Five Serum Trace Elements Associated with Risk of Cardia and Non-cardia Gastric Cancer in a Matched Case-control Study

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Abstract

Background: Serum trace elements have been some time suggested to influence the development of gastric cancer, but evidence is still lacking. **Methods:** In a case-control study from a high risk region of East China, all newly diagnosed patients with gastric cancer were compared with healthy controls 1:1 matched by sex, age (± 3 years) and place of residence during 2013- 2015. **Results:** A total of 299 cases of gastric cancers, which included 122 cases of gastric cardia cancer (GCC) and 177 gastric non-cardia cancer (NCGC), and 299 healthy controls were included. Positive associations were found between serum copper and copper/zinc ratio and risk of overall gastric cancer (OR4th vs. 1st quartile: 2.42, 95% CI: 1.10-5.32 for copper, p for trend=0.003; OR4th vs. 1st quartile: 11.7, 95% CI: 3.83-35.6 for copper/zinc ratio, p for trend<0.0001), and for both GCC and NCGC subtypes. Serum selenium inversely associated with risk of NCGC (OR4th vs. 1st quartile: 0.18, 95% CI: 0.07-0.51, p for trend=0.0001), while molybdenum seem to reduce risk of GCC (OR4th vs. 1st quartile: 0.12, 95% CI: 0.03-0.47, p for trend<0.0001). Strong inverse associations were also detected between serum calcium and risk of all groups of gastric cancer (all p for trend<0.05). Although intermediate serum level of zinc and iron had lower risk of gastric cancer, no linear association was however found (all p for trend>0.05). **Conclusions:** The current study present evidences for positive associations between copper, copper/zinc ratio and gastric cancer, as well as for inverse associations between serum selenium, molybdenum and calcium and risk of gastric cancer.

Background

Although the incidence has been decreasing in the past decades, gastric cancer remains globally the third most frequent cause of death from cancer. Incidence varies greatly among populations and is higher in Asian, Central and South America countries but low in North America and Western Europe.(1) Migration studies found lower incidence of gastric cancer among immigrants moved from high risk region to low risk region, which indicate that environmental factors play an important role in the development of gastric cancer.(2, 3) Furthermore, poor socioeconomic condition has been widely proven to relate with risk of gastric cancer. Higher incidence of gastric cancer were usually found in those regions with more infertile lands, high mountains, or excessive heavy metals levels in soil, cereals and drinking water. Previous study confirmed that these environmental pollution by trace elements might contributed partly to their high incidence of gastric cancer.(4)

Some early studies have investigated the role of trace elements in the development of gastric cancer, however the results were inconsistent and mechanisms of effect of trace metal on gastric cancer are still far from being elucidated completely. Copper (Cu), zinc (Zn) and selenium (Se), iron (Fe), molybdenum (Mo), magnesium (Mg), and calcium (Ca) are essential trace metals and components of critical enzyme systems, playing key roles in maintaining DNA integrity by preventing oxidative DNA damage or affecting gene mutations.(5-7) The variations in these mineral levels are related to the incidence of several cancers. For example, Se has been studied for antioxidant and anticancer properties,(8, 9) especially against

gastric cancer.(10) Zn is trace element found in blood, and most of it is contained in bones, skins, and hair (approximate 70%). Inverse associations were also obtained between serum Zn and risk of gastric cancer in some early studies, (11, 12) but not all.(13) Higher level of Cu alone was also found significantly higher in cancerous human stomach in compared to normal samples.(14) More importantly, Zn and Cu are known as key cofactors in several enzymes, which include Cu/Zn superoxide dismutases (SODs) decomposing superoxide radicals to H_2O_2 and playing a significant role against oxidant stress in several DNA repair proteins.(15, 16) Previous study has demonstrated that high serum levels of Cu/Zn superoxide dismutase can be related with an increased gastric cancer risk.(17) A recent systematic review and meta-analysis gathered information on Fe (intake and serum status), and various cancer risk. Although it seems biologically plausible for Fe to be associated with gastric carcinogenesis, the evidence is rather heterogeneous and insufficients to lead to any conclusions.(18) Molybdenum (Mo) is an essential trace element that enters the body primarily from dietary sources. In humans, Mo is a cofactor for three enzyme classess-sulfite oxidase, aldehyde dehydrogenase, and xanthine oxidate.(19) There has been some evidences suggested that Mo deficiency could increase the risk of carcinogenesis especially in the gastrointestinal tract, but epidemiological evidences from humans are still lacking.(20)

In China, there is an obvious clustering of the geographical distribution of gastric cancer, with the highest mortality being mostly located in rural areas. Xianyou County in Putian City locates between east longitude 118°27'-118°56' and north latitude 25°11'-25°43' on the southeast coast of Fujian province, China, and has an estimated population of 2.1 million in the year 2012. In this county, gastric cancer was the dominant leading cause of death, explaining 30.43% of total cancer death, and the death rate reached as high as 73.93% with an average survival time of 22 months in 2012.(21) The standardize mortality rate of gastric cancer was 49.47/100,000 in Xianyou County, which was 2.23-fold higher than the Chinese national average (21.9/100,000).(21) Standardized mortality rate of gastric cancer in Xianyou County closely related to geographical distributions of mountain, plain and coastal regions. Mountain areas were observed with the highest mortality rate (94.4/100,000), in compared to the lowest rate observed in coastal regions (26.4/100,000) (data not shown). More interesting, we recently detected discrepancies of Cu, Zn, Se, Mo, Fe, Ca, and Mg in soil, water or rice between these various regions with different gastric cancer incidences in Xianyou County, which indicate the potential associations between risk of gastric cancer and environmental trace elements.(22) In line with this finding, an earlier study also confirmed lower serum level of Zn and higher level of Cu/Zn ratio in healthy population in Putian City in compared to that of other regions in Fujian province with lower incidence of gastric cancer.(23) These evidences indicate that trace elements variations might play an important role in the development of gastric cancer in this area. However, to date, no large-scale epidemiological studies have proved the association between serum level of these trace elements and risk of gastric cancer in Xianyou County. Thus, the aim of this study was to investigate whether the serum concentrations of trace elements Cu, Zn, Se, Mo, Fe, Ca, Mg and Cu/Zn ratio are associated with gastric cancer risk, thus explain partly the high incidence of gastric cancer in Xianyou County.

Methods

Study Design and Population

A population-based case-control study was conducted in Xianyou County between March 2013 and December 2015. All newly diagnosed cases of gastric cancer in Xianyou County Hospital were identified. All individuals who were aged ≥ 18 years and have lived in Xianyou for at least 10 years were eligible and recruited in Xianyou County Hospital. Gastric cancer was defined according to the International Classification of Diseases, 10th Revision (ICD-10) code, and was sub-classified as gastric cardia cancer (GCC) if they involved the esophagogastric junction and as non-cardia gastric cancer (NCGC) if they did not. Patients who had a history of any cancer, mental illness, and those unable to complete the questionnaire were excluded. Controls were individually matched (1:1) to cases based on sex, age (± 3 years), and place of residence. They were required to live in the same town or a town with a similar incidence of gastric cancer, and were required to be living in Xianyou County for at least 10 years with no prior history of gastric cancer. Matched controls were randomly selected by the Health department of Xianyou County Government, who was responsible to send study invitation letters to eligible controls. Data was collected from all consenting and participating subjects at Xianyou County Hospital. The study was approved by the ethics committees at Xianyou County Hospital and Fujian Medical University. Written consents were obtained from all participants at the study enrollment.

Data Collection

All cases and controls were personally face-to-face interviewed by trained research nurses from Xianyou County Hospital. A structured and standard questionnaire consisting of 337 items under 8 categories was used for the data collection. The questionnaires included following categories: (i) demographic factors (16 items), including the subject's age, height, weight, marital status, education level, monthly income, etc.; (ii) occupational history (17 items), including profession, pesticide exposure at work, etc.; (iii) lifestyles (70 items), including smoking habits, use of alcohol, tea consumption, etc.; (iv) dietary intake (140 items); (v)) personal medical history (33 items), including personal disease records, medication, etc.; (vi) family cancer history (18 items), including family history (first and second-degree relatives) of any cancer, gastric cancer and esophageal cancer;(vii) mental health (9 items);(viii) cancer related diagnoses and treatment (34 items), including surgery, chemotherapy, and radiotherapy, etc. Smoking habit was defined as consuming at least one cigarette per day during the last 6 months or longer.

Laboratory Test

Approximately 5 mL of venous blood was collected into an element-free vacuum blood tube without anticoagulation in Xianyou County Hospital. After centrifugation, serum samples were prepared and pre-

served at a temperature of -20°C prior to analyses. The serum concentration of all trace elements were analyzed using in-ductively coupled plasma mass spectrometry (ICP-MS; Elmer NexION 350X, USA).

Statistical Analysis

Since most of the serum concentrations of trace elements were not normally distributed, we used median and interquartile range to describe them. Chi-square test was used for the comparisons of categorical variables. The T-test (normally distributed) and Wilcoxon rank-sum test (non-normally distributed) were used for the comparisons of continuous variables. To assess the effect of trace elements on the risk of gastric cancer, conditional logistic regression was used to estimate crude and adjusted odd ratios (ORs) and their 95% confidence intervals (CIs). Serum concentrations of trace elements were classified into quartile based on levels of controls. We adjusted for potential confounding factors including age group (<45, 45-55, 56-65, 66-75, ≥76 years), sex (male, female), body mass index (BMI, <18.5, 18.5-24.9, ≥25 kg/m²), education level (≤7, 7-9, ≥10 years), occupation (farmer, industrial worker, office worker, others), household income (<600, 600-1000, ≥1000 Chinese Yuan/month/person), smoking (yes, no), chronic atrophic gastritis (yes, no) and family history of gastric cancer (yes, no). We also conducted subgroup analysis according to tumor localization: GCC vs. NCGC using conditional logistic regression, and applied the Wald test to assess heterogeneity of the ORs. Two-sided $p < 0.05$ was regarded as having statistical significance. SAS 9.4 was used for all statistical analyses.

Results

Baseline

A total of 299 cases of gastric cancer were included in the final analysis, consisting of GCC (n=122) and NCGC (n=177) patients. The number of sex, age and resident town-matched controls was 299 (Table 1). Cases had lower BMI than controls (all $p < 0.05$). Meanwhile, they were observed with lower education level but higher household income in compared to controls (all $p < 0.05$). Higher prevalence of chronic atrophic gastritis was observed in both GCC (23.8%) and NCGC (18.6%) in compared to GCC (7.4%) (all $p < 0.05$).

Distributions of Trace Elements

Median serum concentration of Cu was observed much lower in control groups (927.7 µg/L, interquartile range 799.1-1312.5 µg/L) in compared to both GCC (1014.6 µg/L, interquartile range 874.4-1309.2 µg/L) and NCGC (1000.2 µg/L, interquartile range 821.4-1226.3 µg/L) (all $p < 0.05$) (Table 2). Cu/Zn ratio was also observed higher in GCC (median=2.16) and NCGC (median=1.91) than controls (median=1.43) (all $p < 0.05$). Median serum concentrations of Mo (1.8 µg/L in controls vs. 0.93 µg/L in GCC and 0.93 µg/L in NCGC) and Ca (85.1 µg/L in controls vs. 66.7 µg/L in GCC, and 68.9 µg/L in NCGC) observed in controls were significantly lower than all gastric cancer groups (all $p < 0.05$). Meanwhile, the distributions of Zn, Se and Mg were similar between controls and gastric cancer cases (all $p > 0.05$).

Serum Trace Elements in Relation to Gastric Cancer

Highest level of Cu was observed with an OR of 2.42 (95% CI: 1.10-5.32) for overall gastric cancer risk in compared to the lowest group (Table 3). Although higher point estimates were also observed in the highest level of Cu among patients with either GCC or NCGC subtypes, no statistically significance was observed. Dose-response correlations were observed between serum level of Cu and all subtypes of gastric cancer (all p for trend <0.05). Study subjects with intermediate level of Zn (quartile 3: 650.2-844.5 $\mu\text{g/L}$) were observed with lower risk of gastric cancer combined (OR=0.25, 95% CI: 0.10-0.61), GCC (OR=0.15, 95% CI: 0.03-0.43) and NCGC (OR=0.34, 95% CI: 0.13-0.98). However, no dose-response association was observed in any of case groups (all p >0.05).

Highest serum level of Se inversely associated with risk of gastric cancer combined (OR=0.33, 95% CI: 0.15-0.75), and this association seems to limit in NCGC subtype (OR=0.18, 95% CI: 0.07-0.51). A dose-response association was observed between serum Se concentration and gastric cancer combined (p for trend=0.009), as well as NCGC (p for trend=0.0001). No associations were observed for GCC (p for trend=0.43). Comparing the highest to the lowest quartiles of Mo biomarkers, we found statistically inverse associations between serum Mo and overall risk of gastric cancer (OR=0.06, 95% CI: 0.02-0.20) and GCC (OR=0.12, 95% CI: 0.03-0.47). Dose-response associations were found for all three case groups (all p for trend <0.05).

No dose-response association was found for Fe in relation to any gastric cancer groups. However, higher serum Fe was observed with more than 50% reduction in gastric cancer combined in compared to the reference level (OR_{3rd quartile}=0.27, 95% CI: 0.11-0.68; OR_{4th quartile}=0.41, 95% CI: 0.18-0.91). More specific, this risk reduction by Fe might only affect GCC patients (OR_{3rd quartile}=0.18, 95% CI: 0.05-0.70). Higher serum concentration of Ca was inversely associated with risk of all gastric cancer groups. In compared to the lowest quartile of Ca, the OR calculated in the highest quartile was 0.04 (95% CI: 0.01-0.11) for risk of gastric cancer combined, 0.06 (95% CI: 0.01-0.26) for GCC risk, and 0.02 (95% CI: 0.00-0.17) for NCGC. Dose-response was observed between serum level of Ca and all gastric cancer groups (all p for trend <0.05). No significant association was observed for serum level of Mg in relation to gastric cancer risk. Study subjects with the highest Cu/Zn ratio had more than 10-fold increased risk of gastric cancer combined (OR=11.70, 95% CI: 3.83-35.6), GCC (OR=9.03, 95% CI: 2.29-35.6) and NCGC (OR=18.82, 95% CI: 3.50-96.01) subtypes. Dose-response was observed for all gastric cancer groups (all p for tend <0.05).

Discussion

The current study mainly reveals the role of eight serum trace elements in the development of gastric cancer. The results demonstrated strong inverse associations between serum concentrations of Se, Mo, Ca and risk of gastric cancer. Meanwhile, high serum concentrations of Cu and Cu/Zn ratio related to increased risk of gastric cancer. Although decreased risks of gastric cancer were also observed in higher serum level of Zn and Fe, no dose-response associations were found.

The data from the present study are consistent with the impression that high serum Se may be associated with decreased risk of gastric cancer. Previous studies have shown that Se may have anti-carcinogenic effects, especially against cancers of lung, prostate, skin and gastrointestinal system.(24, 25) One study observed significant lower serum levels of Se in esophageal cancer group, as compared with normal healthy controls.(26) A nutrition intervention trial conducted in Linxian County among general population observed lower mortality of gastric cancer in participant groups receiving supplementation with vitamins and minerals (including Se) than individuals who did not receive the supplements.(27) Of note, Linxian County is also a rural area with poor socioeconomic status and well-known for its high incidence of gastric cancer, which is very similar to the study region of the current study.(28) Furthermore, it worth mention that, the inverse association between Se level and gastric cancer may only occur in populations with low Se levels.(29) Serum level of Se below 70µg/L is usually considered as deficiency.(30) According to this standard, 25.4% of our controls (76/299) and 34.1% of all gastric cancer cases (102/299) were considered to be Se deficient. It could be inferred that low level of Se might contribute partly to high incidence of gastric cancer found in Xianyou County.

Essential elements Cu and Zn have a role in many biochemical reactions as a micro-source.(31) Zn alone has been associated with risk of gastric cancer, but the epidemiological finding have been quite limited and controversial. Zhang *et al.* have also observed higher serum concentration of Zn in gastric cancer cases than healthy controls.(12) However, a Japanese study found no excess risk of gastric cancer among those with the lowest levels of Zn (OR=1.2).(13) Although in our study, decreased risks of gastric cancer were found in the third quartile of serum Zn, the linear association was not statistically significant. On the other hand, higher level of Cu was also observed associated with higher risk of gastric cancer in the current study. Our result is partly supported by previous studies which detected higher level of Cu in gastric cancer cases in compared to health controls.(32) It has been known that Cu participates in the reductive activation of H₂O₂, causing damage to cellular DNA, proteins and lipids. The interaction of H₂O₂ with Cu generates more reactive oxygen species, such as hydroxyl radicals. These reactive oxygen species have been considered as being responsible for the process of carcinogenesis.(33) More importantly, our results demonstrated that high Cu/Zn ratio could be a strong risk factor of gastric cancer. Disturbances of blood Zn and Cu concentrations have been previously observed in many types of cancers including head and neck, gastrointestinal, and bladder carcinomas, and the Cu/Zn ratio was also suggested as a biochemical marker of cancer prognosis.(32, 34-37)

Our results found limited evidences for the association between serum Fe status and GCC and all gastric cancer combined, which is partly consistent with those from previous studies. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, inverse dose-response associations were found between serum Fe and overall risk of gastric cancer ($OR_{4th \text{ vs. } 1st \text{ quartile}}=0.62$, 95% CI: 0.44-0.88, p for trend=0.034), as well as NCGC ($OR_{4th \text{ vs. } 1st \text{ quartile}}=0.54$, 95% CI: 0.33-0.89, p for trend=0.029).(38) Similarly, a Finnish men cohort study also found serum Fe had an inverse, or possibly “u” shaped relationship with this malignancy in the NCGC site ($OR_{3rd \text{ vs. } 1st \text{ quartile}}=0.39$, 95% CI: 0.19-0.78, p for trend=0.06), although the trend analysis was not statistically significant.(38) In another Finnish cohort study, which involved 120 incident male gastric cancers and 76 female gastric cancers, the relationship in males was statistically significant ($RR_{4th \text{ vs. } 1st \text{ quartile}}=0.60$, p for trend<0.01).(39)

The present study also observed lower risk of gastric cancer, regardless of GCC or NCGC, in the higher quartile of serum Mo, indicating the protective role of Mo in the development of these diseases. However, little evidence exists to explain the effects of serum Mo on gastric cancer. An earlier Chinese study showed that serum Mo levels of 33 patients with gastric cancer were significantly lower than those of 77 healthy controls.(20) Similarly, a recent published Chinese study found that serum Mo concentration tended to be lower in *H.pylori*-infected participants than those un-infected, although no statistically significant differences were actually observed.(40) Due to the scarce epidemiological data, the appropriate amount of serum Mo in human warrants further investigations.

Extensive epidemiological evidence has indicated the protective role of dietary calcium or Vitamin D against gastrointestinal cancer, including gastric cancer.(41, 42) However, how serum calcium influence gastric cancer remains indefinite. A large scale Swedish cohort study evaluated serum calcium status in 782 incident gastric cancers.(43) Although lower ORs were observed in the higher quartiles of serum calcium, no statistically significant association was found.

Some methodological issues of our study deserve attention. The strengths include the large sample size; individual matching for age, sex and resident region by gastric cancer risk; the standardized tumor classification; the personal interviews with all study participants in a standard and strict manner; and stratified analysis by different gastric cancer localization. Meanwhile, our study has some limitations that should be described. A major concern is that we were not able to adjust to *H. pyloric* infection, which might potentially cause confounding effect on the association between serum trace elements and gastric cancer, especially NCGC. However, the availability of chronic atrophic gastritis help to reduce the potential confounding effect, as *H. pyloric* infection was believed to cause NCGC through causing gastritis and gastric atrophy. (44) Another concern is the possibility of exposure misclassification using a

questionnaire that has not been validated. However, the reliability rate of our questionnaire was estimated as high as 97%, suggesting the potential of high accuracy in measuring risk factors (data not shown).

Conclusions

The presence of associations between gastric cancer and serum level of trace elements is observed in the present study. We suggest that lower levels of Se, Mo and Ca and higher levels of Cu and Cu/Zn ratio may play an important role in gastric cancer induction. High risk regions, especially those found with environmental contamination of toxic trace elements should enhance preventive implement against gastric cancer. It need to be noted that, due to inevitable bias in retrospective study, further prospective studies are required to clarify the association between the different stages of gastric cancer and serum trace element levels.

Abbreviations

GCC: Gastric cardia cancer

NCGC: Non-cardia gastric cancer

Declarations

Ethics Approval and Consent to Participate

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Xianyou County Hospital and Fujian Medical University. Written informed consent was obtained from all subjects/patients at the study enrollment.

Consent to Publish

Not applicable

Availability of Data and Materials

The dataset analysed during the current study are not publicly available due to that it concern personal information of study participants, but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no conflict of interest.

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Authors' Contributions

The authors' contributions to this study were as follow: study design, CW, WY, SG, and BL; data collection, CW, WY, SG, and BL; statistical analysis YL; interpretation of results and manuscript writing by all authors. None of authors had a personal or financial conflict of interest.

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Tables

Table 1. Baseline characteristics of gastric cancer and control participants in a Chinese matched case-control study.

Characteristics	Control (%) (n=299)	Cardia (%) (n=122)	Non-cardia (%) (n=177)	<i>p</i> value ¹	<i>p</i> value ²
Sex				0.45	0.57
Male	231 (77.3)	90 (73.4)	141 (79.7)		
Female	68 (22.7)	32 (26.2)	36 (20.3)		
Age (mean±std, years)	65.7±9.3	68.0±8.7	65.0±10.0	0.05	0.40
Age group (years)				0.01	0.57
<55	35 (11.5)	3 (2.5)	24 (13.6)		
55-64	108 (36.3)	44 (36.0)	67 (37.9)		
65-74	107 (35.9)	47 (38.5)	53 (29.9)		
≥75	49 (16.3)	28 (23.0)	33 (18.6)		
Body mass index (kg/m ²)				0.003	<0.0001
<18.5	27 (9.0)	21 (17.2)	44 (24.8)		
18.5-24.9	229 (76.5)	94 (77.1)	125 (70.6)		
≥25	43 (14.5)	7 (5.7)	8 (4.6)		
Educational level (years)				0.0003	0.13
<7	200 (66.8)	104 (85.3)	134 (75.7)		
7-9	60 (20.1)	15 (12.3)	28 (15.8)		
≥ 10	39 (13.1)	3 (2.5)	15 (8.5)		
Occupation				0.05	0.95
Farmer	210 (70.2)	97 (79.5)	121 (68.8)		
Industrial worker	28 (9.4)	8 (6.6)	20 (11.4)		
Office worker	58 (19.4)	17 (13.9)	34 (19.3)		
Others	3 (1.0)		1 (0.6)		
Household income (Yuan/month/person)				<0.0001	<0.0001
<600	138 (46.2)	25 (20.7)	44 (25.0)		
600-1000	77 (25.8)	41 (33.9)	53 (30.1)		
≥1000	84 (28.1)	55 (45.5)	79 (44.9)		
Tobacco smoking				0.67	0.02
Yes	154 (51.5)	60 (49.2)	111 (63.1)		
No	145 (48.5)	62 (50.8)	65 (36.9)		
Alcohol drinking					
Yes	46 (15.4)	11 (9.0)	36 (20.5)	0.11	0.12
No	253 (84.6)	111 (91.0)	140 (79.5)		
Family history of gastric cancer				1.00	0.88
Yes	53 (32.9)	17 (33.3)	22 (34.4)		
No	108 (67.1)	34 (66.7)	42 (65.6)		
Chronic atrophic gastritis				<0.0001	0.0001
Yes	22 (7.4)	29 (23.8)	33 (18.6)		
No	277 (92.6)	93 (76.2)	144 (81.4)		
Reflux				0.03	0.07
Yes	78 (30.2)	47 (83.9)	21 (20.8)		
No	180 (69.8)	9 (16.1)	80 (79.2)		

1. *P* value between control and cardia groups using chi-square test.

2. *P* value between control and noncardia groups using chi-square test.

Table 2. Distribution of serum level (median and interquartile) of trace elements in study population (n=594).

	Controls	Cardia GC	Non-cardia GC	<i>p</i> value ¹	<i>p</i> value ²
Cu (median, IQR, µg/L)	927.7 (799.1-1312.5)	1014.6 (874.4-1309.2)	1000.2 (821.4-1226.3)	0.0003	0.0009
Zn (median, IQR, µg/L)	605.3 (492.76-804.54)	545.6 (394.9-729.5)	554.6 (428.8-761.7)	0.47	0.52
Se (median, IQR, µg/L)	92.14 (69.9-116.7)	90.9 (63.5-112.6)	90.2 (65.5-112.6)	0.28	0.16
Mo (median, IQR, µg/L)	1.8 (0.93-2.79)	0.93 (0.72-1.14)	0.93 (0.78-1.14)	<0.0001	<0.0001
Fe (median, IQR, µg/L)	1109.8 (890.0-1454.3)	925.4 (809.6-1121.0)	936.6 (780.3-1164.8)	<0.0001	0.97
Ca (median, IQR, mg/L)	85.1 (74.9-103.2)	66.7 (59.1-75.2)	68.9 (60.3-78.5)	<0.0001	<0.0001
Mg (median, IQR, mg/L)	19.1 (17.9-20.6)	20.1 (18.1-21.9)	19.8 (18.2-22.1)	0.08	0.11
Cu/Zn ratio (median, IQR)	1.43 (1.07-1.84)	2.16 (1.51-2.98)	1.91 (1.53-2.46)	<0.0001	<0.0001

1. P value comparing mean value of trace elements between controls and gastric cardia cancers; normal distribution use T-test, not-normal distribution used Wilcoxon rank-sum test.
2. P value comparing mean value of trace elements between controls and gastric noncardia cancers; normal distribution use T-test, not-normal distribution used Wilcoxon rank-sum test.

Table 3. Risk assessment of gastric cancer, expressed as odd ratio (OR) and 95% confidence interval (CI) in a Chinese case-control study.

Variables	Controls	All gastric cancer		Gastric cardia cancer		Gastric non-cardia cancer	
	N (%)	N (%)	OR ¹ (95% CI)	N (%)	OR ¹ (95% CI)	N (%)	OR ¹ (95% CI)
Cu (quartile)							
Q1:<799.1	74 (24.75)	55 (18.39)	1.00	20 (16.39)	1.00	35 (19.77)	1.00
Q2:799.1-927.9	77 (25.76)	51 (17.06)	0.73 (0.30-1.83)	21 (17.2)	0.62 (0.18-2.21)	30 (16.95)	0.74 (0.22-2.48)
Q3:927.9-1059.8	74 (24.75)	61 (20.40)	1.01 (0.44-2.34)	23 (18.9)	0.48 (0.15-1.56)	38 (21.47)	1.39 (0.48-3.90)
Q4:>1059.8	74 (24.75)	132 (44.15)	2.42 (1.10-5.32)	58 (47.5)	2.48 (0.94-6.54)	74 (41.81)	2.39 (0.84-6.86)
<i>p for trend</i>			<i>0.003</i>		<i>0.01</i>		<i>0.03</i>
Zn (quartile)							
Q1:<492.8	74 (24.75)	114 (38.13)	1.00	53 (43.44)	1.00	61 (34.46)	1.00
Q2:492.8-650.2	76 (25.42)	75 (25.08)	0.45 (0.18-1.09)	24 (19.67)	0.40 (0.12-1.36)	51 (28.81)	0.50 (0.18-1.42)
Q3:650.2-844.5	74 (24.75)	44 (14.72)	0.25 (0.10-0.61)	17 (13.93)	0.15 (0.03-0.43)	27 (15.25)	0.34 (0.13-0.98)
Q4:>844.2	75 (25.08)	66 (22.07)	0.59 (0.26-1.35)	28 (22.95)	0.82 (0.29-2.24)	38 (21.47)	0.56 (0.19-1.59)
<i>p for trend</i>			<i>0.22</i>		<i>0.54</i>		<i>0.25</i>
Se (quartile)							
Q1:<69.6	73 (24.41)	97 (32.44)	1.00	39 (31.97)	1.00	58 (32.77)	1.00
Q2:69.6-92.1	74 (24.75)	67 (22.41)	0.47 (0.20-1.08)	25 (20.49)	0.95 (0.34-2.68)	42 (23.73)	0.20 (0.06-0.62)
Q3:92.1-118.8	76 (25.42)	67 (22.41)	0.44 (0.19-1.01)	31 (25.41)	0.55 (0.17-1.70)	36 (20.34)	0.38(0.14-1.01)
Q4:>118.8	76 (25.42)	68 (22.74)	0.33 (0.15-0.75)	27 (22.13)	0.72 (0.25-2.02)	41 (23.16)	0.18 (0.07-0.51)
<i>p for trend</i>			<i>0.009</i>		<i>0.43</i>		<i>0.001</i>
Mo (quartile)							
Q1:<0.93	60 (20.00)	143 (47.83)	1.00	60 (49.18)	1.00	83 (46.89)	1.00
Q2:0.93-1.81	89 (29.83)	137 (45.82)	0.86 (0.43-1.73)	54 (44.26)	0.78 (0.31-1.83)	83 (46.89)	0.87 (0.37-2.06)
Q3:1.81-2.79	69 (23.05)	12 (4.01)	0.07 (0.02-0.21)	3 (2.46)	0.04 (0.004-0.29)	9 (5.08)	0.10 (0.02-0.41)
Q4:>2.79	81 (27.12)	7 (2.34)	0.06 (0.02-0.20)	5 (4.10)	0.12 (0.03-0.47)	2 (1.13)	-
<i>p for trend</i>			<i><0.0001</i>		<i><0.0001</i>		<i>0.001</i>
Fe (quartile)							
Q1:<890.0	74 (24.75)	122 (72.58)	1.00	51 (41.80)	1.00	71 (40.11)	1.00
Q2:890.0-1109.8	75 (25.08)	84 (28.09)	0.43 (0.19-0.95)	34 (27.87)	0.37 (0.13-1.07)	50 (28.25)	0.53 (0.20-1.37)
Q3:1109.8-1454.3	73 (24.41)	38 (12.71)	0.27 (0.11-0.68)	16 (13.11)	0.18 (0.05-0.70)	22 (12.43)	0.41 (0.14-1.21)
Q4:>1454.3	77 (25.76)	55 (18.39)	0.41 (0.18-0.91)	21 (17.21)	0.44 (0.16-1.21)	34 (19.21)	0.52 (0.19-1.37)
<i>p for trend</i>			<i>0.07</i>		<i>0.17</i>		<i>0.25</i>
Ca (quartile)							
Q1:<75.3	74 (24.75)	217 (72.58)	1.00	91 (74.59)	1.00	126 (71.19)	1.00
Q2:75.3-86.1	76	38	0.06 (0.02-	14	0.05 (0.01-	24	0.06 (0.02-

	(25.42)	(12.71)	0.16)	(11.48)	0.22)	(13.56)	0.27)
Q3:86.1-104.5	74	31	0.09 (0.03-	11	0.04 (0.01-	20	0.13 (0.04-
	(24.75)	(10.37)	0.25)	(9.02)	0.20)	(11.30)	0.40)
Q4:>104.5	75	13	0.04 (0.01-	6 (4.92)	0.06 (0.01-	7 (3.95)	0.02 (0.00-
	(25.08)	(4.35)	0.11)		0.26)		0.17)
<i>p for trend</i>			<i><0.0001</i>		<i><0.0001</i>		<i><0.001</i>
Mg (quartile)							
Q1:<17.8	74	56	1.00	22	1.00	34	1.00
	(24.75)	(18.73)		(18.03)		(19.21)	
Q2:17.8-19.2	75	61	0.65 (0.27-	27	0.99 (0.30-	34	0.49 (0.16-
	(25.08)	(20.40)	1.58)	(22.13)	3.22)	(19.21)	1.51)
Q3:19.2-20.8	76	69	1.06 (0.43-	23	1.28 (0.36-	46	1.02 (0.34-
	(25.42)	(23.08)	2.66)	(18.85)	4.49)	(25.99)	3.01)
Q4:>20.8	74	111	1.31 (0.59-	50	1.68 (0.56-	63	1.26 (0.48-
	(24.75)	(37.79)	2.91)	(40.98)	4.84)	(35.59)	3.33)
<i>p for trend</i>			<i>0.23</i>		<i>0.25</i>		<i>0.28</i>
Cu/Zn (quartile)							
Q1:<1.08	74	19	1.00	6 (4.92)	1.00	13 (7.34)	1.00
	(24.75)	(6.35)					
Q2:1.08-1.44	76	48	1.75 (0.54-	23	0.65 (0.12-	25	4.02 (0.74-
	(25.42)	(16.05)	5.74)	(18.85)	3.52)	(14.12)	22.01)
Q3:1.44-1.86	75	63	1.91 (0.57-	17	1.07 (0.22-	46	3.87 (0.67-
	(25.08)	(21.07)	6.37)	(13.93)	5.20)	(25.99)	22.9)
Q4:>1.86	74	169	11.70 (3.83-	76	9.03 (2.29-	93	18.82 (3.50-
	(24.75)	(56.52)	35.6)	(62.32)	35.6)	(52.54)	96.01)
<i>p for trend</i>			<i><0.0001</i>		<i><0.0001</i>		<i><0.0001</i>

Adjusted for age group (<45, 45-55, 56-65,66-75, ≥76 years), sex (male, female), body mass index (<18.5, 18.5-24.9, ≥25 kg/m²), education level (<7, 7-9, ≥10 years), occupation (farmer, industrial worker, office worker, others), household income (<600, 600-1000, ≥1000 Chinese Yuan/month/person), smoking (yes, no), and family history of gastric cancer (yes, no)