Chinese Medical Journal®

Dietary intake and serum levels of copper and zinc and risk of hepatocellular carcinoma: A matched case-control study

Xiaozhan Liu[1], Yaojun Zhang[2], Dinuerguli Yishake[1], Yan Luo[1], Zhaoyan Liu[1], Yuming Chen[1], Huilian Zhu[1], Aiping Fang[1]

[1]Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong 510080, China;
[2]State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, China.

Abstract

Background: Copper and zinc are involved in the development of multiple malignancies; yet, epidemiological evidence on hepatocellular carcinoma (HCC) is limited. This study aimed to investigate the association between dietary intake and serum levels of copper and zinc with the risk of HCC.

Methods: A total of 434 case-control pairs matched for sex and age (±1 year) were included in this study. Cases with newly diagnosed HCC were from the Guangdong Liver Cancer Cohort (GLCC) study, and healthy controls were from the Guangzhou Nutrition and Health Study (GNHS). A semi-quantitative 79-item food frequency questionnaire (FFQ) was used to assess habitual dietary intakes of copper and zinc. Serum levels of copper and zinc were measured by using inductively coupled plasma mass spectrometry. The copper (Cu)/ zinc (Zn) ratio was computed by dividing copper levels by zinc levels. Conditional logistic regression models were performed to calculate the odds ratio (OR) and 95% confidence intervals (CI) for per 1 standard deviation increase (per-SD increase) in copper and zinc levels.

Results: Higher dietary intake ($OR_{per\text{-}SD\ increase}$ = 0.65, 95% CI: 0.44, 0.96, P_{trend} = 0.029) and serum levels of zinc ($OR_{per\text{-}SD\ increase}$ = 0.11, 95% CI: 0.04, 0.30, P_{trend} <0.001) were both associated with a lower risk of HCC. Subgroup analyses showed that the inverse association was only pronounced in men but not in women ($P_{interaction}$ = 0.041 for dietary zinc intake and 0.010 for serum zinc levels). Serum copper levels ($OR_{per\text{-}SD\ increase}$ = 2.05, 95% CI: 1.39, 3.03, P_{trend} = 0.020) and serum Cu/Zn ratio ($OR_{per\text{-}SD\ increase}$ = 6.53, 95% CI: 2.52, 16.92, P_{trend} <0.001) were positively associated with HCC risk, while dietary copper intake and dietary Cu/Zn ratio were not associated with HCC risk.

Conclusion: Zinc may be a protective factor for HCC, especially among men, but the effects of copper on HCC risk are not clear.

Keywords: Copper; Zinc; Hepatocellular carcinoma risk; Case-control studies; Dietary intake; Serum

Introduction

Liver cancer is the seventh most commonly diagnosed cancer and the second leading cause of cancer death worldwide in 2020.[1] The number of new cases and deaths in China were 431,383 and 412,216, respectively.[2] China accounts for about half of the total number of new cases and deaths of liver cancer in the world alone.[3,4] Hepatocellular carcinoma (HCC), the most predominant type of primary liver cancer, is insidious and highly malignant with a 5-year survival rate ranging between 5% and 30%.[5] To prevent the occurrence of HCC, the most effective initiative is to reduce its burden.[6] Accumulating evidence has suggested that dietary risks, which are important modifiable lifestyle factors for deaths,[7] play an important role in the development of HCC.[8]

Copper (Cu) and zinc (Zn) are essential micronutrients derived from a wide variety of animal foods, legumes, grain germ, etc.[9] They are important components of metalloenzymes involved in tissue respiration, energy metabolism, and antioxidant processes, such as Cu/Zn-superoxide dismutase (Cu/Zn-SOD).[10,11] Cu/Zn-SOD is a powerful intracellular antioxidant enzyme that is essential for scavenging reactive oxygen species, but the homeostatic imbalance of copper and zinc will affect Cu/Zn-SOD activity, causing oxidative damage to biomolecules such as DNA, proteins, and lipids, thus promoting the development of cancer.[12,13] In addition, copper and zinc have their own physiological effects in carcinogenesis. Copper is a limiting factor in cancer progression such as tumor growth, angiogenesis, and metastasis.[14] Zinc has important functions such as maintaining immune function and cell structural stability and regulating cell

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002761

Correspondence to: Dr. Aiping Fang, Department of Nutrition, School of Public Health, Sun Yat-sen University, 74 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China
E-Mail: fangaip@mail.sysu.edu.cn

Chinese Medical Journal 2024;137(5)
Received: 17-05-2023; **Online:** 28-11-2023 **Edited by:** Jinjiao Li and Yuanyuan Ji

differentiation and proliferation, DNA/RNA synthesis, and repair.[15]

A case-control study also found that serum copper levels were significantly higher and serum zinc levels were significantly lower in HCC patients than in healthy subjects.[16] And there was significant copper accumulation in surgically resected HCC tissue compared with para-cancerous tissue in HCC patients.[17] These findings suggest that imbalance in copper–zinc homeostasis may be closely related to the incidence of HCC. Nevertheless, there are few epidemiological studies regarding copper and zinc and the risk of HCC. A nested case-control study from the European Prospective Investigation into Cancer and Nutrition (EPIC), including 106 pairs of HCC patients and healthy controls, found that higher serum zinc levels and lower copper-to-zinc ratio are associated with a lower risk of HCC.[18] However, two prospective cohort studies from China reported null association between dietary copper and zinc intakes and the risk of liver cancer.[19] To our knowledge, limited studies have examined the role of copper and zinc, measured both in diets and blood samples, in the development of HCC.

Against this background, this study was designed to investigate the association between dietary intake, serum levels of copper and zinc with the risk of HCC in a 1:1 case-control study.

Methods

Study population

This 1:1 matched case–control study was conducted in Guangdong, China. HCC cases were from the Guangdong Liver Cancer Cohort (GLCC). A prospective cohort study of patients aged 18–80 years with incident liver cancer, was established in September 2013 at Sun Yat-sen University Cancer Center (SYSUCC). In this study, we limited our analyses to cases with newly diagnosed HCC (C22.0 as per the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision* [*ICD-10*], codes), who had not received anti-tumor therapy at recruitment and who had data on both dietary intake and serum levels of copper and zinc. Cases were excluded if they had the following conditions: (1) other serious chronic diseases (including heart failure, liver failure, renal failure, physical or mental disability); (2) significant changes in dietary habits or lifestyles within the previous 5 years (i.e., changing from omnivorous diets to vegetarian diets, abstaining recruited from smoking and alcohol drinking). Healthy controls were recruited from the Guangzhou Nutrition and Health Study (GNHS). The GNHS, established in 2008, is a community-based prospective cohort study. The inclusion and exclusion criteria for cases and controls were the same (except for the diagnosis of HCC). The recruitment and enrollment procedures of the cohorts have been previously described in detail.[20,21]

Ethical approval

The GLCC and GNHS were approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University (Nos. 013[2017] and 048[2018]). Written informed consent was obtained from all participants.

Cases and controls were matched 1:1 according to sex and age (±1 year), and a total of 434 case-control pairs were included in the present analysis. The study protocol was approved by the institutional review board of the School of Public Health at Sun Yat-sen University. Written informed consent was provided by each participant.

Dietary assessment

A semi-quantitative 79-item food frequency questionnaire (FFQ) was used to assess dietary intake during the previous year before HCC diagnosis for cases or prior to the interview for controls. Each food item had five predefined qualitative responses, namely "daily," "weekly," "monthly," "annually," and "not eaten." Participants were asked to report the frequency and intake amount of each food consumed. Photographs of common food portion sizes were provided to help participants to quantify their consumption. Individual food intake was translated into grams per day. Daily dietary intakes of total energy, copper, zinc, and other nutrients were then calculated by multiplying the daily consumption of each food item by the nutrient content obtained from the China Food Composition Table 2009.[22] Copper and zinc intakes were adjusted for total energy intake (i.e., 1922 kcal/day for men and 1688 kcal/day for women) by the residual method.[23] The Healthy Eating Index 2015 (HEI-2015)[24] was also calculated to reflect the diet quality of participants, with higher scores indicating better adherence to dietary guidelines. The validity and reproducibility of the FFQ had been confirmed by six non-consecutive, 3-day dietary records at intervals of 2 months within 1 year and two FFQs administered 1 year apart among 61 healthy women recruited from Guangdong.[25]

Laboratory assays

Blood samples were collected on the second day of the hospital admission for HCC cases and at the first follow-up for controls. Serum samples were separated, aliquoted, and stored in −80°C freezers. To minimize technical variability, 100 μL of serum samples was assayed in batches at the KingMed Diagnostics Laboratory (Guangzhou, China) by blinded laboratory personnel between December 2016 and March 2017. Serum copper and zinc concentrations were quantified by using inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS spectrometer, Agilent Technologies, Germany). Masked, replicate, and quality control samples were interspersed among the samples. The intra-assay coefficients of variance for serum copper and zinc were 7.4% and 6.6%, respectively.

Routine laboratory parameters including seropositivity for hepatitis B surface antigen (HBsAg) were analyzed to a standardized protocol at the Clinical Laboratory of SYSUCC for cases and the KingMed Diagnostics Laboratory for controls.

Covariates

The same structured questionnaire was used to collect the following information from cases and controls through face-to-face interviews: socio-demographic characteristics (i.e., sex, age); lifestyle habits (i.e., diet, smoking status, alcohol drinking status); and relevant diseases (i.e., a history of fatty liver disease, a history of diabetes). Smokers or alcohol drinkers were defined as participants who smoked at least one cigarette per day or drank alcohol at least once a week continuously for at least 6 months. Anthropometric measurements (i.e., height and body weight) were collected by a standard procedure. Body mass index (BMI, kg/m^2) was then calculated by dividing weight (kg) by height squared (m^2).

Statistical analysis

The Cu/Zn ratio was computed by dividing copper levels by zinc levels. Paired student's *t*-test, Wilcoxon signed-rank test, or McNemar χ^2 test were used to compare differences in baseline characteristics between cases and controls. Spearman's rank correlation coefficients were estimated between dietary intake and serum levels of copper or zinc. Cases and controls were divided into three groups according to the tertiles of copper and zinc levels in the control group. Conditional logistic regression models were used to calculate the odds ratio (OR) and 95% confidence intervals (95% CIs). The trend test was performed by assigning the median value for each group as a continuous variable in the regression models. We also calculated OR and 95% CIs for one standard deviation increase (per-SD increase) in copper and zinc levels. Model 1 was adjusted for age and total energy intake; model 2 was additionally adjusted for BMI, smoking status, alcohol drinking status, and HEI-2015 based on model 1; and model 3 was additionally adjusted for HBsAg, history of fatty liver disease, and history of diabetes based on model 2. The interaction of copper and zinc with other established risk factors for HCC including sex, age, smoking status, alcohol drinking status, HBsAg, history of fatty liver disease, and history of diabetes was explored using the multiplicative scale. Stratified analyses were further performed using a binary logistic regression model for all factors except sex and age.

The statistical analyses were performed in SPSS (IBM, Armonk, NY, USA), version 26.0. All tests were two-sided, and a value of P <0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 434 case-control pairs were included in this study. Men accounted for 83.2% (361/434); the mean ± standard deviation (SD) age was 59.8 ± 7.6 years. The baseline characteristics of the case and control groups are shown in Table 1. The case group had a higher proportion of smokers, alcohol drinkers, HBsAg (+), and a history of fatty liver disease but lower HEI-2015 scores than the control group (all P <0.001). No significant differences in BMI, total energy intake, and the proportion with a history of diabetes were found between cases and controls.

As shown in Table 2, dietary intakes of copper (median: 1.56 mg/day *vs.* 2.10 mg/day), zinc (median: 12.3 mg/day *vs.* 13.0 mg/day), and their ratio (median: 0.13 *vs.* 0.16) were significantly lower in cases than in controls (all P <0.001). Regarding serum levels, serum copper (median: 982 μg/L *vs.* 874 μg/L) and Cu/Zn ratio (median: 1.13 *vs.* 0.79) were higher, while serum zinc was lower (median: 871 μg/L *vs.* 1098 μg/L) in cases than in controls (all P <0.001). There was significantly inverse correlation between serum copper levels and dietary copper intake ($r_{spearman}$ = −0.101, P = 0.003), whereas serum zinc levels were positively correlated with dietary zinc intake ($r_{spearman}$ = 0.171, P <0.001).

Association of dietary copper and zinc intake with HCC risk

The associations of dietary intake of copper and zinc and their ratios with HCC risk are shown in Table 3. Higher dietary zinc intake was associated with a lower risk of HCC after adjustment for age and total energy intake (model 1: OR [95% CI] $_{\text{Tertile 3 } vs. \text{ Tertile 1}}$ = 0.39 [0.27, 0.56], P_{trend} <0.001). The inverse association remained significant after further adjustment for BMI, smoking, alcohol drinking, HEI-2015 (model 2: OR [95% CI] $_{\text{Tertile 3 } vs. \text{ Tertile 1}}$ = 0.33 [0.17, 0.63], P_{trend} = 0.001), as well as HBsAg, history of fatty liver disease, and history of diabetes (model 3: OR [95% CI] $_{\text{Tertile 3 } vs. \text{ Tertile 1}}$ = 0.26 [0.08, 0.81], P_{trend} = 0.029). Per-SD increase in dietary zinc intake was associated with a 35% decrease in the risk of HCC (OR [95% CI] $_{\text{per-SD increase}}$ = 0.65 [0.44, 0.96]). An inverse association with HCC risk was also observed for dietary copper intake and dietary Cu/Zn ratio in model 1, however, the association became non-significant after additional adjustment for other risk factors.

Association of serum levels of copper and zinc with HCC risk

The associations of serum levels of copper and zinc and their ratio with HCC risk are shown in Table 4. After adjusting for potential confounders, higher serum zinc levels were associated with a lower risk of HCC (model 3: OR [95% CI] $_{\text{Tertile 3 } vs. \text{ Tertile 1}}$ = 0.02 [0.002, 0.13], P_{trend} <0.001). For each SD increase in serum zinc levels, the risk of HCC was decreased by 89% (model 3: OR [95% CI] $_{\text{per-SD increase}}$ = 0.11 [0.04, 0.30]). However, serum copper levels (model 3: OR [95% CI] $_{\text{per-SD increase}}$ = 2.05 [1.39, 3.03]) and serum Cu/Zn ratio (model 3: OR [95% CI] $_{\text{per-SD increase}}$ = 6.53 [2.52, 16.92]) were positively associated with HCC risk.

Stratified analyses

Supplementary Tables 1 and 2, http://links.lww.com/CM9/B614 show a significant interaction of dietary zinc intake and serum zinc levels with sex, respectively ($P_{interaction}$ = 0.041; 0.010). Higher zinc intake ($OR_{\text{Tertile 3 } vs. \text{ Tertile 1}}$ = 0.54, 95% CI [0.32, 0.92], P_{trend} = 0.038) or higher serum zinc levels ($OR_{\text{Tertile 3 } vs. \text{ Tertile 1}}$ = 0.06, 95% CI [0.01, 0.29], P_{trend} <0.001) were associated with a lower risk of HCC in men, while no significant association was observed in

Table 1: Baseline characteristics of HCC cases and matched controls.

Characteristics	HCC cases (*n* = 434)	Matched controls (*n* = 434)	Statistics	*P* values
Sex			0†	1.000
Women	73 (16.8)	73 (16.8)		
Men	361 (83.2)	361 (83.2)		
Age (years)	59.8 ± 7.6	59.9 ± 7.5	1.98*	0.048
BMI (kg/m^2)	22.8 ± 3.2	22.8 ± 2.8	0.35*	0.727
Smoking			117.44†	<0.001
No	169 (38.9)	327 (75.3)		
Yes	265 (61.1)	107 (24.7)		
Alcohol drinking			73.36†	<0.001
No	253 (58.3)	367 (84.6)		
Yes	181 (41.7)	67 (15.4)		
BCLC stage			–	–
0	41 (9.4)	–		
A	165 (38.0)	–		
B	42 (9.7)	–		
C	186 (42.9)	–		
D	–	–		
HBsAg (+)			448.00†	<0.001
No	66 (15.2)	366 (84.3)		
Yes	368 (84.8)	51 (11.8)		
Missing	–	17 (3.9)		
History of fatty liver disease			14.34†	<0.001
No	339 (78.1)	375 (86.4)		
Yes	95 (21.9)	52 (12.0)		
Missing	–	7 (1.6)		
History of diabetes			1.63†	0.257
No	378 (87.1)	390 (89.9)		
Yes	56 (12.9)	44 (10.1)		
HEI-2015	55.5 ± 7.0	65.1 ± 6.3	21.01*	<0.001
Total energy intake (kcal/day)	1912 ± 610	1951 ± 585	0.97*	0.334

Values are shown as *n* (%) or mean ± standard deviation. *t value, †chi-squared value. BCLC stage: Barcelona Clinic Liver Cancer stage; BMI: Body mass index; HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma; HEI-2015: Healthy Eating Index 2015. –: Not available.

Table 2: Dietary intakes and serum levels of copper and zinc of HCC cases and matched controls.

Characteristics	HCC cases (*n* = 434)	Matched controls (*n* = 434)	Statistics	*P* values
Dietary copper intakes (mg/day)*	1.56 (1.21, 2.04)	2.10 (1.63, 2.89)	−8.26	<0.001
Dietary zinc intakes (mg/day)*	12.3 (11.1, 13.6)	13.0 (12.0, 14.0)	−5.48	<0.001
Dietary Cu/Zn ratio*	0.13 (0.10, 0.16)	0.16 (0.13, 0.23)	−7.15	<0.001
Serum copper (μg/L)	982 (838, 1200)	874 (802, 955)	−9.26	<0.001
Serum zinc (μg/L)	871 (773, 980)	1098 (968, 1257)	−14.85	<0.001
Serum Cu/Zn ratio	1.13 (0.94, 1.42)	0.79 (0.66, 0.93)	−15.67	<0.001

Values are shown as median (IQR). *Intakes of copper and zinc were adjusted for sex-specific mean total energy intake (i.e., 1922 kcal/day for men and 1688 kcal/day for women) using the Residual Method. Cu/Zn: Copper/zinc; HCC: Hepatocellular carcinoma; IQR: Interquartile range.

women. There was no significant interaction between zinc and the remaining risk factors, including age, smoking, alcohol drinking, HBsAg (+), a history of fatty liver disease, and a history of diabetes (all $P_{interaction}$ >0.05). In Supplementary Tables 3 and 4, http://links.lww.com/CM9/B614, there was no significant interaction between dietary intake or serum levels of copper and the stratified factors (all $P_{interaction}$ >0.05).

Table 3: OR (95% CI) of the risk of HCC by tertiles of dietary copper and zinc intakes.

	OR (95% CI)				OR (95% CI)
Characteristics	Tertile 1	Tertile 2	Tertile 3	P_{trend} values	per-SD increase
Dietary copper intake*	≤1.76 mg/day	>1.76–2.52 mg/day	>2.52 mg/day		per 2.02 mg/day
Cases/control	273/144	93/145	68/145		434/434
Model 1†	1.00 (ref‖)	0.31 (0.21, 0.45)	0.25 (0.17, 0.37)	<0.001	0.60 (0.49, 0.74)
Model 2‡	1.00 (ref‖)	1.08 (0.58, 2.00)	0.66 (0.36, 1.22)	0.140	0.87 (0.67, 1.12)
Model 3§	1.00 (ref‖)	1.15 (0.40, 3.29)	0.94 (0.37, 2.38)	0.834	0.86 (0.59, 1.26)
Dietary zinc intake*	≤12.35 mg/day	>12.35–13.68 mg/day	>13.68 mg/day		per 1.81 mg/day
Cases/controls	229/144	102/145	103/145		434/434
Model 1†	1.00 (ref‖)	0.38 (0.27, 0.55)	0.39 (0.27, 0.56)	<0.001	0.70 (0.61, 0.81)
Model 2‡	1.00 (ref‖)	0.23 (0.11, 0.46)	0.33 (0.17, 0.63)	0.001	0.68 (0.54, 0.86)
Model 3§	1.00 (ref‖)	0.19 (0.06, 0.63)	0.26 (0.08, 0.81)	0.029	0.65 (0.44, 0.96)
Dietary Cu/Zn ratio*	≤0.14	>0.14–0.20	>0.20		per 0.19
Cases/controls	267/145	99/144	68/145		434/434
Model 1†	1.00 (ref‖)	0.32 (0.22, 0.47)	0.25 (0.17, 0.37)	<0.001	0.70 (0.57, 0.86)
Model 2‡	1.00 (ref‖)	1.09 (0.59, 2.05)	0.65 (0.35, 1.23)	0.110	0.95 (0.74, 1.23)
Model 3§	1.00 (ref‖)	0.86 (0.30, 2.47)	0.88 (0.36, 2.16)	0.799	0.97 (0.68, 1.39)

*Intakes of copper and zinc were adjusted for total energy intake (i.e., 1922 kcal/day for men and 1688 kcal/day for women) using the residual method. †Model 1: Adjusted for age and total energy intake. ‡Model 2: Adjusted for covariates in model 1 + BMI, smoking status, alcohol drinking status, and HEI-2015. §Model 3: Adjusted for covariates in model 2 + HBsAg, history of fatty liver disease, and history of diabetes. ‖"ref" refers to the inclusion of copper and zinc indicators as categorical variables in the model, using Tertile 1 as a reference to calculate the OR (95% CI) for Tertile 2 and Tertile 3, respectively. BMI: Body mass index; CI: Confidence interval; Cu/Zn: Copper/zinc; HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma; HEI-2015: Healthy Eating Index 2015; OR: Odds ratio; SD: Standard deviation.

Table 4: OR (95% CI) of the risk of HCC by tertiles of serum copper and zinc levels.

	OR (95% CI)				OR (95% CI)
Characteristics	Tertile 1	Tertile 2	Tertile 3	P_{trend} values	per-SD increase
Serum copper levels	≤825 μg/L	>825–921 μg/L	>921 μg/L		per 122 μg/L
Cases/controls	100/144	74/146	260/144		434/434
Model 1*	1.00 (ref§)	0.76 (0.51, 1.14)	2.51 (1.78, 3.55)	<0.001	1.56 (1.40, 1.73)
Model 2†	1.00 (ref§)	0.75 (0.38, 1.47)	1.48 (0.82, 2.68)	0.100	1.33 (1.14, 1.55)
Model 3‡	1.00 (ref§)	0.68 (0.19, 2.38)	2.57 (0.90, 7.32)	0.020	2.05 (1.39, 3.03)
Serum zinc levels	≤1020 μg/L	>1020–1186 μg/L	>1186 μg/L		per 404 μg/L
Cases/controls	361/144	57/146	16/144		434/434
Model 1*	1.00 (ref§)	0.10 (0.06, 0.17)	0.03 (0.01, 0.06)	<0.001	0.05 (0.03, 0.58)
Model 2†	1.00 (ref§)	0.16 (0.07, 0.37)	0.03 (0.01, 0.09)	<0.001	0.09 (0.04, 0.20)
Model 3‡	1.00 (ref§)	0.43 (0.13, 1.44)	0.02 (0.002, 0.13)	<0.001	0.11 (0.04, 0.30)
Serum Cu/Zn ratio	≤0.71	>0.71–0.87	>0.87		per 0.21
Cases/controls	17/144	56/145	361/145		434/434
Model 1*	1.00 (ref§)	3.04 (1.50, 6.14)	24.86 (12.34, 50.07)	<0.001	4.05 (3.03, 5.40)
Model 2†	1.00 (ref§)	7.27 (2.43, 21.80)	26.06 (9.16, 74.12)	<0.001	3.97 (2.56, 6.16)
Model 3‡	1.00 (ref§)	9.17 (1.41, 59.65)	65.42 (8.90, 480.83)	<0.001	6.53 (2.52, 16.92)

*Model 1: Adjusted for age and total energy intake. †Model 2: Adjusted for covariates in model 1 + BMI, smoking status, alcohol drinking status, and HEI-2015. ‡Model 3: Adjusted for covariates in model 2 + HBsAg, history of fatty liver disease, and history of diabetes. §"ref" refers to the inclusion of copper and zinc indicators as categorical covariates in the model, using Tertile 1 as a reference to calculate the OR (95% CI) for Tertile 2 and Tertile 3, respectively. BMI: Body mass index; CI: Confidence interval; Cu/Zn: Copper/zinc; HBsAg: Hepatitis B surface antigen; HEI-2015: Healthy Eating Index 2015; HCC: Hepatocellular carcinoma; OR: Odds ratio; SD: Standard deviation.

Discussion

In this 1:1 matched case-control study, we found that higher dietary intake and serum levels of zinc were associated with a lower risk of HCC, and the inverse association between zinc and HCC risk was only predominant in men. In addition, serum levels of copper and serum Cu/Zn ratio were positively associated with HCC risk, while dietary copper intake and dietary Cu/Zn ratio were not associated with HCC risk in model 3.

In line with our findings, a nested case-control study conducted within the EPIC showed an inverse association between serum zinc levels and HCC risk ($n = 106$).[18] However, the results from the Shanghai Women's and Men's Study observed no significant association between dietary zinc intake and liver cancer risk,[19] which were inconsistent with our study. In this study, we found both dietary zinc intake and serum zinc levels were inversely associated with HCC risk, even after additional adjustment for other risk factors for HCC including hepatitis B virus infection. Our findings are supported by several studies with zinc supplementation in patients with chronic liver diseases (CLDs). Randomized controlled trials have showed that zinc supplementation significantly increased serum zinc levels and decreased levels of liver enzymes and oxidative stress in patients with non-alcoholic fatty liver disease.[26,27] A retrospective study reported that zinc supplementation maintained liver function and reduced the incidence of HCC in 267 patients with CLDs, particularly in those with post-treatment serum zinc levels ≥700 μg/L.[28]

In stratified analyses, we also found that the inverse association between dietary intake and serum levels of zinc and the risk of HCC was only pronounced in men but not in women. The exact mechanism for the sex differences remains to be elucidated. Animal studies have shown that sexually dimorphic liver gene expression is modulated by regulator of sex-limitation 1 (Rsl1), one of the Krüppel-associated box zinc finger proteins (KRAB-ZFPs), and thus contribute to the hepatic sex differences in liver disease pathogenesis.[29,30] This may partially explain why zinc is a protective factor for HCC in men but not in women.

HBV infection and alcohol drinking are well-established risk factors for HCC. Although the case group had a higher proportion of participants with HBsAg (+) and alcohol drinkers than the control group, the association between serum levels and dietary intake of zinc and HCC risk was not modified by HBV infection and alcohol drinking status in our study. However, the limited number of HBsAg(−) participants and non-alcohol drinkers preclude drawing a firm conclusion.

Copper intake were not observed to be associated with the risk of HCC in this study, which was in accord with the findings from the Shanghai Women's and Men's Study.[19] However, we found a positive association between serum copper levels and HCC risk. Given the fact that dietary copper levels were lower while serum copper levels were higher in HCC cases than in healthy controls in our study, we speculate that the increase in serum copper levels in patients with HCC is likely due to the redistribution of hepatic copper caused by the pathological inflammation in the liver rather than excessive copper intake.[31] Redistribution of hepatic copper includes excessive activation of ceruloplasmin and release of hepatocyte necrosis, which leads to excessive copper release from the liver into the blood.[32,33] Therefore, high serum copper levels may be a consequence of abnormal liver function in patients with HCC, rather than an independent risk factor for HCC. Consistent with the assumption, a nested case-control study within the EPIC showed null association between serum copper levels and HCC risk.[18] Nevertheless, our previous study found that higher serum copper levels were associated with a poorer prognosis in HCC patients,[20] which may suggest a more important role of copper in the progression of HCC.

Previous studies have found a positive correlation between serum Cu/Zn ratio and the overall severity of viral hepatitis and liver cirrhosis,[16,34] and Cu/Zn ratio may serve as a reference indicator to predict the incidence of HCC.[35] We also observed a positive association between serum Cu/Zn ratio and HCC risk, but in consideration of copper not being an independent risk factor for HCC, the positive association may be mainly attributed to zinc alone.

Zinc, a core component of Cu/Zn-SOD, is a key factor in reducing cellular oxidative stress and inhibiting cancer initiation.[13,36] It was found that zinc supplementation in healthy elderly subjects increased their serum zinc levels and significantly increased the antioxidant activity of circulating Cu/Zn-SOD, while zinc deficiency at baseline was associated with lower activity of circulating Cu/Zn-SOD.[37,38] Zinc deficiency also decreases glutathione levels, which in turn increases hepatic genotoxicity.[39] Zinc is particularly important for cellular and humoral immunity in humans. Zinc is involved in the proliferation and differentiation of immune cells, as well as in the regulation of a series of cytokines such as interleukins, interferons, and tumor necrosis factors.[15,40] Zinc deficiency decreases the quantity of granulocytes and natural killer cells as well as the phagocytic capacity of macrophages and also affects the quantity and function of T and B lymphocytes in the thymus and marrow, thereby increasing the risk of various cancers.[41,42] Meanwhile, zinc binds to the oncogene *p53* in the form of zinc-binding protein, performing the functions of monitoring cell cycle and DNA repair and inhibiting excessive cell proliferation. Zinc deficiency decreases the level of binding to *p53*, causes misfolding of p53, and impairs DNA repair functions, thus promoting cancer development.[39,43] In addition, zinc is a physiological trigger for metallothioneins (MTs), which have a high affinity for heavy metals and prevent oxidative stress and DNA damage caused by heavy metals.[36] Thus, the mechanistic aspect supports our view that high serum zinc levels contribute to the prevention of HCC.

Strengths of this study are that, first, we measured zinc and copper in both diet and serum in the same population, which allows us to explore the link between dietary intake and serum levels. Second, the sample size

of HCC cases is relatively large and all HCC cases were newly diagnosed and previously untreated to avoid the confounding effect of anti-tumor therapy on dietary intake and serum levels of copper and zinc. Third, cases and controls were matched by sex and age (±1 year), which increases the statistical power. Fourth, dietary information was collected by the same FFQ, and serum levels of zinc and copper were quantified using ICP-MS at the same laboratory concurrently for cases and controls, which increases comparability of exposures between the groups. Finally, a wide range of covariates was collected, including sex, age, total energy intake, BMI, smoking status, alcohol drinking status, HEI-2015, HBsAg seropositivity, the history of fatty liver disease, and diabetes.

Nevertheless, several limitations of this study are needed to be noticed. First, dietary information within the previous year before HCC diagnosis and blood samples from cases were collected at the diagnosis of HCC. Therefore, the possibility of reverse causality cannot be ruled out due to the case-control design. Second, we have only one measurement of dietary intake and serum levels of copper and zinc, which cannot reflect the long-term nutritional status of copper and zinc in the study population. Third, the FFQ used in this study were only validated in the healthy subjects, and recall bias is inevitable. To minimize the bias, we only included participants whose dietary habits without marked change in the past 5 years and patients with newly diagnosed HCC. In addition, photographs were provided to help participants assess food consumption. Fourth, hepatitis C virus (HCV) infection was not considered in the analysis. However, since the prevalence of HCV infection in China is low,[44] its impact on the results may be negligible. Lastly, the study population was all of Asians living in southern China, and caution should be taken when generalizing the findings to different regions and ethnicities.

In summary, higher dietary intakes and serum levels of zinc were associated with a lower risk of HCC, especially in men, suggesting that zinc may protect against HCC. Although serum copper was significantly higher in HCC than in healthy controls, copper may not be an independent risk factor for HCC, as it is likely due to the redistribution of hepatic copper caused by the pathological inflammation of the liver. Large-scale prospective cohort studies or randomized controlled studies are warranted.

Acknowledgements

The authors thank the office and field staff of the Sun Yat-Sen University Cancer Center and School of Public Health for data collection, processing, and preparation. We thank all participants from Guangdong Liver Cancer Cohort (GLCC) study and Guangzhou Nutrition and Health Study (GNHS) for their contributions to this study.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (No. 81803219); the Natural Science Foundation of Guangdong Province, China (Nos. 2022A1515011744, 2020A1515110682, and 2018A030310335); and the China Postdoctoral Science Foundation (No. 2020M683135).

Conflicts of interest

None.

References

1. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chin Med J 2021;134:783–791. doi: 10.1097/CM9.0000000000001474.
2. Xia C, Dong X, Li H, Cao M, Sun D, He S, Yang F, Yan X, Zhang S, Li N, Chen W. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J 2022; 135:584–590. doi: 10.1097/CM9.0000000000002108.
3. Petrick JL, Florio AA, Znaor A, Ruggieri D, Laversanne M, Alvarez CS, *et al*. International trends in hepatocellular carcinoma incidence, 1978–2012. Int J Cancer 2020;147:317–330. doi: 10.1002/ijc.32723.
4. Zheng RS, Zhang SW, Zeng HM, Wang SM, Sun KX, Chen R, *et al*. Cancer incidence and mortality in China, 2016. J Natl Cancer Cent 2022;2:1–9. doi: 10.1016/j.jncc.2022.02.002.
5. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, *et al*. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): Analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 2018; 391:1023–1075. doi: 10.1016/S0140-6736(17)33326-3.
6. Arnold M, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA, *et al*. Global burden of 5 major types of gastrointestinal cancer. Gastroenterology 2020;159:335–349.e15. doi: 10.1053/j.gastro.2020.02.068.
7. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020;396:1223–1249. doi: 10.1016/S0140-6736(20)30752-2.
8. Yang WS, Zeng XF, Liu ZN, Zhao QH, Tan YT, Gao J, *et al*. Diet and liver cancer risk: A narrative review of epidemiological evidence. Br J Nutr 2020;124:330–340. doi: 10.1017/S0007114520001208.
9. King JC. Zinc: An essential but elusive nutrient. Am J Clin Nutr 2011;94:679S–684S. doi: 10.3945/ajcn.110.005744.
10. Osredkar J, Sustar N. Copper and zinc, biological role and significance of copper/zinc imbalance. J Clini Toxicol 2011; 3: 0495. doi: 10.4172/2161-0495.S3-001.
11. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on dietary reference values for copper. EFSA J 2015;13:4253. doi: 10.2903/j.efsa.2015.4253.
12. Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. Mol Aspects Med 2005;26:268–298. doi: 10.1016/j.mam.2005.07.015.
13. Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME. Zinc and human health: An update. Arch Toxicol 2012;86:521–534. doi: 10.1007/s00204-011-0775-1.
14. Denoyer D, Masaldan S, La Fontaine S, Cater MA. Targeting copper in cancer therapy: 'Copper That Cancer'. Metallomics 2015;7:1459–1476. doi: 10.1039/C5MT00149H.
15. Skrajnowska D, Bobrowska-Korczak B. Role of zinc in immune system and anti-cancer defense mechanisms. Nutrients 2019;11:2273. doi: 10.3390/nu11102273.
16. Lin CC, Huang JF, Tsai LY, Huang YL. Selenium, iron, copper, and zinc levels and copper-to-zinc ratios in serum of patients at different stages of viral hepatic diseases. Biol Trace Elem Res 2006;109:15–24. doi: 10.1385/BTER:109:1:015.
17. Ebara M, Fukuda H, Hatano R, Saisho H, Nagato Y, Suzuki K, *et al*. Relationship between copper, zinc and metallothionein in hepatocellular carcinoma and its surrounding liver parenchyma. J Hepatol 2000;33:415–422. doi: 10.1016/S0168-8278(00)80277-9.
18. Stepien M, Hughes DJ, Hybsier S, Bamia C, Tjønneland A, Overvad K, *et al*. Circulating copper and zinc levels and risk of hepatobiliary cancers in Europeans. Brit J Cancer 2017; 116:688–696. doi: 10.1038/bjc.2017.1.

19. Ma X, Yang Y, Li HL, Zheng W, Gao J, Zhang W, *et al*. Dietary trace element intake and liver cancer risk: Results from two population-based cohorts in China. Int J Cancer 2017;140: 1050–1059. doi: 10.1002/ijc.30522.
20. Fang AP, Chen PY, Wang XY, Liu ZY, Zhang DM, Luo Y, *et al*. Serum copper and zinc levels at diagnosis and hepatocellular carcinoma survival in the Guangdong Liver Cancer Cohort. Int J Cancer 2019;144:2823–2832. doi: 10.1002/ijc.31991.
21. Zhang ZQ, He LP, Liu YH, Liu J, Su YX, Chen YM. Association between dietary intake of flavonoid and bone mineral density in middle aged and elderly Chinese women and men. Osteoporosis Int 2014;25:2417–2425. doi: 10.1007/s00198-014-2763-9.
22. Yang YX, Wang GY, Pan XC. China food composition. Beijing: Peking University Medical Press, 2009.
23. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65: 1220S–1228S; discussion 1229S-1231S. doi: 10.1093/ajcn/65.4.1220S.
24. Reedy J, Lerman JL, Krebs-Smith SM, Kirkpatrick SI, Pannucci TE, Wilson MM, *et al*. Evaluation of the healthy eating index-2015. J Acad Nutr Diet 2018;118:1622–1633. doi: 10.1016/j.jand.2018.05.019.
25. Zhang CX, Ho SC. Validity and reproducibility of a food frequency questionnaire among Chinese women in Guangdong province. Asia Pac J Clin Nutr 2009;18:240–250. Available online at: https://apjcn.nhri.org.tw/server/APJCN/18/2/240.pdf. [Last access on Jan. 19th 2023]
26. Fathi M, Alavinejad P, Haidari Z, Amani R. The effect of zinc supplementation on steatosis severity and liver function enzymes in overweight/obese patients with mild to moderate non-alcoholic fatty liver following calorie-restricted diet: A double-blind, randomized placebo-controlled trial. Biol Trace Elem Res 2020; 197:394–404. doi: 10.1007/s12011-019-02015-8.
27. Fathi M, Alavinejad P, Haidari Z, Amani R. The effects of zinc supplementation on metabolic profile and oxidative stress in overweight/obese patients with non-alcoholic fatty liver disease: A randomized, double-blind, placebo-controlled trial. J Trace Elem Med Biol 2020;62:126635. doi: 10.1016/j.jtemb.2020.126635.
28. Hosui A, Kimura E, Abe S, Tanimoto T, Onishi K, Kusumoto Y, *et al*. Long-term zinc supplementation improves liver function and decreases the risk of developing hepatocellular carcinoma. Nutrients 2018;10:1955. doi: 10.3390/nu10121955.
29. Krebs CJ, Khan S, MacDonald JW, Sorenson M, Robins DM. Regulator of sex-limitation KRAB zinc finger proteins modulate sex-dependent and -independent liver metabolism. Physiol Genomics 2009;38:16–28. doi: 10.1152/physiolgenomics.90391.2008.
30. Krebs CJ, Schultz DC, Robins DM. The KRAB zinc finger protein RSL1 regulates sex- and tissue-specific promoter methylation and dynamic hormone-responsive chromatin configuration. Mol Cell Biol 2012;32:3732–3742. doi: 10.1128/MCB.00615-12.
31. Balter V, Nogueira da Costa A, Bondanese VP, Jaouen K, Lamboux A, Sangrajrang S, *et al*. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. Proc Natl Acad Sci USA 2015;112:982–985. doi: 10.1073/pnas.1415151112.
32. Deshmukh VK, Raman PH, Dhuley JN, Naik SR. Role of ceruloplasmin in inflammation: Increased serum ceruloplasmin levels during inflammatory conditions and its possible relationship with anti-inflammatory agents. Pharmacol Res Commun 1985; 17:633–642. doi: 10.1016/0031-6989(85)90070-0.
33. Araya M, Pizarro F, Olivares M, Arredondo M, González M, Méndez M. Understanding copper homeostasis in humans and copper effects on health. Biol Res 2006;39:183–187. doi: 10.4067/S0716-97602006000100020.
34. Nangliya V, Sharma A, Yadav D, Sunder S, Nijhawan S, Mishra S. Study of trace elements in liver cirrhosis patients and their role in prognosis of disease. Biol Trace Elem Res 2015;165:35–40. doi: 10.1007/s12011-015-0237-3.
35. Poo JL, Rosas-Romero R, Montemayor AC, Isoard F, Uribe M. Diagnostic value of the copper/zinc ratio in hepatocellular carcinoma: A case control study. J Gastroenterol 2003;38:45–51. doi: 10.1007/s005350300005.
36. Michalczyk K, Cymbaluk-Płoska A. The role of zinc and copper in gynecological malignancies. Nutrients 2020;12:3732. doi: 10.3390/nu12123732.
37. Mariani E, Mangialasche F, Feliziani FT, Cecchetti R, Malavolta M, Bastiani P, *et al*. Effects of zinc supplementation on antioxidant enzyme activities in healthy old subjects. Exp Gerontol 2008;43:445–451. doi: 10.1016/j.exger.2007.10.012.
38. Andriollo-Sanchez M, Hininger-Favier I, Meunier N, Toti E, Zaccaria M, Brandolini-Bunlon M, *et al*. Zinc intake and status in middle-aged and older European subjects: The ZENITH study. Eur J Clin Nutr 2005;59:S37–S41. doi: 10.1038/sj.ejcn.1602296.
39. Romualdo GR, Goto RL, Henrique Fernandes AA, Cogliati B, Barbisan LF. Dietary zinc deficiency predisposes mice to the development of preneoplastic lesions in chemically-induced hepatocarcinogenesis. Food Chem Toxicol 2016;96:280–289. doi: 10.1016/j.fct.2016.08.020.
40. Wessels I, Haase H, Engelhardt G, Rink L, Uciechowski P. Zinc deficiency induces production of the proinflammatory cytokines IL-1β and TNF-α in promyeloid cells via epigenetic and redox-dependent mechanisms. J Nutr Biochem 2013;24:289–297. doi: 10.1016/j.jnutbio.2012.06.007.
41. Haase H, Rink L. Zinc signals and immune function. Biofactors 2014;40:27–40. doi: 10.1002/biof.1114.
42. Rolles B, Maywald M, Rink L. Influence of zinc deficiency and supplementation on NK cell cytotoxicity. J Funct Foods 2018;48:322–328. doi: 10.1016/j.jff.2018.07.027.
43. Loh SN. The missing zinc: p53 misfolding and cancer. Metallomics 2010;2:442–449. doi: 10.1039/c003915b.
44. Wu J, Zhou Y, Fu X, Deng M, Zheng Y, Tian G, *et al*. The burden of chronic hepatitis C in China from 2004 to 2050: An individual-based modeling study. Hepatology 2019;69:1442–1452. doi: 10.1002/hep.30476.

How to cite this article: Liu XZ, Zhang YJ, Yishake D, Luo Y, Liu ZY, Chen YM, Zhu HL, Fang AP. Dietary intake and serum levels of copper and zinc and risk of hepatocellular carcinoma: A matched case-control study. Chin Med J 2024;137:596–603. doi: 10.1097/CM9.0000000000002761