and control subjects. The median level of serum Cu/Zn SOD was significantly higher in cases than in controls across each age category. Serum levels of Cu/Zn SOD in controls did not differ significantly across each age group.

No significant differences were observed in serum levels of Cu/Zn SOD between males and females, and analysis by sex showed similar ORs, so we have presented the combined results for males and females. The risk of gastric cancer is positively associated with serum levels of

Table III. Odds Ratios for Intestinal and Diffuse Type of Gastric Cancer According to the Quartile of Serum Cu/Zn SOD Levels

	Cases		Controls		OR	95% CI
	N	%	N	%		
Intestinal						
0–6.4	2	2.3	30	25.2	1.00	
6.5–8.8	4	4.5	30	25.2	1.56	0.25–9.84
8.9–11.5	16	18.2	30	25.2	6.18	1.20–31.90
11.6–	66	75.0	29	24.4	23.75	4.93–114.49
					<i>P</i> for trend <0.01	
Diffuse						
0–6.4	7	6.0	30	25.2	1.00	
6.5–8.8	9	7.8	30	25.2	1.27	0.37–4.36
8.9–11.5	22	19.0	30	25.2	3.54	1.14–11.01
11.6–	78	67.2	29	24.4	11.20	4.05–35.52
					<i>P</i> for trend <0.01	

Adjusted for sex, age stratified by 10 years, cigarette smoking and *Helicobacter pylori* infection.

Table IV. Odds Ratios for Early and Advanced Gastric Cancer According to the Quartile of Serum Cu/Zn SOD Levels

	Cases		Controls		OR	95% CI
	N	%	N	%		
Early						
0–6.4	4	4.5	30	25.2	1.00	
6.5–8.8	4	4.6	30	25.2	0.87	0.18–4.22
8.9–11.5	13	14.8	30	25.2	2.85	0.73–11.09
11.6–	67	76.1	29	24.4	15.58	4.34–55.91
					<i>P</i> for trend <0.01	
Advanced						
0–6.4	5	4.2	30	25.2	1.00	
6.5–8.8	9	7.6	30	25.2	1.62	0.44–6.00
8.9–11.5	27	22.7	30	25.2	5.22	1.56–17.42
11.6–	78	65.5	29	24.4	14.11	4.44–44.83
					<i>P</i> for trend <0.01	

Adjusted for sex, age stratified by 10 years, cigarette smoking and *Helicobacter pylori* infection.

Cu/Zn SOD, and the trend is statistically significant (trend $P < 0.01$) (Table II). Compared with the lowest quartile, the OR was 4.54 (95% CI, 1.62–12.66) for the third quartile and 15.75 (95% CI, 5.84–42.46) for the highest quartile after adjustment for age and *H. pylori* infection. Since there were age differences between cases and controls, we calculated the ORs and 95% CIs for each 10-year age category. The positive associations were similarly observed among each age category (data not shown). In both intestinal and diffuse type, the higher the serum levels of Cu/Zn SOD, the higher the risk (trend $P < 0.01$) (Table III). The OR is much larger for the intestinal type than for the dif-

fuse type. There was a significant association between serum levels of Cu/Zn SOD and an increased risk of both early and advanced gastric cancers (Table IV).

In this case-control study, we found that serum levels of Cu/Zn SOD were significantly higher in gastric cancer patients than in apparently healthy controls. Even after adjustment for confounding factors such as sex, age, cigarette smoking and *H. pylori* infection, the risk of gastric cancer increased substantially as serum Cu/Zn SOD levels increased. Compared with the lowest quartile, the OR was 15.12 for the highest quartile, which was comparable to that for *H. pylori* infection.

The development of enzyme-linked immunoassays has facilitated an accurate and reproducible determination of the protein levels of Cu/Zn SOD in serum. Oka *et al.* showed that serum Cu/Zn SOD levels were significantly elevated in 66 patients with gastric cancer (mean level, 46.9 $\mu\text{g/liter}$) compared with 154 healthy controls (14.0 $\mu\text{g/liter}$).¹⁰⁾ This was consistent with our findings. Measurement of Cu/Zn SOD in erythrocytes has yielded similar results. Magalova *et al.* reported that Cu/Zn SOD measured in erythrocytes was significantly increased in patients with gastric cancer compared with randomly selected healthy controls.¹¹⁾

One concern in interpreting our finding is that the observed association of gastric cancer and Cu/Zn SOD may be a result of the disease rather than a causal factor. If the observed association were a result of the disease, the production of Cu/Zn SOD should substantially increase as the disease advanced. In this situation, a larger OR could be expected for the association of serum Cu/Zn SOD with advanced gastric cancer, as compared with early gastric cancer. However, we found no significant difference in ORs between early and advanced cancer, suggesting that the possibility of elevation in serum Cu/Zn SOD caused by tumor itself is minimal. Our finding indicated that Cu/Zn SOD might not reflect disease progression, but could be involved in the genesis of early gastric cancer.

The mechanism by which Cu/Zn SOD influences gastric carcinogenesis has not been clarified. Two possibilities could be considered to interpret the putative association. First, Cu/Zn SOD is a primary enzyme in the intracellular antioxidant defense system and its activity may be altered by the production of ROS. ROS production has been shown to increase in the gastric mucosa of persons infected with *H. pylori*.¹²⁾ Using biopsy specimens of *H. pylori*-associated mucosa, Gotz *et al.* found that the amount and activity of Mn SOD was increased 2–3 fold, whereas Cu/Zn SOD showed a slight decrease in gastric mucosa and corpus, compared with the normal mucosa of patients without *H. pylori* infection.¹³⁾ Experiments by Smoot *et al.* showed that total SOD activity increased 156% after exposure to CagA+ strains, but Cu/Zn SOD protein levels were not significantly altered.⁶⁾ Unlike cytokine-inducible Mn SOD, Cu/Zn SOD may not increase in response to *H. pylori* infection. Indeed, we did not find a statistically significant difference in serum levels of Cu/Zn SOD between control subjects infected with *H. pylori* and those not infected with *H. pylori*. However, the possibility remains that a large amount of ROS was generated in response to an active inflammatory reaction in the stomach, even among those without *H. pylori* infection. High levels of ROS are toxic to cells through reactions with many intracellular targets, including proteins, lipids, and DNA. Levels of antioxidant Cu/Zn SOD would be correspondingly increased in order to protect cells

against the toxic effects of ROS. In other words, elevated levels of Cu/Zn SOD could be the result of a requirement for protection from the toxic effects of high levels of ROS.

Second, in order to protect against the toxic effects of ROS and to modulate the physiological effects of ROS, the cell has developed an intricately regulated antioxidant defense system that includes low-molecular-weight antioxidant compounds (vitamins E, C, and A), and primary (Mn SOD, Cu/Zn SOD, catalase, glutathione peroxidase) and secondary antioxidant enzymes (such as glutathione reductase and glucose-6-phosphate dehydrogenase). It is possible that the effect of other antioxidants, such as glutathione peroxidase and catalase, may be weak, since the lack of balance among these antioxidants is thought to play an important role in multistage carcinogenesis.¹⁴⁾ This issue should be addressed in future studies.

In conclusion, our study showed that, compared with apparently healthy controls, serum levels of Cu/Zn SOD were significantly elevated in gastric cancer patients, and increased serum levels of Cu/Zn SOD may be associated with an increased risk of gastric cancer in those infected with *H. pylori*. Our finding has clinical implications, in that the emphasis should be placed on those who have *H. pylori* infection and elevated serum Cu/Zn SOD in the early diagnosis of gastric cancer.

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