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ORIGINAL PAPER

Toenail zinc level and gastric cancer risk in Cali, Colombia

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Abstract
Purpose To examine the associations between gastric cancer (GC) risk and the zinc levels in toenail clippings, we conducted a hospital-based case-control study during the period from 2000 to 2002 in Cali, Colombia.
Methods Toenail clippings and information on lifestyles including dietary habits were obtained from 156 GC patients newly diagnosed in three hospitals in Cali and 287 controls selected from non-cancer patients who were hospitalized in the same hospitals as GC patients. Zinc concentrations in toenail clippings were examined using inductively coupled plasma mass spectrometry.
Results An inverse association was observed between toenail zinc level and GC risk (P for trend = 0.039). When we examined this association separately for current and former smokers and non-smokers, only current-smokers showed a significant inverse association (P for trend = 0.035). Histology specific analysis revealed that this inverse association was stronger when we limited GC cases with intestinal-type and their matched controls (P for trend < 0.001). This association was also observed in the carcinomas located in the upper two-thirds of the stomach (P for trend = 0.004) but not in carcinomas in the lower-third of the stomach (P for trend = 0.727).
Conclusions There was an inverse association between toenail zinc level and GC risk. However, the association was limited to smokers, intestinal-type GC, and tumors in the upper two-thirds of the stomach. Further studies seem warranted to confirm our findings.

Keywords Gastric cancer · Zinc · Toenail · Colombia

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Introduction

Zinc has been suspected to be a protective factor of cancer (Ho 2004) since it is known to be an essential component of DNA-binding proteins with zinc finger (Sarkar 1995; Witkiewicz-Kucharczyk and Bal 2006; Prasad and Kucuk 2002) involving DNA transcriptions and repairs, and copper–zinc-superoxide dismutase (SOD), an important free radical scavenger (Powell 2000; Taniguchi 1992). Furthermore, zinc has a wide range of roles in anti-angiogenic activity of endostatin (Boehm et al. 1998; Tjin Tham Sjin et al. 2005), cell proliferations and signaling (Ho and Ames 2002; Oteiza et al. 2001), and regulation of immune responses (Kitamura et al. 2006).

Zinc deficiency has been under intense study to examine its effect on cancer risk and its association with biological effects on such as oxidative stress and DNA damage/repair. In animal models using rats, Fong et al. (1996, 1997) have

shown that dietary zinc deficiency increases susceptibility to carcinogenic compounds, especially *N*-nitrosomethylbenzylamine. In humans, an association of zinc deficiency with cancer risk has been suggested in several dietary studies (Zhang et al. 1997a, b; Lee et al. 2004; 2005a, b; Zhou et al. 2005), case-control comparisons using serum clippings collected after cancer diagnosis (Atukorala et al. 1979; Mellow et al. 1983), and studies nested in a cohort, where prediagnostic sera were collected and stored (Kok et al. 1988; Leone et al. 2006). Particularly of interest is prostate cancer, which is related to decreased zinc levels in prostate, where zinc levels are known to be higher than in any other soft tissues (Costello and Franklin 2006; Platz and Helzlsouer 2001).

On the other hand, epidemiological findings of the association between zinc level and gastric cancer (GC) risk have been quite limited. In a case-control study using interview data on dietary habits, a lower zinc intake was associated with the increased risk of adenocarcinomas of the esophagus and gastric cardia (Zhang et al. 1997a, b). Lee et al. (2005a, b) also reported a significant inverse association between upper-digestive tract cancer risk and dietary zinc levels among women in a prospective study using a food-frequency questionnaire. However, no association between serum zinc levels and GC risk was observed in a case-control study nested in a prospective cohort (Kabuto et al. 1994). On the other hand, a case-control study using serum samples collected after cancer diagnosis reported a significant elevation of serum copper/zinc SOD level among GC patients compared with healthy controls (Lin et al. 2002). It should be pointed out, however, that zinc concentration in the red cells is much higher than that in plasma (Hinks et al. 1983), and serum zinc levels are relatively constant due to homeostatic mechanisms (Lee et al. 1993). In addition, serum zinc levels are dependent on both the affinity of albumin for zinc and serum albumin concentration (Foote and Delves 1983), which is usually low among the patients with advanced cancer. Therefore, serum samples may not be an ideal specimen to examine the association between marginal zinc deficiency and cancer risk.

In the present study, we examined relationships between GC risk and zinc levels in toenail clippings among residents in Cali, Colombia.

Materials and methods

Study subjects

We conducted a hospital-based case-control study in Cali, Colombia, to examine histology and tumor-location specific risk factors of GC, and the details of the methods for the recruitment of the study subjects were described elsewhere (Campos et al. 2006). In brief cases, 395 GC patients were newly diagnosed during the period between September 2000 and October 2002 in three reference hospitals in Cali, Colombia: Instituto de los Seguros Sociales "Rafael Uribe", Hospital Universitario del Valle, and Hospital San Juan de Dios. We identified 223 GC patients after excluding inappropriate cases because of disease recurrence, less than 5-year residents of the study area, and loss of contact. Interview data and toenail clippings were obtained from 216 GC cases since seven GC patients (2%) refused to participate in the present study. The major reason for refusal for participation was because of their severe clinical conditions. Two controls for each GC case were selected from hospitalized patients diagnosed neither as malignant diseases nor gastric illnesses matching on gender, age (5-year category), and hospital. Among 528 patients as potential controls, 67 patients were excluded because they had lived in Valle del Cauca less than 5 years. Twenty-nine patients (5%) refused to participate in the study, including 19 patients in severe clinical conditions. After interview, one patient turned out to be inappropriate as a control because he had been diagnosed as GC before. Thus, the total number of controls was 431. All the subjects were interviewed during the period from September 2000 to December 2002. We used a standardized questionnaire to obtain information on lifestyles, dietary intake, culinary use, and residential, medical and family history (Campos et al. 2006).

Of the original study subjects, 216 GC cases and 431 controls, we could collect toenail clippings from 158 GC cases (73%) and 315 controls (73%). Two GC cases and 28 controls were excluded from the data analysis because of the following reasons; (1) a small amount of toenail for measurement of metals (1 GC case and 7 controls), and (2) more than 10% of relative standard deviation among zinc measurements repeated thrice (1 GC case and 21 controls). Thus, the total numbers of GC cases and controls were 156 and 287, respectively, in the present study.

The Institutional Review Board of the Faculty of Health, Universidad del Valle, Cali, Colombia, approved this study and all the subjects gave their informed consent.

Analytical methods

Each toenail sample was put in a separate plastic envelope with the identification number of the corresponding subject. The envelope was sealed and stored at room temperature until use. The toenail clippings were processed for analysis using inductively coupled plasma mass spectrometry as described before (Anwar 2005). In brief, after cleaning by a neutral detergent and rinsed with de-ionized water, the toenails were dried at room temperature. To dissolve them into constituent elements wet-digestion was then carried out with 1 ml of pure water and 1 ml of nitric acid by micro-

wave-accelerated reaction system, using MDS-200, CEM, USA. The digested solution was used to measure the species of trace elements using the system of inductively coupled plasma mass spectrometry (Model POEMS 3, Thermo Jarrell-Ash, USA). A multi-element standard solution for calibration was prepared by mixing eight species of standard solutions of single elements for atomic absorption spectroscopy. An internal standard solution for the mass weight of analytical elements was prepared for four species of standard solutions of single elements for atomic absorption spectroscopy. Two standard solutions were measured after every five samples for quality control. Toenail clippings obtained from GC cases and controls were analyzed together by laboratory technicians who were blinded to the case or control status during all the zinc assay procedures.

Statistical analysis

As the distribution of zinc concentration was skewed and had a long upper tail, we calculated the geometric means for toenail zinc concentration and their corresponding 95% confidence intervals (CIs).

Although we recruited two control subjects for one GC case matching on gender, age (5-year category), and hospital, we failed to collect information of the zinc levels from 60 GC cases and 144 controls, leaving 156 cases and 287 controls for statistical analysis. In addition, if the case-control matching were to be maintained, we lost additional 26 GC cases and 58 controls in statistical analysis since their corresponding controls and cases lacked information on the zinc levels. In order to permit the inclusion of all subjects with toenail zinc measurements, we used unconditional (conventional) logistic regression models, ignoring the matching scheme.

The zinc values were categorized into quartiles based on the distribution among the controls. These quartiles were entered into logistic regression models as indicator variables or as ordered categorical variables for trend test. Dummy variables for seasons of toenail collection were also included in the models as covariates. The date of sample collection was categorized into four seasons, December–February, March–May, June–August, September–November, since, in general, Colombia has two rainy seasons in March–May and September–November.

For tumor-location and histology specific analyses, we applied conditional logistic regression models as the information of tumor-location and histological type was limited to GC cases. We rematched the GC cases and controls so that we could use as many subjects with zinc data as possible for conditional logistic regression analysis since keeping the originally matched pairs could lose 26 GC cases and 58 controls for analysis as mentioned above. For most of GC cases (75%), we recruited 1–2 controls for each GC case matching on gender, age (±5 years), and hospital. Regarding the remaining GC cases except two, we assigned 3–6 controls for each GC case in order to recover controls that lost their originally matched cases. We used only one control for the last two GC cases since these cases harbored the same clinico-pathological features including tumor location, and there was no more than one adequate control for those GC cases. We could not find adequate controls for two GC cases.

The location of a tumor, defined as the predominant location of the tumor, was divided into the following three categories: the upper-third, middle-third and lower-third parts according to the guidelines of the Japanese Research Society for Gastric Cancer (Japanese Research Society for Gastric Cancer 1995a, b). We could not obtain information on tumor location in 17 cases, and those GC cases and their corresponding controls were excluded from the tumor-location specific analysis. Thus, the total numbers of GC cases and controls were 137 and 252, respectively, for tumor-location specific analyses.

We could retrieve formalin-fixed paraffin-embedded blocks of 125 GCs, mainly surgically resected tumors. Thus, the 125 GC cases and their corresponding 230 controls were used for histology-specific analysis. The histological diagnosis was made on the basis of Japanese classification (Japanese Research Society for Gastric Cancer 1995a, b), and the details were reported elsewhere (Campos et al. 2006). After classification by the Japanese guidelines, we categorized them into two histological types, intestinal and diffuse types according to Lauren's classification (Lauren 1965).

The maximum likelihood estimates of odds ratios (ORs) and corresponding 95% CIs were calculated. All the *P* values presented are two-sided.

Results

Table 1 shows the distribution of matched factors and other important covariables to be used in statistical analysis. There was no significant case-control difference in the distributions of matching factors at control recruitment (age, gender and hospital). However, the cases and controls had significantly different distributions of toenail-sampling season and smoking status. Thirty-five percent of controls were interviewed and their toenails were collected during the period from December to February while the toenails of nearly 30% of cases were collected from June to August. Most of the tobacco used among the study subjects was cigarette smoking. The GC cases tended to be ex-smokers and not non-smokers. However, the GC cases and the controls had similar proportions of current smokers.

Table 1 Distribution of matched factors and other major covariables to be used in statistical analysis

	Number (%)		*P* value*
	Control	Case	
All	287 (100)	156 (100)	
Age			*P* for trend = 0.865
<50	58 (20)	31 (20)	
50–59	60 (21)	34 (22)	
60–69	83 (29)	41 (26)	
70	86 (30)	50 (32)	
Gender			0.276
Male	195 (68)	98 (63)	
Female	92 (32)	58 (37)	
Hospital			0.384
ISS	103 (36)	48 (31)	
HUV	133 (46)	73 (47)	
HSJD	51 (18)	35 (22)	
Season of sample collection			<0.001
December–February	101 (35)	33 (21)	
March–May	36 (13)	41 (26)	
June–August	67 (23)	47 (30)	
September–November	83 (29)	35 (22)	
Smoking			0.020
Non-smoker	133 (46)	53 (34)	
Ex-smoker[a]	89 (31)	67 (43)	
Smoker	65 (23)	36 (23)	

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* *P* values were calculated by Chi-square test. A *P* value for trend test was obtained by a logistic regression model

[a] Subjects who quit smoking 1-year before their interview

The geometric mean levels of zinc in toenails are shown in Table 2. The zinc concentrations tended to decrease with age in both GC cases and controls (*P* for trend = 0.026 and 0.118, respectively, in multivariate regression models adjusting for the effect, gender and hospital). The male subjects showed higher zinc levels in both GC cases and controls (*P* = 0.360 and 0.057, respectively, by multivariate logistic regression models adjusting for the effect of age and hospital). In the GC cases, the lowest geometric mean zinc concentration was observed in current smokers. In the controls, however, the current smokers had a higher average of zinc concentration than did the ex-smokers and non-smokers.

In the following analysis, we applied unconditional (conventional) logistic regression models to permit the inclusion of all subjects with toenail zinc concentration (Table 3). This was because, if we used conditional logistic regression models, 26 GC cases without any matched controls, and 58 controls without matched cases would be dropped from the analysis. There was an inverse relationship between a GC risk and the toenail zinc level. Although the observed trend was not monotonic as shown in Table 3, a marginally significant *P* value for trend was obtained after adjusting for the effects of age, gender, hospital, smoking, and season of toenail collection (*P* for trend = 0.056). The magnitudes of these ORs were not evidently affected by adjusting for the possible effects of suspected GC risk factors such as salt intake, consumption of fruits and vegetables, fried foods, and cooking with coal. The inverse association was statistically significant after adjusting for these factors (Table 3, model 2). Since the patients with cardiovascular diseases were recruited as controls in the present study (Campos et al. 2006), and there was a possible inverse association between zinc levels and the risk of cardiovascular diseases (Lee et al. 2005a, b), we re-calculated the risk estimates for GC after excluding the 136 patients with cardiovascular diseases. The magnitudes of GC risk slightly increased toward the unity in all the quartiles, and the statistical significance of the inverse association diminished (Table 3, model 3).

The association between the toenail zinc level and GC risk was modified by a smoking status (Table 4). Only the current smokers showed a significant inverse association between toenail zinc level and GC risk (*P* for trend = 0.035). On the other hand, the GC risk among non-smokers and ex-smokers did not significantly decrease in relation to zinc levels. However, the difference between the three smoking status groups was not statistically significant (*P* = 0.275). After excluding the subjects with cardiovascular diseases, a significant inverse association between the toenail zinc level and GC risk was still observed among the current smokers (*P* for trend = 0.021).

Table 5 shows the associations between the toenail zinc level and GC risk by the histological type (intestinal and diffuse) of tumors. Since the information on the histological type was limited to GC cases, we applied conditional logistic regression models for histology-specific analysis. In intestinal-type tumors, the GC risk significantly decreased with toenail zinc level (*P* for trend < 0.001). On the other hand, diffuse-type GC risk was not significantly associated with the toenail zinc level (*P* for trend = 0.859). In other words, there was a difference between the intestinal and the diffuse types with respect to the association of GC risk with the toenail zinc level, and this difference was statistically significant (*P* = 0.014). We observed a significant inverse association between the zinc level in toenails and the intestinal type of GC even after excluding the subjects with cardiovascular diseases (*P* for trend = 0.033).

We also conducted a tumor-location specific analysis by conditional logistic regression models (Table 6). For tumors in the upper two-thirds of the stomach, there was a

Table 2 Geometric mean levels of zing in toenails according to age, gender, season of toenail collection, and smoking habit

	Controls		Cases	
	Number (%)	Mean, ppm (95%CI)	Number (%)	Mean, ppm (95%CI)
Total	287 (100)	132.0 (123.8–140.8)	156 (100)	124.1 (112.0–137.6)
Age				
<50	58 (20)	132.5 (114.3–153.6)	31 (20)	157.3 (120.5–205.4)
50–59	60 (21)	137.1 (117.5–160.1)	34 (22)	120.3 (99.2–145.8)
60–69	83 (29)	136.4 (119.5–155.7)	41 (26)	118.0 (93.1–149.5)
70	86 (30)	124.3 (112.6–137.2)	50 (32)	114.1 (97.5–133.7)
Gender				
Male	195 (68)	137.8 (126.1–150.6)	98 (63)	129.8 (112.4–149.8)
Female	92 (32)	120.6 (112.7–129.1)	58 (37)	115.1 (100.4–132.0)
Hospital				
ISS	103 (36)	142.6 (126.1–161.3)	48 (31)	126.8 (107.0–150.1)
HUV	133 (46)	122.8 (113.1–133.3)	73 (47)	124.3 (106.5–145.0)
HSJD	51 (18)	136.6 (116.9–159.7)	35 (22)	120.3 (93.5–154.9)
Season of toenail collection				
December–February	101 (35)	130.8 (116.4–147.0)	33 (21)	123.0 (96.4–157.1)
March–May	36 (13)	121.9 (107.2–138.6)	41 (26)	114.2 (96.2–135.5)
June–August	67 (23)	142.0 (121.4–166.0)	47 (30)	118.3 (99.0–141.4)
September–November	83 (29)	130.4 (117.1–145.3)	35 (22)	147.3 (112.9–192.1)
Smoking				
Non-smoker	133 (46)	130.6 (120.1–142.2)	53 (34)	128.6 (108.1–152.9)
Ex-smoker[a]	89 (31)	127.6 (114.4–142.4)	67 (43)	137.6 (115.6–163.9)
Smoker	65 (23)	141.4 (118.8–168.3)	36 (23)	97.3 (82.4–114.9)

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[a] Subjects who quit smoking more than 1-year before their interview

significant inverse relationship between GC risk and the toenail zinc level (P for trend = 0.004), and the association was observed even after excluding the subjects with cardiovascular diseases (P for trend = 0.005). However, the risk of carcinomas located in the lower-third of the stomach was not significantly associated with the toenail zinc level (P for trend = 0.727). The tumor locations, i.e., the upper two-thirds versus the lower-third of the stomach, modified the association of GC risk with toenail zinc level with a marginally statistical significance (P = 0.102).

Discussion

In the present study, we observed a significant inverse association between the zinc levels in toenail clippings and GC risk (P for trend = 0.039 after adjusting the effects of age, gender, hospital, smoking, season of toenail collection, and potential risk factors relating to diets). The association was particularly evident among current smokers, intestinal-type GCs, and carcinomas in the upper two-thirds of the stomach. Our findings are consistent with observations in dietary studies of GC (Zhang et al. 1997a, b; Lee et al. 2005a, b) although epidemiological findings on the association between the zinc level in biological specimens and GC risk are quite limited. On the other hand, a large-scale chemoprevention trial in Linxian, China, revealed that a preventive effect of supplemental zinc on GC risk was marginal (Taylor et al. 1994).

In the present study, only current smokers showed a significant inverse association between the zinc level and GC risk. This finding is consistent with the results obtained from a case-control study of lung cancer reported by Zhou et al. (2005). They examined the association between the dietary zinc levels and lung cancer risk, and also found a stronger inverse association between the zinc level and lung cancer risk in current smokers than in the ex-smokers, suggesting a protective role of zinc in the carcinogenesis among current smokers. One of the possible factors confounding our results may be the use of nutritional supplements containing zinc, especially among ex-smokers, since they might have had some clinical symptoms of pre-malignant conditions and quit smoking more than 1 year before our interview. Although we did not have information on

Table 3 Risk estimates for GC according to quartile level of toenail zinc by unconditional logistic regression models

Zinc	Number (%)		Model 1[a]	Model 2[b]	Model 3[c]
	Control	Case	OR (95%CI)	OR (95%CI)	OR (95%CI)
Quartile of toenail zinc (boundaries in ppm)					
1 (≤95.3)	72 (25)	57 (37)	1.0 (referent)	1.0 (referent)	1.0 (referent)
2 (≤114.8)	72 (25)	42 (27)	0.7 (0.38–1.13)	0.7 (0.37–1.15)	0.8 (0.39–1.54)
3 (≤151.7)	72 (25)	19 (12)	0.3 (0.15–0.55)	0.3 (0.15–0.56)	0.3 (0.13–0.67)
4 (152.1≤)	71 (25)	38 (24)	0.7 (0.42–1.28)	0.7 (0.38–1.22)	1.0 (0.50–2.02)
P for trend			0.056	0.039	0.434
P for heterogeneity			0.001	0.003	0.003

OR odds ratio, *CI* confidence interval

[a] ORs and corresponding 95%CIs were obtained by logistic regression model after adjusting for the effects of age, gender, hospital, smoking, and season of toenail collection

[b] ORs and corresponding 95%CIs were obtained by logistic regression model after adjusting for adjusting for salt intake, consumption of fruits and vegetables, frying foods, and cooking with coal in addition to the factors using in model 1

[c] ORs and corresponding 95%CIs were obtained by model 2 but the controls with cardiovascular diseases (n = 136) were excluded from this analysis

Table 4 Risk estimates for GC according to quartile level of toenail zinc by smoking habit

	Number (%)		OR (95%CI)[a]	OR (95%CI)[b]
	Control	Case		
Non-smoker				
Zinc level				
1 (low)	29 (22)	18 (34)	1.0 (referent)	1.0 (referent)
2	39 (29)	13 (25)	0.5 (0.17–1.31)	0.6 (0.18–2.00)
3	35 (26)	7 (13)	0.2 (0.08–0.80)	0.2 (0.05–0.90)
4	30 (23)	15 (28)	0.8 (0.28–2.08)	1.0 (0.30–3.12)
P for trend			0.388	0.714
P for heterogeneity			0.089	0.114
Ex-smoker[c]				
Zinc level				
1 (low)	23 (26)	23 (34)	1.0 (referent)	1.0 (referent)
2	18 (20)	17 (25)	1.1 (0.39–2.97)	1.2 (0.32–4.82)
3	27 (30)	9 (13)	0.4 (0.13–1.13)	0.5 (0.10–2.02)
4	21 (24)	18 (27)	1.3 (0.47–3.67)	12.9 (1.78–93.4)
P for trend			0.841	0.045
P for heterogeneity			0.139	0.005
Current-smoker				
Zinc level				
1 (low)	20 (31)	16 (44)	1.0 (referent)	1.0 (referent)
2	15 (23)	12 (33)	0.9 (0.28–2.76)	0.7 (0.16—3.32)
3	10 (15)	3 (8)	0.3 (0.07–1.56)	0.3 (0.03–1.92)
4	20 (31)	5 (14)	0.3 (0.08–1.11)	0.2 (0.03–0.96)
P for trend			0.035	0.021
P for heterogeneity			0.177	0.139

OR odds ratio, *CI* confidence interval

[a] ORs and corresponding 95%CIs were obtained by logistic regression model using covariates in model 2 of Table 3 except smoking

[b] ORs and corresponding 95%CIs were obtained by logistic regression model using covariates in model 2 of Table 3 except smoking after excluding the controls with cardiovascular diseases (n = 136)

[c] Subjects who quit smoking more than 1-year before their interview

supplement use, it is unlikely that a substantial number of our study subjects used supplements containing high zinc levels since our study subjects were low and middle income groups. In addition, although subjects who started to use supplements might be conscious of their diets, there was no significant difference in the frequency of the subjects who have changed their diets in the last 10 years among the three groups of smoking status (data not shown).

Table 5 Histology-specific risk estimates for GC according to quartile level of toenail zinc by conditional logistic regression models

	Number (%)[a]		OR (95%CI)[c]	OR (95%CI)[d]
	Control[b]	Case[b]		
Intestinal type				
Zinc level				
1 (low)	28 (22)	27 (42)	1 (reference)	1 (reference)
2	33 (26)	19 (29)	0.7 (0.24–2.04)	0.3 (0.05–1.91)
3	34 (26)	7 (11)	0.1 (0.01–0.29)	0.04 (0.001–1.14)
4	34 (26)	12 (18)	0.1 (0.04–0.52)	0.2 (0.03–1.18)
P for trend			<0.001	0.033
P for heterogeneity			<0.001	0.064
Diffuse type Zinc level				
1 (low)	29 (29)	21 (35)	1 (reference)	1 (reference)
2	31 (31)	18 (30)	0.6 (0.21–1.68)	0.5 (0.11–2.11)
3	25 (25)	6 (11)	0.8 (0.24–2.68)	0.6 (0.12–3.40)
4	16 (16)	15 (25)	1.1 (0.36–3.61)	2.2 (0.45–10.6)
P for trend			0.859	0.437
P for heterogeneity			0.694	0.342

OR odds ration, *CI* confidence interval

[a] Twenty nine GC cases and their corresponding 57 controls were excluded from this analysis since histological classification could not be confirmed in these GC cases

[b] Cases and controls were matched on the gender, age (±5 years), and hospital

[c] ORs and corresponding 95%CIs were obtained by conditional logistic regression model using season of toenail collection, smoking, salt intake, consumption of fruits and vegetables, frying foods, and cooking with coal as covariates

[d] ORs and corresponding 95%CIs were obtained by conditional logistic regression model using season of toenail collection, smoking, salt intake, consumption of fruits and vegetables, frying foods, and cooking with coal as covariates after excluding the controls with cardiovascular diseases (n = 136)

We also found that the concentration of zinc in toenail clippings was only related to the intestinal type of Lauren classification (P for trend <0.001). One of the possible explanations is the difference in contributions of *Helicobacter pylori* (*H. pylori*) infection to two histological types of GC. Although *H. pylori* infection was reported to be related to both intestinal- and diffuse-type GCs in some reports (Parsonnet et al. 1997; Uemura et al. 2001; Kikuchi 2002), microscopic studies suggest that *H. pylori* infection seems to be more strongly related to intestinal-type tumors rather than diffuse-type tumors (Kikuchi 2002). *H. pylori* infection was reported to cause an increase of reactive oxygen species (Zhang et al. 1997a, b) and the total SOD activity was increased in GC tissue with *H. pylori* infection (Smoot et al. 2000; Noguchi et al. 2002). However, those studies showed that the elevation of total SOD was mainly explained by an increase of magnesium SOD, but not by copper/zinc SOD levels, which was not strongly affected by *H. pylori*, infection. The notion was supported by Janssen et al. (2000), who reported a significantly increased level of magnesium SOD in GC tissues but not in the level of copper/zinc SOD in comparison with those in adjacent normal tissues.

Although we could not examine the interaction between zinc level and *H. pylori* infection on GC risk because of a small number of GC cases with information on *H. pylori* infection, the strong inverse association between the toenail zinc levels and intestinal-type GC risk was even more evident in the upper two-thirds of the stomach than that in the lower-third (data not shown) although the positive association between GC risk and *H. pylori* is known to be stronger in the antrum rather than in the non-antrum. This finding, thus, suggests that the strong inverse relationship between toenail zinc levels and intestinal-type GC risk in the present study cannot be explained by only *H. pylori* infection in relation to oxygen stress. The other roles of zinc in carcinogenesis such as the effects on DNA transcriptions and repairs, cell proliferation, or signaling should also be considered.

Tumor-location specific analysis revealed that the toenail zinc levels were strongly associated with tumors in the upper two-thirds of the stomach (P for trend = 0.002). This inverse association was observed both in the upper and the middle-third part of the stomach. Although the significance of the trend was relatively small in carcinomas located in the

Table 6 Tumor-location specific risk estimates for GC according to quartile level of toenail zinc by conditional logistic regression models

	Number (%)[a]		OR (95%CI)[c]	OR (95%CI)[d]
	Control[b]	Case[b]		
Upper two-thirds				
Zinc level				
1 (low)	21 (21)	22 (42)	1 (reference)	1 (reference)
2	26 (25)	15 (28)	0.9 (0.29–2.63)	0.7 (0.10–4.40)
3	25 (25)	6 (11)	0.1 (0.03–0.64)	0.1 (0.02–1.14)
4	30 (29)	10 (19)	0.2 (0.06–0.74)	0.1 (0.03–0.72)
P for trend			0.004	0.005
P for heterogeneity			0.009	0.032
Lower-third				
Zinc level				
1 (low)	43 (29)	30 (36)	1 (reference)	1 (reference)
2	43 (29)	23 (27)	0.6 (0.26–1.56)	0.4 (0.08–1.48)
3	38 (25)	9 (11)	0.3 (0.11–0.92)	0.3 (0.07–1.67)
4	26 (17)	22 (26)	1.2 (0.46–3.12)	20.2 (1.61–255)
P for trend			0.727	0.115
P for heterogeneity			0.070	<0.001

OR odds ration, *CI* confidence interval

[a] Seventeen GC cases and their corresponding 35 controls were excluded from this analysis since information of tumor location was missing in these GC cases

[b] Cases and controls were matched on the gender, age (±5 years), and hospital

[c] ORs and corresponding 95%CIs were obtained by conditional logistic regression model using season of toenail collection, smoking, salt intake, consumption of fruits and vegetables, frying foods, and cooking with coal as covariates

[d] ORs and corresponding 95%CIs were obtained by conditional logistic regression model using season of toenail collection, smoking, salt intake, consumption of fruits and vegetables, frying foods, and cooking with coal as covariates after excluding the controls with cardiovascular diseases (n = 136)

upper-third of the stomach (P for trend = 0.212), this might be due to a small number of carcinomas in the upper-third of the stomach (n = 17) since the OR was similar to each other. Proximally located GCs are known to have etiological backgrounds and clinico-pathological features different from more distally located stomach carcinomas (Correa and Chen 1994). According to the report by Inoue et al. (1994), smoking is a potential GC risk factor of the upper-third of the stomach. However, when our analysis was limited to non-smokers, the association of GC risk with the toenail zinc level was still different in the upper two-thirds and lower-third of the stomach (data not shown).

Exposure assessment of micronutrients based on a food-frequency questionnaire is considered inadequate since their concentrations in foods can vary geographically. In most analytical epidemiological studies, therefore, micronutrient exposures have been assessed using biological specimens such as serum, nails, and hair. Unfortunately, the present study could not use biological specimens collected before cancer diagnosis. It should be of note, however, that Garland et al. (1993) reported the relatively high degree of reproducibility over a 6-year period of toenail zinc.

In addition to the fact that we could not obtain toenail samples in advance to cancer development, there are two major limitations in the present study. One of them was the use of hospitalized controls. We recruited the controls from hospitalized non-cancer patients in the present study, and the major causes of hospitalization of our control subjects were cardiovascular diseases (46%), trauma (29%), and infectious diseases or urological disorders (24%). Since there was a possible inverse association between the zinc levels and the risk of cardiovascular mortality (Lee et al. 2005a, b), we re-calculated the risk estimates for GC after excluding the 136 patients with cardiovascular diseases. Just opposite to what we expected, the magnitudes of GC risk slightly increased toward the unity in all quartiles, and the statistical significance of the inverse association diminished (Table 3, model 3). There might be some selection bias in the recruitment of our controls. However, the significant inverse associations between the toenail zinc level and GC risk among current smokers, intestinal-type GCs, and carcinomas in the upper two-thirds of the stomach were still observed (Tables 4, 5, 6).

The second problem is the use of unconditional (conventional) logistic regression models to permit the inclusion of

all subjects with toenail zinc in the present study even though we originally designed this study as a matched case-control study which requires conditional logistic analysis. However, the analysis of matched data using conditional logistic models still gave an inverse association between toenail zinc level and GC cancer risk, and the magnitudes of the risk were not evidently changed; ORs (95%CIs) were 0.6 (0.30–1.14), 0.3 (0.12–0.64), and 0.7 (0.36–1.26) for the second lowest, the second highest, and the highest quartiles of zinc levels, respectively, after adjusting the effects of potential risk factors used in the model 2 of Table 3.

In conclusion, a protective effect of zinc on GC risk was suggested especially among current smokers, intestinal-type GCs, and carcinomas in the upper two-thirds of the stomach. Further studies seem warranted to confirm our findings.

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