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Original Article

Polycyclic aromatic hydrocarbons and risk of gastric cancer in the Shanghai Women's Health Study

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Abstract: Purpose: Polycyclic aromatic hydrocarbons (PAHs) are byproducts of incomplete combustion of organic materials. Sources include tobacco smoke, charbroiled meat, and air pollution. Indirect evidence suggests that PAHs may be associated with carcinogenesis, but the association with gastric cancer is unclear. Methods: Using a nested case-control study design, we examined prediagnostic urinary concentrations of 1-hydroxypyrene glucuronide (1-OHPG), a PAH metabolite, in 153 gastric cancer cases and 306 matched controls within the Shanghai Women's Health Study. Conditional logistic regression adjusted for potential risk factors was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs). Results: Urinary 1-OHPG concentrations were slightly higher among cases than controls, with medians of 0.29 μmol/mol Cr (interquartile range, 0.16-0.48) and 0.24 μmol/mol Cr (interquartile range, 0.12-0.45), respectively. Increasing concentrations of 1-OHPG appeared to be associated with elevated risk of gastric cancer, but not within the highest category of 1-OHPG (Q4 vs Q1: OR = 1.4; 95% CI = 0.8-2.5). Conclusions: Our findings suggest that higher concentrations of 1-OHPG are related to gastric cancer risk, but no clear dose-response relationship was observed.

Keywords: 1-hydroxypyrene glucuronide, polycyclic aromatic hydrocarbons, gastric cancer, China

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are large aromatic planar compounds that are formed by incomplete combustion of organic material [1, 2]. PAHs are ubiquitous, and major sources of exposure include tobacco smoke [3], charbroiled meat [4], ambient air pollution [5], and occupational exposure [6]. Several of the more than 100 identified PAHs, including benzo(a)pyrene (BaP), are considered carcinogenic to animals [7]. In humans, there is also evidence supporting a causal association between PAHs or PAH-containing substances and cancers of the skin, scrotum, lung, and bladder [6, 8]. However, the association between PAHs and gastric cancer is less clear.

Gastric cancer is the fourth most common type of incident cancer and the second leading cause of cancer death in the world [9]. There are some suggestions for a role of PAH in gastric carcinogenesis. Animal studies have shown that a diet contaminated with coal or BaP strongly increases the risk of forestomach cancers [7]. Indirect evidence in humans also suggests that gastric cancer may be associated with higher exposure to PAHs. For example, in a meta-analysis of gastric cancer and tobacco smoking, a major source of PAHs, a higher risk of gastric cancer was reported [10]. Exposure to diesel exhaust has also been associated with gastric cancer risk [11]. Based on the few studies that have evaluated PAHs and gastric cancer indirectly, the association remains unclear. However, to our knowledge, the association between a biomarker of PAH exposure and gastric cancer has not yet been examined in a prospective study.

Urinary 1-hydroxypyrene glucuronide (1-OHPG), a metabolite of pyrene, has been established

as a biomarker of PAH exposure [12, 13]. To test whether urinary 1-OHPG is associated with risk of subsequent gastric cancer, we conducted a nested case-control study in a large prospective cohort study in Shanghai, China.

Materials and methods

Study population

Cases and controls were selected from participants of the Shanghai Women's Health Study (SWHS), a population-based prospective cohort study of women residing in Shanghai, China. The design of the study has been described elsewhere [14]. Women aged 40-70 years who were permanent residents of Shanghai were invited to participate in the study. A total of 74,942 women agreed to participate and completed the baseline survey between 1996 and 2000. Data collected included demographics, personal habits, dietary habits, occupational history, family history of cancer, disease and surgery history, reproductive history, and weight history. *H. pylori* infection was determined using *H. pylori* enzyme-linked immunosorbent assay kits (Biohit ELISA kit, Finland) to detect serum IgG antibodies. All study participants provided written informed consent prior to interview and the study protocols were approved by the institutional review boards of the National Cancer Institute, Vanderbilt University, and the Shanghai Cancer Institute in accordance with the Declaration of Helsinki.

Of the eligible participants, 65,574 (87%) provided spot urine samples, which was collected into a sterilized 100-mL cup containing 125 mg ascorbic acid. Samples were kept in a portable insulated bag with ice packs (0-4°C) and processed within 6 h for long-term storage at −70°C. Among the participants who provided urine samples, 153 incident gastric cancer cases were diagnosed through December 2005. Incident gastric cancer cases were identified through in-person follow-up interviews and by linking to the Shanghai Cancer Registry and the Shanghai Vital Statistics Unit. Two controls were selected for each case matched by age at sample collection (± 2 years), menopausal status, time of sample collection (morning or afternoon), date of sample collection (± 1 month), and time interval since last meal (± 2 hours). Controls were also free of any cancer at the time of cancer diagnosis for their corresponding case.

Urinary 1-hydroxypyrene glucuronide assay

Urinary concentration of 1-OHPG is a PAH derivative that may be a reasonable reflection of both usual and recent PAH exposure [13]. 1-OHPG was measured using immunoaffinity chromatography and synchronous fluorescence spectroscopy [15]. Assays were performed in batches of 20 that included laboratory QC samples and blinded duplicate samples. The coefficient of variation (CV) for replicate measurements of urinary 1-OHPG concentration across batches using aliquots from a single quality control pool was 10.6%. The Spearman rank correlation coefficient for paired 1-OHPG measurements in 30 blinded duplicate samples was 0.93. The assay limit of detection was 0.1 pmol/mL. For measurements below the limit of detection (9.1% and 10.1% of cases and controls, respectively), a value of 0.05 pmol/mL was assigned.

Statistical analysis

Conditional logistic regression, adjusted for matching and potential risk factors for gastric cancer: age, body mass index (BMI: kg/m^2), education level, fruit and vegetable intake, *H. pylori* status and smoking status (ever/never), was used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between creatinine-adjusted urinary 1-OHPG concentration (henceforth referred as 1-OHPG) and gastric cancer. Sensitivity analyses using 1-OHPG concentrations without correction for creatinine and with creatinine concentration included as an independent variable in the statistical model were also conducted, but did not have any noticeable effects on the results. The distribution of 1-OHPG among controls was used to determine cut points for quartiles. We also evaluated 1-OHPG as a continuous variable. A one unit change was defined as half the distance between the 25th and 75th percentiles among controls. *P*-values for trend were calculated by modeling the median of each quartile. Sensitivity analyses included analyses excluding 32 matched sets of controls and cases diagnosed within the first two years of follow-up after sample collection, excluding 22 ever smokers, without adjustment for smoking and *H. pylori,* and stratified analyses by the median duration of follow-up time. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

Table 1. Distribution of selected characteristics among gastric cancer cases and control in the Shanghai Women's Health Study

Characteristics	Cases (n = 153)	Controls (n = 306)
Age in years, mean (SD)	58.7 (8.7)	58.7 (8.6)
Fresh Vegetable Intake (g/day)	270.0 (162.2)	296.5 (186.9)
Fresh Fruit intake (g/day)	223.1 (161.0)	252.9 (172.5)
Education, n (%)		
College	9 (5.9)	30 (9.8)
High school	28 (18.3)	64 (20.9)
Middle school	39 (25.5)	83 (27.1)
Elementary or less	77 (50.3)	129 (42.2)
BMI (kg/m^2), n (%)		
< 25	84 (54.9)	182 (59.5)
25- < 30	60 (39.2)	101 (33.0)
≥ 30	9 (5.9)	23 (7.5)
Ever smokers, n (%)	9 (5.9)	13 (4.3)
Ever exposed to passive smoking, n (%)	75 (64.7)	204 (74.2)
Ever drank alcohol, n (%)	3 (1.9)	4 (1.3)
H. pylori positive, n (%)[a]	136 (96.5)	260 (92.2)

Note: Continuous variables are displayed as means (standard deviation) and frequencies are displayed as counts (percentages). [a]Available on only 423 subjects.

Results

The characteristics of the 153 gastric cancer cases (11 cardia and 142 non-cardia) and 306 control subjects in this study are provided in **Table 1**. Cases and controls were similar in age, fruit and vegetable intake, education level, and BMI. Few of the women smoked (4% and 6% among controls and cases, respectively) or drank alcohol (1% and 2% among controls and cases). A large proportion of both cases and controls tested positive for *H. pylori* IgG antibodies. Urinary 1-OHPG concentration were slightly higher but not statistically different between gastric cancer cases and controls, with medians of 0.29 and 0.24 μmol/mol creatinine, respectively (Wilcoxon *p*-value = 0.13).

Overall, urinary 1-OHPG concentration was not clearly associated with risk of gastric cancer (**Table 2**). Increasing concentrations of 1-OHPG appeared to be associated with elevated risk of gastric cancer, but not within the highest category of 1-OHPG. Compared to the lowest quartile of 1-OHPG, those in the third quartile of 1-OHPG were associated with an OR of 1.93 (95% CI: 1.07-3.49) but those in the highest quartile of 1-OHPG were associated with an OR of 1.40 (95% CI: 0.77-2.54; p-trend = 0.30). A continuous association (where values were standardized so that a change of one unit corresponds to a change of 25% of the control distribution) was also suggestive but not statistically significant (OR = 1.06, 95% CI: 0.97-1.16). Additional adjustment for *H. pylori* status (one of the main risk factors for gastric cancer), which was only available on a subset of the samples (n = 282 subjects), did not change the estimates. Also, effect estimates did not change substantially when adjustment for smoking status was removed (Q4 vs Q1 OR = 1.35, 95% CI: 0.73-2.52), exclusion of ever smokers (n = 22; Q4 vs Q1 OR = 1.25, 95% CI: 0.67-2.34), or after exclusion of cases diagnosed within two years of sample collection (n = 32; Q4 vs Q1 OR = 1.35, 95% CI: 0.67-2.75). We also did not observe a difference in risk when stratified by the median duration of follow-up time (3.75 years; data not shown).

Discussion

In this nested case-control study within the SWHS cohort, we found that higher concentrations of 1-OHPG may be associated with gastric cancer risk, but no clear dose-response relationship was observed. Although there have been some studies that have suggested a role of PAHs in gastric cancer carcinogenesis, using a well-established biomarker of PAH exposure, we were unable to detect a clear trend with increasing urinary 1-OHPG concentrations.

1-OHPG has been associated with recent consumption of charbroiled food [16] and recent occupational exposure to PAHs [15]. However, Cross et al. recently estimated BAP intake from charbroiled meats through a dietary questionnaire and did not observe an association with gastric cancer in a large prospective study [17]. Friesen et al. also found no evidence that oil-based metal working fluids (PAH component) were associated with risk of stomach cancer in an auto worker cohort [18]. In comparison, our

Table 2. Association between creatinine-adjusted urinary 1-OHPG concentration and risk of gastric cancer

1-OHPG (μmol/mol Cr)	Cases (N)	Controls (N)	Unadjusted	Adjusted[a]	Adjusted[b]
			OR (95% CI)	OR (95% CI)	OR (95% CI)
Q1: < 0.12	28	76	1.00	1.00	1.00
Q2: ≥ 0.12 to < 0.24	32	78	1.10 (0.61-1.99)	1.15 (0.63-2.09)	1.13 (0.59-2.14)
Q3: ≥ 0.24 to < 0.45	50	76	1.90 (1.06-3.41)	1.93 (1.07-3.49)	1.91 (1.02-3.60)
Q4: ≥ 0.45	43	76	1.56 (0.87-2.80)	1.40 (0.77-2.54)	1.34 (0.72-2.50)
P trend			0.13	0.30	0.40
Per 1-unit increase in 1-OHPG[c]			1.07 (0.98-1.17)	1.06 (0.97-1.16)	1.05 (0.96-1.15)

[a]Adjusted for age, education, smoking status (ever/never), fruit intake, vegetable intake, and body mass index (BMI). [b]Same adjustments as above but additionally adjusted for H. pylori status within 423 subjects (141 cases and 282 controls). [c]A 1-unit increase was defined as half of the difference between the 25th and 75th percentiles for creatinine-adjusted 1-OHPG measurements among controls.

study suggests a possible association with gastric cancer by using a direct biomarker of PAH exposure. In a secondary analysis evaluating the relations between urinary 1-OHPG concentrations and potential determinants of PAH exposure among a combined sample of controls from this study and a related study of colorectal cancer, active and passive smoking, coal as a cooking fuel, eating cooked well done foods, and recent consumption of fried dough (e.g., yóutiáo) were associated with elevated concentrations of 1-OHPG [19]. Only active smoking and fried dough consumption achieved statistical significance in multivariate analyses. Although PAHs are a complex mixture, urinary 1-OHPG concentration appears to be a useful in assessing human PAH exposure.

To the best of our knowledge, this is the first study to evaluate urinary 1-OHPG concentrations and risk of gastric cancer. This study has several strengths which include its prospective design, collection of prediagnostic urine samples from study subjects when they were healthy, long-term follow-up, collection of data on potential confounders, and rigorous methods to ascertain outcomes. There are also several limitations in this study. Our results should be interpreted as preliminary given our relatively small sample size, which also limited our ability to evaluate potential interactions with other gastric cancer risk factors. Urine samples were collected as spot samples and not 24 hour urines; in addition, urine samples were only collected once at baseline and may not reflect long-term exposure or an etiologically relevant time period.

In summary, the results of this study do not support a clear association between 1-OHPG and risk of gastric cancer. As our study was limited by a small sample size, this association should be further explored in a larger study to confirm findings.

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Disclosure of conflict of interest

No potential conflicts of interest were disclosed.

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