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Urinary isothiocyanates level and liver cancer risk: a nested case-control study in Shanghai, China

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Abstract

Experimental studies have provided evidence that isothiocyanates (ITCs) from cruciferous vegetables may modulate carcinogen metabolism and facilitate carcinogen detoxification and reduce cancer risk. However, no epidemiological studies on liver cancer were reported. This study investigates the association between urinary ITCs levels and liver cancer risk among men and women in Shanghai, China. A nested case-control study of 217 incident cases of liver cancer and 427 matched controls identified from the Shanghai Women's Health Study and Shanghai Men's Health Study was conducted. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) summarizing the association between urinary ITCs levels and liver cancer risk. Compared to those with undetectable ITCs, non-significantly inverse association was observed among detectable (OR = 0.80; 95% CI = 0.51–1.26), below-median (OR = 0.76; 95% CI = 0.47–1.24), and above-median concentration (OR = 0.86; 95% CI = 0.52–1.41) with liver cancer risk. Similar patterns were observed when urinary ITCs levels were categorized into tertiles or quartiles. Although our study firstly focused on the association between urinary ITCs exposure and liver cancer risk, we did not find significant results. Future multicenter prospective, different population studies are warranted to validate our findings.

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Keywords

isothiocyanates; liver neoplasms; urinary biomarkers; nested case-control study

Introduction

Liver cancer in men is the fifth most frequently diagnosed cancer worldwide but the second most frequent cause of cancer death. In women, it is the ninth most commonly diagnosed cancer and the sixth leading cause of cancer death (1). It was estimated that 782,451 new cases and 745,517 deaths of liver cancer occurred worldwide in 2012. Among these, almost 50.5% of cases and 51.4% of deaths occurred in China (2). Hepatocellular carcinoma (HCC), the major histological subtype, account for 70% to 85% of the total liver cancer burden worldwide (1).

The prevalence of hepatitis B virus (HBV) infection is higher in Asia population, which has been attributed to one important risk factor of HCC (3, 4). Besides HBV infection, alcohol drinking and consumption of foods contaminated with aflatoxin have been consistently associated with an increased risk of HCC (3, 4). Although the World Cancer Research Fund and the American Institute of Cancer Research have both considered vegetable intake as a limited-no conclusion factor for HCC (4), several observational studies still suggested an inverse association between aforementioned variable and risk of HCC (5-8). Cruciferous vegetables (CV) have been of specific interest because of their content in a variety of anticancer constituents such as glucosinolates, the precursors of isothiocyanates (ITCs) as well as indole-3-carbinol (I3C), both of which may contribute to against development of cancer (9, 10). Previous epidemiologic studies have indicated that ITCs have antioxidative properties and chemopreventive effects on the development of cancers of lung, gastric, breast, and colorectum (11-17). Experimental studies have provided evidence that ITCs modulate carcinogen metabolism and facilitate carcinogen detoxification via altering Phase I and Phase II enzyme systems, thus inhibiting carcinogenesis (18-20). In long-term feeding experiments, ITCs significantly reduced the formation of liver cancer by 3′-methyl-4-dimethylaminoazobenzene, ethionine, and *N*-2-fluorenylacetamide in male Wistar rats in a dose-dependent manner (20). In addition, sulforaphane (a form of ITCs) may decrease the secretion of inflammatory signaling molecules by white blood cells and to decrease DNA binding of nuclear factor-kappa B (NF-κB), a proinflammatory transcription factor, which plays an important role in the development of liver cancer (9, 21-23). Although these studies of mechanisms demonstrated the potential association between ITCs exposure and liver cancer risk. However, to our knowledge, no epidemiologic studies have evaluated the aforementioned relationship.

Herein, we assessed the associations between urinary ITCs levels and liver cancer risk in a nested case-control study within two large, population-based, prospective cohorts of women and men from China.

Materials and methods

Study Participants

The details of the study design and methods of Shanghai Men's Health Study (SMHS) and Shanghai Women's Health Study (SWHS) have been published elsewhere (24, 25). The SMHS and SWHS are two ongoing population-based prospective cohorts with a primary focus on the relationships of dietary intake with cancer and other chronic diseases. These two studies were approved by the relevant Institutional Review Boards for human research in Shanghai Cancer Institute, Vanderbilt University, and National Cancer Institute and written informed consent was obtained from all study participants. Briefly, from 1997 to 2000, the SWHS recruited 74,941 women aged 40–70 years, residing in 7 urban communities of Shanghai, with a response rate of 92.7%. From 2002 to 2006, the SMHS recruited 61,491 men who were aged 40–74 years, were free of cancer, and lived in 8 selected urban communities in Shanghai, with a response rate of 74.1%.

At baseline, in-person interviews were conducted to obtain information on demographics, lifestyle and dietary habits, medical history, and other characteristics through structured questionnaires (24, 26). Anthropometric measurements, including current weight, height, and circumferences of the waist and hips were also taken. Of all participants, 65,754 (87.7%) of the SWHS and 55,802 (90.7%) of the SMHS provided a spot urine sample. Urine samples were collected into a sterilized cup containing 125 mg ascorbic acid to prevent oxidation of labile metabolites, and 56,831 (75.8%) and 46,332 (75.3%) participants of the SWHS and SMHS provided a 10-ml blood sample which was drawn into an ethylenediaminetetraacetic acid vacutainer tube. After collection, the samples were kept in a portable styrofoam box with ice packs (at approximately 0–4 °C) and processed within 6 h for long-term storage at −70 °C. From mouth-rinse samples, cell pellets were stored for future studies at −70 °C. At the time of sample procurement, a biospecimen collection form was completed for each donated participant.

Follow-up and Outcome Ascertainment

These two cohort members have been followed up for occurrence of cancer and other chronic diseases by a combination of active surveys conducted every 3 yr and annual record linkage with database of the Shanghai Cancer Registry and Shanghai Vital Statistics. All possible matches identified via record linkages were verified by home visits and reviewing medical charts from their diagnostic or/and treatment hospitals. The SWHS underwent 4 times in-person follow-up survey between 2000 and 2011. Follow-up rates for the first (2000–2002), second (2002–2004), third (2004–2007), and fourth (2008–2011) surveys were 99.8%, 98.7%, 96.7%, and 92.0%, respectively. For the SMHS, the response rates for the first (2004–2008) and second (2008–2011) follow-up surveys were 97.6% and 93.6%, respectively.

Nested Case-Control Design

The nested case-control study described in this report included 217 incident liver cancer cases (131 from the SMHS, 86 from the SWHS) who donated a urine and blood sample at baseline and in whom cancer was diagnosed before December 31, 2008. Liver cancer cases

were defined as having an *International Classification of Diseases*, 9th rev. and code 155 for cases.

For each case, 2 controls were randomly selected from the cohorts who donated a urine and blood sample at baseline. Two controls were matched to the index case by gender, age at baseline (± 2 years), and date (± 30 days) of biospecimen collection. Three controls (2 from SMHS, 1 from SWHS) were the same controls of different liver cancer cases. Seven controls from SWHS were excluded because they were other cancer cases at the time of cancer diagnosis for the index liver cancer case. The final analytic data set included 217 liver cancer cases and 427 matched controls (262 from the SMHS, 165 from the SWHS).

Urinary Isothiocyanates Assay

The urinary level of ITCs was analyzed according an established protocol previously reported (27, 28). Urinary creatinine was measured by the Jaffé alkaline picrate procedure (29). Urinary total ITCs levels are expressed as μmol/g creatinine. Urine samples and standards were assayed in triplicate. The average of 3 measurements for each participant was used in the analysis. Three representative standards and a reagent blank were included in all analytic runs. The laboratory coefficient of variation for quality control of ITCs was 4.6%. To control for batch-to-batch variability, samples for each case-control set were analyzed in the same laboratory run. Laboratory staff was blinded to the case-control status of the urine samples and the identity of the quality control samples. All lab assays were performed in 2010 and 2011.

Statistical Analyses

Urinary ITCs levels were standardized to urinary creatinine levels (μmol/g creatinine), which were categorized as 2 (nondetected, detected), 3 (nondetected, below median, median or above), 3 (tertiles of control group distribution), and 4 (quartiles of control group distribution) categories. Median and percentages of selected baseline characteristics for cases and controls were calculated. Pairwise comparisons for urinary ITCs concentrations and other continuous variables were conducted using Wilcoxon's signed-rank test. Conditional logistic regression was used to estimate the odds ratios (ORs) of developing liver cancer and their 95% confidence intervals (CIs) associated with urinary ITCs concentration and to adjust for potential confounders. In multivariable models, potential confounders were adjusted for education level (4 categories: elementary school or less, middle school, high school, and college or above); family history of liver cancer (yes or no); chronic liver disease or cirrhosis (yes or no); cholelithiasis or cholecystectomy (yes or no). Body mass index, family income level, physical activity, smoking, and alcohol consumptions were not associated with liver cancer risk in our study participants; therefore, we did not adjust for them in the final model.

Tests for linear trend were performed by assigning an ordinal value (1, 2, and 3) to each tertile (T1, T2, and T3) or an ordinal value (1, 2, 3, and 4) to each quartile (Q1, Q2, Q3, and Q4) of exposure and treating it as a continuous variable in the model. All statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Inc, Cary, NC). All *P*

values were calculated by 2-sided tests and were considered statistically significant if *P* was less than 0.05.

Results

The distribution of baseline characteristics in this study participant is presented in Table 1. Compared with controls, cases appeared to have less education and more likely to have a history of chronic liver disease or cirrhosis and family history of liver cancer than controls. No difference was seen for body mass index, household income, most lifestyle characteristics, and history of diabetes. Median urinary ITC did not significantly differ between cases and controls (Table 1).

Table 2 presents the associations between urinary ITCs and liver cancer risk. A detectable amount of ITC in the urine was associated with a non-significant decrease in liver cancer risk in all the participants after adjustment for the potential confounders. Compared with those with undetectable ITCs, individuals with a detectable but below-median concentration of ITCs or above-median concentration yielded similar non-significant results. When dividing the ITCs levels into tertile or quartile according to distribution of control group, compared to the lowest category, results of the highest category of tertile or quartile did not show statistical significance, with all 95% confidence intervals (CIs) including the null value of 1.0.

In the stratified analyses by sex, we did not observe significant protective effect of urinary ITC levels among men and women and the test for multiplicative interaction did not show statistical significance, which indicated that sex does not influence the association between urinary ITCs and liver cancer (data not shown). Furthermore, results from analyses of all participants that excluded cases diagnosed in the first year of follow-up were similar to the results presented in Table 2 (data not shown).

Discussion

In this nested case-control study including men and women, generally, as the first urine-based biomarker study of ITCs exposure and liver cancer, the association was not significant either in the main analyses or in the analyses stratified by gender in this population after adjusting for potential confounders. Moreover, we did not observe the dose-response relationships for any of the associations.

It has been hypothesized that glucosinolates as the most frequently attributable anticancer constituent of cruciferous vegetables, the precursors of ITCs and I3C, may contribute to reduce risk of cancer. Although the inverse association between dietary CV and ITCs intake and cancer risk has already been reported by previous epidemiologic studies (13-15, 30-32), our study first explored the relationship between urinary ITCs levels and liver cancer risk. Recently, evidence from animal studies has indicated that the joint induction of Phase I (i.e., cytochrome P450s) and Phase II enzymes [e.g., glutathione S-transferases (GST)] by a variety of CV results in a favorable metabolic profile for the elimination of certain chemical carcinogens (18, 19). Animal experiments have shown that ITCs, when administered in vivo, are inhibitors of isozymes that metabolize carcinogens such as 4-(methylnitrosamino)-

l-(3-pyridyl)-l-butanone in liver of rats and mice (33). Furthermore, phenethyl isothiocyanate had inhibitory activity against liver cancer induction by N-nitrosodiethylamine in the mice (19). Studies developed in vitro have also demonstrated that ITCs induce apoptosis, which could be linked to their chemopreventive activity in the postinitiation phase (10). Sulforaphane, one of the most extensively studied ITCs, may have a potential effect on decreasing the secretion of inflammatory signaling molecules by white blood cells and DNA binding of nuclear factor-kappa B (NF-κB), which is one of the early key events involved in neoplastic progression of the liver (21-23). Furthermore, several studies have suggested that IκB kinase complex inhibition and/or signal transducer and activator of transcription 3 downregulation may attribute to ITCs-induced apoptosis (10, 34). Although ITCs are metabolized and disposed in a time-dependent fashion, total urinary ITCs levels was still considered as the best biomarker of human exposure to dietary ITCs recently (35). As the first epidemiologic study that demonstrated a link between urinary ITCs levels and cancer risk, London et al. (16) reported in a nested case-control study within a prospective Chinese cohort that having detectable levels of urinary ITCs at baseline was inversely related with subsequent risk of lung cancer in men. Lately, Spitz et al. (36), Moy et al. (12), Yang et al. (11), Fowke et al. (17), and Epplein et al. (37) also have observed the similar protective effect of ITCs among lung, gastric, breast and colorectal cancers. However, Fowke et al. (38) did not find evidence that urinary ITCs levels were significantly associated with lower lung cancer risk among nonsmoking women, regardless of exposure to environmental tobacco smoke or menopausal status.

There are several strengths of this study. Our study included incident liver cancer cases and matched controls from the ongoing prospective cohorts of SWHS and SMHS, which ruled out the possibility of recall bias and minimized selection bias. All blood and urine samples of including participants were collected prior to the diagnosis of liver cancer, which minimized the concern over a possible impact of clinical manifestation of cancer on the metabolism of ITCs, resulting in altered levels of ITCs in urine, among cases with a short follow-up duration (12). Furthermore, the almost complete follow-up for incident cancer and death minimized the potential bias on results due to the loss to follow-up. Because the potential seasonal and storage effects on concentrations of urinary ITCs, we also matched cases and controls on the date of specimen collection to minimize the difference of the stability of the ITCs between cases and controls. In addition, using the complete information that was collected before the cancer diagnosis, we were better able to adjust for the potential factors that might confound the ITCs-liver cancer association.

This study also has several potential limitations. First, because ITCs are metabolized and disposed in a time-dependent fashion, continuous urine collection over a certain time period after dietary ITC intake, e.g., 8–24 h, may be necessary to detect the majority of urinary equivalent (35). For that reason, it cannot be assumed that ITCs levels in a randomly timed, single void urine sample correlate with usual intake of dietary ITCs for the individuals. However, Seow et al. (39) have demonstrated among Chinese in Singapore, a population that shares a similar cultural and dietary heritage as our study population in Shanghai, a close and statistically significant correlation between dietary ITCs ascertained from a validated food frequency questionnaire and total ITCs levels in randomly timed spot urine. Moreover, London et al. (16) have established an inverse association between dietary intake

of ITC and lung cancer in men using this same biomarker approach based on single-spot urine. Fowke et al. have found that habitual CV intake estimated from FFQ significantly increased with urinary ITC levels (17, 40), reflecting a traditional diet with strong links to regional agriculture. In addition, limited by the short follow-up period of SMHS, we could not well investigate whether the sample storage time might modify the association between urinary ITCs levels and liver cancer risk. Extended follow-up of this cohort would allow us to evaluate this issue further in the future.

Secondly, although we have adjusted the history of chronic liver disease or cirrhosis in the multivariable analyses, we cannot completely rule out confounding from unmeasured confounders of HBV infection, HCV infection, and aflatoxin exposure, whereas the latter two exposures are very low in Shanghai (41). Thirdly, it has been hypothesized that individuals that are homozygous for deletion of either the GSTM1 or GSTT1 gene may metabolize and eliminate ITC at a slower rate and therefore may be more intensely exposed to ITCs after consumption of CV (42). However, we did not evaluate the potential modifying effect of GST genotypes on the association. The analysis is warranted to explore this modifying effect in future studies. Last but not least, considering about the limited numbers of liver cancer cases were included in the study, thus restricted statistical power should be considered in the analyses.

In summary, this nested case-control study within two large, population-based, prospective cohort studies, first using urinary biomarker of dietary ITCs to analyze the relationship between urinary ITCs levels and risk of liver cancer but did not provide enough evidence to support the results of experiment studies. Furthermore, though most of the findings showed inverse association in the stratified analyses none of them were statistically significant. Future multicenter prospective investigations should focus on the relationship between CV consumption and liver cancer risk in multicenter or different population studies.

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Table 1

Baseline characteristics of liver cancer cases and controls (the Shanghai Men's Health Study and the Shanghai Women's Health Study)

Characteristics	All subjects		
	Cases (n=217)	Controls (n=427)	*P*
Age at interview (y) †	61 (51, 67)	61 (50, 67)	0.83
Body mass index (kg/m^2)	23.6 (21.3, 26.0)	24.1 (21.9, 25.9)	0.17
Education level, No. (%)			0.03
Elementary school or less	63 (29.3)	115 (27.0)	
Middle school	69 (32.1)	148 (34.7)	
High school	62 (28.8)	91 (21.4)	
College or above	21 (9.8)	72 (16.9)	
Household income, No. (%)			0.29
Low	126 (58.1)	220 (51.6)	
Middle	66 (30.4)	146 (34.3)	
High	25 (11.5)	60 (14.1)	
Ever smoked, No. (%)	93 (42.9)	173 (40.5)	0.57
Ever drank alcohol, No. (%)	45 (20.7)	98 (23.0)	0.52
Physical activity (MET-hours/week) ‡	72.1 (51.0, 105.6)	78 (52.7, 107.4)	0.41
Family history of liver cancer, No. (%)	28 (12.9)	18 (4.2)	<0.001
History of diabetes, No. (%)	25 (11.5)	35 (8.2)	0.17
History of chronic liver disease or cirrhosis, No. (%)	69 (31.8)	18 (4.2)	<0.001
Urinary ITCs (μmol/g creatinine)	1.49 (0.49, 3.49)	1.44 (0.46, 3.95)	0.83

† Median; interquartile range (between the 25th and the 75th percentiles) in parentheses (all such values).

‡ Physical activity level was measured by metabolic equivalent (MET)-hours per week per year.

Table 2

Association of urinary isothiocyanates (ITC) levels with liver cancer risk in multivariable analyses

Urinary ITC (μmol/g Creatinine)	Range	Cases	Control	Multivariate OR[‡] (95% CI)
Non-detected [†]		35	64	1.00 (Ref)
Detected		182	363	0.80 (0.51, 1.26)
Non-detected [†]		35	64	1.00 (Ref)
Below median	< 1.93	86	181	0.76 (0.47, 1.24)
Median or above	≥ 1.93	96	182	0.86 (0.52, 1.41)
T1	< 0.72	74	142	1.00 (Ref)
T2	0.72-2.80	75	142	0.98 (0.65, 1.48)
T3	≥ 2.80	68	143	0.86 (0.55, 1.33)
P for trend				0.4895
Q1	< 0.46	54	106	1.00 (Ref)
Q2	0.46-1.44	53	107	0.83 (0.52, 1.33)
Q3	1.44-3.95	61	107	0.95 (0.58, 1.54)
Q4	≥ 3.95	49	107	0.87 (0.53, 1.43)
P for trend				0.6994

[†]Undetectable ITC value was less than 0.1 μmol/g.

[‡]Odds ratios (ORs) were estimated by using multivariable conditional logistic regression models, adjusted for education level, family history of liver cancer, history of chronic liver disease or cirrhosis, history of cholelithiasis or cholecystectomy, and intake of total energy and non-cruciferous vegetables.