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Urinary Prostaglandin E_2 metabolite and Gastric Cancer Risk in the Shanghai Women's Health Study

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Abstract

Chronic inflammation has been implicated in the etiology of gastric cancer. Prostaglandin-E_2(PGE_2) is one of the major end products of the COX-2 pathway, an enzyme that is an important mediator of inflammation. Using a novel method of quantifying the primary urinary metabolite of PGE_2(PGE-M, 11 alpha-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid), we evaluated urinary PGE-M concentrations in association with subsequent risk of development of gastric cancer in the Shanghai Women's Health Study, a large population-based prospective cohort, using a nested case-control study design. Controls were matched(1:1) to 153 gastric cancer cases by menopausal status; age, time and date of sample collection; time interval since last meal and availability of urine sample. Odds ratios(OR) and 95% confidence intervals(95%CI) were calculated using conditional logistic regression adjusted for potential confounders. Baseline urinary PGE-M levels were slightly higher among gastric cancer cases with a median of 6.4 ng/mg creatinine (interquartile range: 3.4–11.2) compared to 5.4 among controls (interquartile range: 2.8–9.0) but this difference was not statistically significant (Wilcoxon p-value=0.34). With increasing quartiles of urinary PGE-M levels, the ORs for risk of gastric cancer increased in quartiles 2, 3, and 4: 1.00 (95% CI:0.48–2.08), 1.40 (95%CI:0.67–2.91) and 1.98 (95%CI:0.95–4.13), with a statistically significant test for trend (p=0.04). The association persisted after additional adjustment for *H. pylori* status, and was slightly strengthened among non-NSAID users, subjects with positive *H. pylori* status, and for cases diagnosed within 46 months after study enrollment. Our findings suggest that higher levels of urinary PGE-M, a marker of inflammation, may be associated with gastric cancer risk.

Keywords

gastric cancer; prostaglandins; inflammation; urinary marker

Introduction

Regular use of non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with a reduced risk of cancer at several sites, predominantly gastrointestinal cancers (1,2). A growing number of epidemiologic studies conducted thus far support an inverse association between

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NSAID use and risk of gastric cancer (3–7), including three recent reviews/meta-analyses (8–10). The primary mechanism through which NSAIDs may exert a chemopreventive effect is through the inhibition of cyclooxygenase-2 (COX-2) production, an enzyme that is an important mediator of inflammation through the synthesis of prostaglandins from arachidonic acid (11,12). This mechanism may be particularly relevant for gastric cancer since *H. pylori* infection and subsequent persistent inflammation and oxidative stress are well established risk factors in the initiation and progression of gastric cancer (13). COX-2 is not highly expressed in the gastric mucosa, but an increased expression of COX-2, further induced by growth factors and cytokines, has been detected in gastric tumors (14,15).

Prostaglandin-E_2 (PGE) is an end product of the COX-2 pathway. This metabolite has been reported to induce cell proliferation and motility, inhibit apoptosis, and have inflammatory effects (16–18). A novel method of quantifying the primary urinary metabolite of PGE_2 (PGE-M, 11 alpha-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid) was recently developed (19). The measurement of urinary metabolites is considered to be a more complete capture of prostaglandin production as it will reflect a combination of prostaglandins from both the blood stream, as well as the kidney (19,20). To test whether urinary PGE-M was associated with risk of gastric cancer, we conducted a nested case-control study in a large prospective cohort study in Shanghai, China.

Materials and Methods

Study population

The Shanghai Women's Health Study is a population-based prospective cohort study of women residing in Shanghai, China. A detailed description of the study methodology has been published elsewhere (21). In brief, from 1996 to 2000, 74,942 women aged 40–70 years residing in Shanghai were recruited into the study. At baseline, detailed in-person interviews were conducted by trained interviewers to collect questionnaire information, yielding a response rate of 93%. Data was collected on demographic characteristics, personal habits, dietary habits, water drinking, physical activity, residential history, occupational history, family history of cancer, disease and surgery history, menstrual history, reproductive history and hormone use, and weight history. Body measurements were also taken. Among cohort members, 56,831 (76%) women provided a blood sample and 65,754 (88%) women provided a spot urine sample, which was collected into a sterilized 100-ml cup containing 125 mg of ascorbic acid. Samples were kept in a portable insulated bag with ice packs (0–4°C) and processed within 6 hours for long-term storage at −70°C. Each woman also filled out a biospecimen collection form at the time of sample collection, which included information on the date and time of sample collection, time of last meal and use of any medications over the previous 24 hours and week. *H. pylori* infection was determined using *H. pylori* enzyme-linked immunosorbent assay kits (Biohit ELISA kit, Finland) to detect serum IgG antibodies. In follow-up surveys, interviewers were able to interview and follow-up with 99.8% (2000–2002), 98.7% (2002–2004), and 96.7% (2004–2007) of cohort members or their next of kin.

Included in the nested case-control study are 153 incident gastric cancer cases and individually matched controls that provided a urine sample at the baseline survey. 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. Controls were randomly selected from cohort members and matched to cases by age at sample collection (±2 years), menopausal status, time of sample collection (morning or afternoon), date of sample collection (±1 month), time interval since last meal (±2 hours), and availability of urine and plasma sample. Controls were also free of any cancer at the time of cancer diagnosis for their corresponding case. No subjects were allowed to be sampled multiple times.

Urinary PGE-M measurement

Measurement of urinary PGE-M (11 alpha-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid) using a liquid chromatography/tandem mass spectrometric method has been described in detail elsewhere (19,22). In brief, 0.75 ml of urine was pH adjusted with HCl and endogenous PGE-M was converted to the *O*-methyloxime derivative. After incubating for an hour, methoximated PGE-M was extracted, applied to a C-18 Sep-Pak, and eluted with ethyl acetate. Using an internal standard of [$^2H_6$]*O*-methyloxime PGE-M (6.2 ng in 10μg ethanol), samples were analyzed by liquid chromatography on a Zorbax Eclipse XDB-C18 column attached to a ThermoFinnigan Surveyor MS Pump (Thermo Finnigan, San Jose, CA). For endogenous PGE-M, the predominant product ion *m/z* 336 representing [M-(OCH_3+H_2O)]$^-$ and the analogous ion, *m/z* 339 [M-OC ($^2H_3$+H_2O)]$^-$, for the deuterated internal standard were monitored in the selected reaction monitoring mode. Quantification of endogenous PGE-M was based on the ratio of the mass chromatogram peak areas of the *m/z* 336 and *m/z* 339 ions. Urinary creatinine levels were measured using a kit (Sigma Company, St Louis, MO). Staff was blinded to case/control status and duplicate quality control samples interspersed among samples. The intraclass correlation among 15 duplicate QC samples was 89%. We successfully measured 144 of 153 case/control pairs.

Statistical analyses

Urinary PGE-M levels were standardized using the urinary creatinine levels of each sample and are expressed as ng/mg creatinine. Values were also log-transformed due to a skewed distribution, and differences between cases and controls were estimated using paired t-tests and Wilcoxon signed rank tests. The distribution of PGE-M levels among controls was used to determine cut points for quartiles. Conditional logistic regression, adjusted for body mass index (BMI: kg/m^2), education, fruit and vegetable intake, smoking status, recent NSAID use, and family history of gastric cancer, was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for the association between urinary PGE-M levels and gastric cancer. Tests for trend were calculated by modeling a variable coded 0, 1, 2 and 3. Sensitivity analyses including additional adjustments for *H. pylori*, and stratified analyses by recent NSAID use, *H. pylori* status, and the time between urine collection and cancer development were also conducted. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Baseline characteristics of 144 successfully measured pairs of matched gastric cancer cases and controls are shown in Table 1. There were no significant differences between cases and controls in age, BMI, education, and family history of gastric cancer. Few individuals smoked, drank alcohol, used NSAIDs or multivitamins in this cohort. Controls had a slightly higher intake of fruits and vegetables than cases with borderline significance. Most cases and controls (~95%) tested positive for *H. pylori* IgG antibodies. A median of 46.3 months passed between urine sample collection and gastric cancer diagnosis.

Baseline urinary PGE-M levels were slightly higher among gastric cancer cases (geometric mean =5.52 ng/mg creatinine; median =6.4 ng/mg creatinine) than controls (geometric mean =4.86 ng/mg creatinine; median =5.4 ng/mg creatinine) but this difference was not statistically significant (paired t-test p-value=0.30; Wilcoxon p-value=0.34).

When urinary PGE-M levels were considered as a categorical variable, the odds ratios for women in the highest versus the lowest quartile were associated with an approximate two-fold increase in risk of gastric cancer (OR: 1.98, 95% CI: 0.95–4.13; Table 2) and had a significant test for trend across quartiles (p = 0.04). Restricting the analysis to pairs that were positive for *H. pylori* (n=121 pairs, OR: 2.22, 95% CI: 0.97–5.09; p for trend=0.05) or those who did not

report recent NSAID use (n=119 pairs, OR: 2.57, 95% CI: 1.14–5.80; p for trend=0.02) slightly increased the risk estimates associated with the highest quartile of urinary PGE-M levels.

We further evaluated the association between urinary PGE-M and gastric cancer by stratifying on the median time (46.3 months) between urine collection and cancer development. Although both strata show increased risk with increasing PGE-M quartiles, the positive association appears to be more pronounced among those who were diagnosed with gastric cancer within 46 months (OR:2.75, 95% CI: 0.84–8.94) than those diagnosed more than 46 months (OR: 1.48, 95% CI: 0.52–4.20; Table 3). To further evaluate whether this association may be due to undiagnosed early gastric cancers, we excluded cases diagnosed within the first year (n=17 cases) and the first two years (n=30 cases) of follow-up after urine collection. The ORs associated with the highest quartile of urinary PGE-M levels were 1.46 (95% CI: 0.67–3.16; p for trend=0.20) and 1.74 (95% CI: 0.77–3.93; p for trend=0.16), respectively, demonstrating a continued increase in risk after excluding potential undiagnosed early gastric cancers within the first two years.

Discussion

In this prospective study, we found that higher concentrations of urinary PGE-M were associated with higher risk of gastric cancer. The positive association with urinary PGE-M levels persisted after additional adjustment for important gastric cancer risk factors and exclusion of one or two years of initial follow-up, which should exclude occult gastric cancers. Risk associated with the highest level of PGE-M appeared stronger for cases diagnosed within the first four years of follow-up in particular. Our findings suggest that higher levels of this urinary metabolite, a marker of inflammation, are associated with gastric cancer development.

From our evaluation of risk at different time intervals before cancer diagnosis, it is possible that PGE-M may serve as both a marker of underlying inflammation, as well as a marker that reflects an upregulated COX-2 pathway associated with impending gastric cancer development. The timing of when the sample was taken in relation to cancer development appears to be a factor that should be taken under consideration. However, as an increased risk was still apparent after excluding those who were diagnosed with gastric cancer within the first two years, so undiagnosed gastric cancers probably do not explain this association.

This is the first study to evaluate urinary PGE-M levels in relation to gastric cancer. Epidemiologic studies investigating the role of inflammation have demonstrated a consistently inverse association between NSAID use and gastric cancer (8–10). A number of studies exploring the underlying mechanism have proposed that this association occurs through the inhibition of enzymes in the COX-2 pathway (11,23). Expression of COX-2 appears to be highly elevated in gastric cancer tissues while COX-1 is not, which suggests that COX-2 is the more important enzyme in PGE_2 production in gastric cancer cells (15). Another study has described overexpression of microsomal prostaglandin E synthase-1 (mPGES-1), an inducible enzyme involved in the synthesis of PGE_2, in gastric carcinomas (24). 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a key enzyme in prostaglandin degradation, was also recently identified in gastric carcinomas (25). The expression of 15-PGDH appears to be downregulated by COX-2 in gastric cancer tissues, thus contributing to increasing levels of PGE_2 (25). The results from the current study with gastric cancer are consistent with results from our previous study evaluating urinary PGE-M in relation to risk of colorectal cancer, a tumor that also has a well-established inflammatory mechanism and inverse association with NSAID use (22). The strength of the association with gastric cancer is more modest than that for colorectal cancer and PGE-M seems unlikely to show utility as a screening test for gastric cancer, rather it may provide insight into the mechanism of gastric carcinogenesis.

One of the strengths of our study is that it is nested within a prospective cohort. In addition, there was a high proportion of follow-up (99.8%) and a large proportion of the cohort provided prediagnostic urine samples. A potential source of variation among samples could come from the use of NSAIDs prior to biospecimen collection. It has been previously reported that NSAID use within the previous 48 hours modifies levels of PGE-M and would thus not result in an accurate measurement (26). NSAID use was very low among study participants, but we also took this into account during our analyses through both exclusion and adjustment. We used spot urine samples collected at baseline, but this hypothesis might be bolstered if tested in urine samples collected at several different time points. Another limitation of our study is the small sample size. Additional studies with a larger number of gastric cancer cases are needed to replicate and confirm these findings.

In conclusion, the results from this study suggest that increasing levels of urinary PGE-M, a marker of COX-2 activity, may be associated with an increased risk of gastric cancer among women, which offers further evidence of the importance of COX-2 in gastric cancer development.

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Table 1

Characteristics at baseline for women from the Shanghai Women's Health Study

Characteristics	Case (n=144)	Control (n=144)	p-value*
Age, years	59.0 ± 8.6	59.1 ± 8.4	0.64
Body mass index (kg/m^2)	24.8 ± 3.4	24.7 ± 3.5	0.78
Education, High School or more	34 (23.6)	45 (31.3)	0.15
Family history of gastric cancer, yes	13 (9.0)	14 (9.7)	0.84
Smoking status, ever	9 (6.3)	8 (5.6)	0.80
Alcohol use, ever	3 (2.1)	2 (1.4)	0.65
NSAID/cold medication use, within past day	10 (6.9)	15 (10.4)	0.30
H.pylori positive†	128 (96.2)	126 (94.7)	0.55
Regular multiple vitamin use	9 (6.3)	9 (6.3)	0.99
Fruits/vegetables intake, g/d	490.6 ± 268.7	552.7 ± 297.0	0.05
Fruits, g/d	218.3 ± 159.4	252.9 ± 186.2	0.08
All vegetables, g/d	272.3 ± 162.9	299.9 ± 161.9	0.13
Meat intake, g/d	44.4 ± 33.9	47.3 ± 36.6	0.50

Continuous variables are displayed as means ± standard deviation and frequencies are displayed as counts (percentage)

*P-values are calculated from paired t-test for continuous variables and Chi^2 test for categorical variables

†Data on H. pylori status available only on n=270

Table 2

Association of urinary PGE-M levels and risk of gastric cancer

	PGE-M (quartiles, ng/mg creatinine) 1 (< 2.83)	2 (2.83–5.36)	3 (5.37–9.16)	4 (≥ 9.17)	p for trend*
No. of cases	29	28	39	48	
No. of controls	36	37	37	34	
OR (95% CI)†	1.00	0.96 (0.48–1.92)	1.38 (0.68–2.80)	1.87 (0.93–3.75)	0.05
OR (95% CI)‡	1.00	1.00 (0.48–2.08)	1.40 (0.67–2.91)	1.98 (0.95–4.13)	0.04

*P value for linear trend tested by including a variable coded 0, 1, 2, and 3

†Unadjusted estimates

‡Adjusted for body mass index, education, fruit and vegetable intake, smoking status, NSAID use, and family history of gastric cancer

Table 3

Association of urinary PGE-M levels and risk of gastric cancer stratified by median time interval between urine collection and cancer diagnosis

Time after Urine Collection	PGE-M (quartiles, ng/mg creatinine) 1 (< 2.83)	2 (2.83–5.36)	3 (5.37–9.16)	4 (≥ 9.17)	p for trend*
≤ 46.3 mos (72 pairs)					
No. of cases	15	13	19	25	
No. of controls	19	16	22	15	
OR (95% CI)†	1.00	1.10 (0.38–3.22)	1.26 (0.43–3.66)	2.75 (0.84–8.94)	0.09
> 46.3 mos (72 pairs)					
No. of cases	14	15	20	23	
No. of controls	17	21	15	19	
OR (95% CI)†	1.00	0.99 (0.34–2.90)	1.96 (0.62–6.17)	1.48 (0.52–4.20)	0.29

*P value for linear trend tested by including a variable coded 0, 1, 2, and 3

†Adjusted for body mass index, education, fruit and vegetable intake, smoking status, NSAID use, and family history of gastric cancer