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# A Prospective Study of Urinary Prostaglandin E2 Metabolite, *Helicobacter pylori* Antibodies, and Gastric Cancer Risk

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***Background.*** Previous studies suggest that a stable end-product of prostaglandin E2, the urinary metabolite PGE-M, is associated with colorectal cancer, and 1 study of relatively small sample size found an association with gastric cancer among women. In the present study we further investigate the PGE-M, *Helicobacter pylori*, and gastric cancer association.

***Methods.*** The present analysis included 359 prospectively ascertained gastric cancer cases and 700 individually matched controls from the Shanghai Women's and Men's Health Studies. Urinary PGE-M was measured by a liquid chromatography/tandem mass spectrometric method. Seropositivity to 15 *H. pylori* recombinantly expressed fusion proteins was detected by *H. pylori* multiplex serology.

***Results.*** Adjusting for *H. pylori*, increasing PGE-M was associated with higher risk of gastric cancer (quartile 4 vs 1: odds ratio [OR], 1.76 [95% confidence interval {CI}, 1.17–2.66], $P_{trend}$ = .004). This association remained after excluding those diagnosed within 2 years from sample collection (OR, 1.73 [95% CI, 1.12–2.65], $P_{trend}$ = .007). However it was no longer present among individuals with 10 or more years of follow-up (2–4.9 years: OR, 3.15 [95% CI, 1.11–8.91]; 5–9.9 years: OR, 2.23 [95% CI, 1.22–4.06]; ≥10 years: OR, 0.73 [95% CI, .31–1.70]). Compared to *H. pylori*–negative individuals with below-median PGE-M levels, *H. pylori*–positive individuals with above-median PGE-M levels had a 5-fold increase in the odds of gastric cancer (OR, 5.08 [95% CI, 2.47–10.43]).

***Conclusions.*** In China, higher PGE-M levels may indicate an increased risk of gastric cancer independent of the risk conferred by *H. pylori* infection status, particularly for cancers diagnosed within 10 years of sample collection.

**Keywords.** *Helicobacter pylori*; stomach neoplasms; serology; inflammation.

Multiple lines of evidence have indicated an important role for aberrant arachidonic acid metabolism in both inflammation and carcinogenesis [1, 2]. Cyclooxygenase 2 (COX-2), a rate-limiting enzyme, catalyzes the conversion of arachidonic acid to prostaglandin H2 (PGH2) [3, 4]. PGH2 is converted by microsomal prostaglandin E synthase to a key inflammatory mediator, prostaglandin E2 ($PGE_2$) [5]. Upregulation of COX-2 or overproduction of $PGE_2$ is found in various malignancies and is also associated with poor prognosis [6–9]. Catabolism of $PGE_2$ is initiated by 15-hydroxyprostaglandin dehydrogenase and results in a stable end metabolite, PGE-M (11α-hydroxy-9, 15-dioxo-2, 3, 4, 5-tetranorprostane-1, 20-dioic acid), which is excreted in the urine and used as an index of systemic $PGE_2$ production [10, 11].

Levels of PGE-M in healthy individuals are suppressed by both the nonselective COX inhibitor and the COX-2 selective inhibitor [11]. Previously, our findings suggested a linear association between urinary PGE-M levels and gastric cancer risk in women [12]. Importantly, *Helicobacter pylori* infection is the leading causal factor for gastric cancer, and COX-2 overexpression might be a significant step in *H. pylori*–associated gastric carcinogenesis. Hence, in the present study, with longer follow-up and more cases in both sexes, we investigated this association with consideration of *H. pylori* infection in Shanghai, China, an area with documented high rates of gastric cancer and *H. pylori* infection.

## METHODS

### Study Design and Study Population

The Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS), 2 population-based prospective

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cohort studies located in urban Shanghai, China, provided participants for the current nested case-control study. The SWHS recruited 74 942 Chinese women aged 40–70 years from 1996 to 2000, while the SMHS recruited 61 480 men aged 40–74 years from 2002 to 2006 [13, 14].

Using the *International Classification of Diseases for Oncology* (including codes C16.0–C16.6, and C16.9), incident gastric cancer was ascertained through registry linkage and active follow-ups. Incidence density sampling was used, with 2 controls chosen at random for each gastric cancer case from the appropriate risk sets consisting of all cohort members alive, free of cancer (excluding nonmelanoma skin cancer), and without history of gastrectomy at the time of diagnosis of the index case. Our final analysis included 359 prospectively ascertained gastric cancer cases with 700 controls matched on sex, age, and date of specimen collection. Written informed consent was provided by all participants and this study was approved by the institutional review boards of Vanderbilt University (Nashville, Tennessee); the German Cancer Research Center (Heidelberg, Germany); and the Shanghai Cancer Institute (Shanghai, China).

#### Data Collection and Laboratory Methods

At study enrollment, all participants were interviewed in-person by a trained interviewer using a structured questionnaire. The questionnaire consists of the following sections: demographic background, medical history, personal habits, dietary habits, physical activity, family history of cancer, occupational history, weight and height history, reproductive history, and residential history [14, 15].

Urinary PGE-M was measured by a liquid chromatography/tandem mass spectrometric method, as described previously [11, 16]. In brief, 0.75 mL of urine was acidified to pH 3 with HCl and endogenous PGE-M was converted to the *O*-methyloxime derivative. The methoximated PGE-M was extracted after incubation, applied to a C-18 Sep-Pak, and then eluted with ethyl acetate. With an internal standard of $^{2}H_{6}$-O-methyloxime PGE-M, samples were then analyzed by liquid chromatography on a Zorbax Eclipse XDB-C18 column attached to a ThermoFinnigan Surveyor MS Pump. 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 ($^{2}H_3$ + $H_2O$)]$^-$ for the deuterated internal standard were monitored in the selected reaction monitoring mode. Quantification of endogenous PGE-M was calculated by comparing the ratio of the mass chromatogram peak areas of the *m/z* 336 and *m/z* 339 ions. To account for variations in hydration status, urinary PGE-M levels were standardized using the urinary creatinine levels of each sample and are expressed as nanograms per milligram (ng/mg) creatinine. Urinary creatinine levels were measured using a kit (Sigma). Staff was blinded to case and control status, and duplicate quality-control samples were interspersed among samples. The intra-assay and interassay coefficients of variation for PGE-M levels adjusted for creatinine were 10.8% and 19.9%, respectively.

Based on the glutathione *S*-transferase capture immunosorbent assay combined with fluorescent bead technology (Luminex, Austin, Texas), human immunoglobulin A, M, and G antibodies to 15 *H. pylori* recombinantly expressed fusion proteins (UreA, Catalase, GroEL, NapA, CagA, CagM, Cagδ, HP 0231, VacA, HpaA, Cad, HyuA, Omp, HcpC, and HP 0305) were detected by *H. pylori* multiplex serology simultaneously [17, 18]. Overall, *H. pylori* seropositivity was defined as ≥4 seropositive results of the 15 *H. pylori* antigens assessed, in accordance with previous validation applying commercial serological assay classification [17].

#### Statistical Analyses

The present analysis includes 1059 participants. Quartile cut points were determined using the distribution of PGE-M among controls. Differences of baseline characteristics across quartiles of PGE-M were compared using $\chi^2$ tests for categorical variables and Kruskal-Wallis tests for continuous variables.

To determine the associations between the prevalence of *H. pylori* infection and PGE-M levels, analysis of variance was applied to calculate mean PGE-M levels within groups defined by *H. pylori* dichotomous status, or Omp and HP 0305 status (comparing Omp$^-$ and HP 0305$^-$, Omp$^-$ or HP 0305$^-$, and Omp$^+$ and HP 0305$^+$) [19]. As PGE-M levels were not normally distributed, they were log-transformed. Statistical adjustment was made for education, body mass index (BMI), and smoking status with cases and controls matched by sex, age at enrollment, and date of specimen collection.

Conditional logistic regression models were constructed to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer risk across quartiles of PGE-M. In Table 3, model 1 evaluated the odds of gastric cancer incidence by PGE-M level conditioned on matched case-control sets only. Then in model 2 the potential confounders of education, BMI, and smoking were added. Model 3 adjusted for all variables in model 2 together with *H. pylori* infection status categorized dichotomously. Model 4 then adjusted for *H. pylori* based on seropositivity of combined Omp and HP 0305 status instead of *H. pylori* dichotomously, as these *H. pylori* proteins were previously found to be better markers than *H. pylori* seropositivity of gastric cancer risk in our population [19]. Tests for linear trend were performed by entering the categorical variables as continuous parameters. With the same analysis strategy as in Table 3, stratified analyses were conducted by follow-up period (2–4.9 years, 5–9.9 years, ≥10 years) from sample collection to diagnosis. Again with the same covariates as used in model 2, the combined effect of PGE-M and *H. pylori* was evaluated. We also examined effect modification by *H. pylori* status using a likelihood ratio test to compare models with and without interaction terms. Sensitivity analyses were performed excluding

**Table 1. Distribution of Selected Demographic Characteristics Across Quartiles of Urinary PGE-M Levels at Baseline in the *Helicobacter pylori* Biomarker Cohort Consortium**

| Characteristic | PGE-M (ng/mg Creatinine) | | | | *P* Value |
|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | |
| Age, y, median (IQR) | 55.6 (48.1–64.0) | 60.0 (48.6–65.5) | 61.9 (54.1–66.4) | 63.3 (55.9–67.3) | <.001 |
| Follow-up time, y, median (IQR) | 7.8 (5.1–9.9) | 7.8 (5.1–10.6) | 7.7 (4.1–10.2) | 7.0 (4.7–9.6) | .356 |
| Sex | | | | | .930 |
| Female | 198 (80.8) | 206 (82.7) | 219 (82.0) | 241 (80.9) | |
| Male | 47 (19.2) | 43 (17.3) | 48 (18.0) | 57 (19.1) | |
| Smoking | | | | | |
| Female | | | | | .058 |
| Noncurrent smoker | 193 (97.5) | 203 (98.5) | 208 (95.0) | 227 (94.2) | |
| Current smoker | 5 (2.5) | 3 (1.5) | 11 (5.0) | 14 (5.8) | |
| Male | | | | | .028 |
| Noncurrent smoker | 29 (61.7) | 30 (69.8) | 22 (45.8) | 25 (43.9) | |
| Current smoker | 18 (38.3) | 13 (30.2) | 26 (54.2) | 32 (56.1) | |
| Drinking | | | | | |
| Female | | | | | .288 |
| Never drinker | 195 (98.5) | 201 (97.6) | 212 (96.8) | 239 (99.2) | |
| Ever drinker | 3 (1.5) | 5 (2.4) | 7 (3.2) | 2 (0.8) | |
| Male | | | | | .277 |
| Never drinker | 30 (63.8) | 27 (62.8) | 35 (72.9) | 31 (54.4) | |
| Ever drinker | 17 (36.2) | 16 (37.2) | 13 (27.1) | 26 (45.6) | |
| Education[a] | | | | | <.001 |
| Elementary school or less | 54 (22.0) | 76 (30.5) | 107 (40.1) | 146 (49.0) | |
| Junior high school | 81 (33.1) | 95 (38.2) | 79 (29.6) | 74 (24.8) | |
| High school | 66 (26.9) | 62 (24.9) | 45 (16.9) | 45 (15.1) | |
| Professional education or above | 43 (17.6) | 16 (6.4) | 35 (13.0) | 31 (10.4) | |
| Missing | 1 (0.4) | 0 (10) | 1 (0.4) | 2 (0.7) | |
| BMI, kg/m$^2$ | | | | | .217 |
| <23.0 | 88 (35.9) | 95 (38.1) | 87 (32.6) | 86 (28.9) | |
| 23.0–24.9 | 66 (26.9) | 49 (19.7) | 56 (21.0) | 71 (23.8) | |
| 25.0–27.4 | 53 (21.7) | 56 (22.5) | 67 (25.0) | 75 (25.2) | |
| ≥27.5 | 38 (15.5) | 49 (19.7) | 57 (21.4) | 66 (22.1) | |
| NSAID/cold medication use, within past day | | | | | .612 |
| No | 225 (91.8) | 236 (94.8) | 250 (93.6) | 277 (93.0) | |
| Yes | 20 (8.2) | 13 (5.2) | 17 (6.4) | 21 (7.0) | |
| History of gastritis | | | | | .837 |
| No | 187 (76.3) | 191 (76.7) | 212 (79.4) | 232 (77.9) | |
| Yes | 58 (23.7) | 58 (23.3) | 55 (20.6) | 66 (22.1) | |
| *H. pylori* infection status | | | | | .008 |
| Negative | 44 (18.0) | 34 (13.7) | 28 (10.5) | 26 (8.7) | |
| Positive | 201 (82.0) | 215 (86.3) | 239 (89.5) | 272 (91.3) | |
| Omp and HP 0305 status | | | | | .024 |
| Omp$^-$ and HP 0305$^-$ | 62 (25.3) | 42 (16.9) | 40 (15.0) | 50 (16.8) | |
| Omp$^+$ or HP 0305$^+$ | 75 (30.6) | 81 (32.5) | 88 (33.0) | 81 (27.2) | |
| Omp$^+$ and HP 0305$^+$ | 108 (44.1) | 126 (50.6) | 139 (52.0) | 167 (56.0) | |

Abbreviations: BMI, body mass index; IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drug; PGE-M, prostaglandin E2 metabolite; Q, quartile.

[a]The $\chi^2$ test was done within those nonmissing subjects.

cases diagnosed within 2 years after sample collection, current smokers, or ever drinkers and their matched controls, which were suggested to be associated with PGE-M levels and gastric cancer [20–22]. Statistical analyses were conducted with SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina). *P* values of <.05 (2-sided probability) were interpreted as being statistically significant for all analyses.

## RESULTS

### Study Cohorts' Characteristics

Among 1059 participants, individuals with higher PGE-M quartiles were more likely to be older, current smokers, less educated, and seropositive to *H. pylori* overall and seropositive to both *H. pylori* proteins Omp and HP 0305 specifically (Table 1). The differences among quartiles for PGE-M in follow-up time,

sex, ever drinkers, BMI, nonsteroidal anti-inflammatory drugs (NSAIDs) or cold medication use, and history of gastritis were not statistically significant.

#### *Helicobacter pylori* or Omp and HP0305 Status Associated With PGE-M Levels

Overall, *H. pylori*–seropositive individuals had an average PGE-M level that was 19.4% higher than *H. pylori*–seronegative individuals ($P < .001$) (Table 2). While this trend was apparent separately among both cases and controls, the difference reached significance only among the controls, who had overall lower PGE-M levels than the cases. Comparing PGE-M levels by combined status of seropositivity to Omp and HP 0305 instead of by *H. pylori* dichotomously, individuals in the least virulent group ($Omp^-$ and HP $0305^-$) had an average PGE-M level that was 15.4% lower than those in the most virulent group ($Omp^+$ and HP $0305^+$) ($P = .004$). While there was an overall trend of increasing PGE-M levels with increasing *H. pylori* virulence based on Omp and HP 0305 status, similarly there were no significant differences in mean PGE-M levels among cases.

#### Gastric Cancer Risk by Urinary PGE-M Levels

Increasing level of PGE-M was associated with increasing risk of gastric cancer in all 4 models in this study ($P_{trend} < .01$) (Table 3). After adjustment for *H. pylori* status along with the other risk factors of education, BMI, and smoking status, the odds of gastric cancer for individuals in the highest quartile of PGE-M levels was 72%–76% greater than those in the lowest quartile of PGE-M levels (adjusting for *H. pylori* dichotomously: OR, 1.76 [95% CI, 1.17–2.66], $P_{trend} = .004$; adjusting for *H. pylori* using combined Omp and HP 0305 status: OR, 1.72 [95% CI, 1.13–2.62], $P_{trend} = .004$). If cases diagnosed within 2 years of blood draw together with their matched controls were excluded, similar increasing risks of gastric cancer by PGE-M level were found (adjusting for *H. pylori* dichotomously: OR, 1.73 [95% CI, 1.12–2.65], $P_{trend} = .007$; adjusting for *H. pylori* by Omp and HP 0305 status: OR, 1.70 [95% CI, 1.10–2.63], $P_{trend} = .007$). When analyses were stratified by sex, an increased trend of gastric cancer risk was observed among both men and women, although the strength of the association in men was weaker than that of women, potentially due to the small sample size of male cases (data not shown).

When analyses were stratified by follow-up period from sample collection to diagnosis, the risk of gastric cancer associated with increasing PGE-M levels decreased over time (Table 3). Adjusting for *H. pylori* infection, individuals in the highest PGE-M quartile diagnosed within 2–4.9 years from sample collection had an approximate 3-fold increase in the odds of gastric cancer (adjusting for *H. pylori* dichotomously: OR, 3.15 [95% CI, 1.11–8.91], $P_{trend} = .046$; adjusting for *H. pylori* by Omp and HP 0305 status: OR, 2.95 [95% CI, .98–8.86], $P_{trend} = .071$), while for the group with 5–9.9 years of follow-up, the increased risk associated with high PGE-M levels was only 2-fold (adjusting for *H. pylori* dichotomously: OR, 2.23 [95% CI, 1.22–4.06], $P_{trend} = .006$; adjusting for *H. pylori* by Omp and HP 0305 status: OR, 2.20 [95% CI, 1.19–4.10], $P_{trend} = .007$). For those with ≥10 years of follow-up, no association of PGE-M level and gastric cancer risk was found (adjusting for *H. pylori* dichotomously: OR, 0.73 [95% CI, .31–1.70], $P_{trend} = .775$; adjusting for *H. pylori* by Omp and HP 0305 status: OR, 0.69 [95% CI, .29–1.66], $P_{trend} = .806$).

**Table 2. Association of *Helicobacter pylori* or Omp and HP0305 Status Seroprevalence With PGE-M Levels in the *H. pylori* Biomarker Cohort Consortium**

| | PGE-M Levels (ng/mg Creatinine) | | |
|---|---|---|---|
| *H. pylori* Status | Entire Population (N = 1059) | Cases (n = 359) | Controls (n = 700) |
| *H. pylori*$^-$, geometric mean level (95% CI)[a] | 6.2 (5.6–6.8) | 7.2 (5.5–9.4) | 6.0 (5.4–6.6) |
| *H. pylori*$^+$, geometric mean level (95% CI)[a] | 7.4 (7.1–7.6) | 7.9 (7.4–8.4) | 7.1 (6.8–7.4) |
| Difference between *H. pylori*$^-$ and *H. pylori*$^+$ | | | |
| *P* value[b] | <.001 | .497 | .003 |
| Percentage increase[c] | 19.4% | 9.7% | 18.3% |
| $Omp^-$ and HP $0305^-$, geometric mean level (95% CI)[a] | 6.5 (6.0–7.1) | 7.3 (5.8–9.2) | 6.4 (5.8–6.9) |
| $Omp^-$ or HP $0305^-$, geometric mean level (95% CI)[a] | 7.2 (6.8–7.7) | 7.7 (6.9–8.7) | 7.0 (6.4–7.5) |
| $Omp^+$ and HP $0305^+$, geometric mean level (95% CI)[a] | 7.5 (7.1–7.9) | 8.0 (7.4–8.6) | 7.1 (6.7–7.6) |
| Difference between ($Omp^-$ and HP $0305^-$) and ($Omp^-$ or HP $0305^-$) | | | |
| *P* value[b] | .047 | .662 | .038 |
| Percentage increase[c] | 10.8% | 5.5% | 9.4% |
| Difference between ($Omp^-$ and HP $0305^-$) and ($Omp^+$ and HP $0305^+$) | | | |
| *P* value[b] | .004 | .461 | .038 |
| Percentage increase[c] | 15.4% | 9.6% | 10.9% |
| Difference between ($Omp^-$ or HP $0305^-$) and ($Omp^+$ and HP $0305^+$) | | | |
| *P* value[b] | .380 | .631 | .615 |
| Percentage increase[c] | 4.2% | 3.9% | 1.4% |

Abbreviation: CI, confidence interval; PGE-M, prostaglandin E2 metabolite.

[a]The geometric mean and its 95% CI.

[b]Analysis of variance (ANOVA) with cases and controls matched on sex, age, and date of biological collection, adjusted for education, body mass index, and smoking.

[c]For relative difference.

#### Combined Effect by PGE-M Levels and *H. pylori*

Grouping individuals into categories by both *H. pylori* status and PGE-M level, *H. pylori*–positive participants with above-median PGE-M levels had a 5-fold increased odds for gastric cancer compared to *H. pylori*–negative participants with below-median PGE-M levels (OR, 5.08 [95% CI, 2.47–10.43]) (Table 4). Similarly, when we used Omp and HP 0305 to categorize *H. pylori* status instead, individuals categorized into the highest-risk *H. pylori* group ($Omp^+$ and HP $0305^+$) and with above-median PGE-M levels had a >6-fold increased odds of gastric cancer compared to low-risk *H. pylori* individuals (Omp- and HP 0305-) with below-median PGE-M levels (OR, 6.54 [95% CI, 3.40–12.60]).

**Table 3. Risk of Gastric Cancer by Urinary PGE-M Levels in the *Helicobacter pylori* Biomarker Cohort Consortium**

| PGE-M Level (Quartiles, ng/mg Creatinine) | Cases/Controls, No. | OR (95% CI)[a] | OR (95% CI)[b] | OR (95% CI)[c] | OR (95% CI)[d] |
|---|---|---|---|---|---|
| Overall | | | | | |
| Q1 | 69/176 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Q2 | 75/174 | 1.15 (.77–1.72) | 1.06 (.70–1.60) | 1.05 (.69–1.60) | 0.98 (.64–1.51) |
| Q3 | 92/175 | 1.49 (1.00–2.23) | 1.40 (.93–2.10) | 1.26 (.83–1.91) | 1.22 (.80–1.86) |
| Q4 | 123/175 | 2.03 (1.36–3.01) | 1.92 (1.28–2.88) | 1.76 (1.17–2.66) | 1.72 (1.13–2.62) |
| $P_{trend}$ | … | <.001 | <.001 | .004 | .004 |
| 2–4.9 y of follow-up from sample collection to diagnosis | | | | | |
| Q1 | 11/35 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Q2 | 14/25 | 1.73 (.63–4.70) | 1.86 (.65–5.30) | 1.90 (.62–5.76) | 1.91 (.57–6.42) |
| Q3 | 15/35 | 1.48 (.55–3.98) | 1.54 (.57–4.19) | 1.54 (.55–4.32) | 1.73 (.56–5.39) |
| Q4 | 24/29 | 2.87 (1.10–7.49) | 3.29 (1.21–8.94) | 3.15 (1.11–8.91) | 2.95 (.98–8.86) |
| $P_{trend}$ | … | .044 | .031 | .046 | .071 |
| 5–9.9 y of follow-up from sample collection to diagnosis | | | | | |
| Q1 | 33/96 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Q2 | 34/80 | 1.30 (.73–2.29) | 1.16 (.64–2.11) | 1.10 (.60–2.02) | 1.05 (.57–1.97) |
| Q3 | 41/80 | 1.69 (.95–3.01) | 1.59 (.88–2.89) | 1.42 (.78–2.62) | 1.41 (.75–2.63) |
| Q4 | 64/81 | 2.69 (1.53–4.73) | 2.47 (1.37–4.45) | 2.23 (1.22–4.06) | 2.20 (1.19–4.10) |
| $P_{trend}$ | … | <.001 | .002 | .006 | .007 |
| ≥10 y of follow-up from sample collection to diagnosis | | | | | |
| Q1 | 21/37 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Q2 | 22/54 | 0.73 (.34–1.57) | 0.63 (.28–1.42) | 0.66 (.29–1.53) | 0.54 (.23–1.29) |
| Q3 | 29/42 | 1.34 (.62–2.87) | 1.22 (.55–2.72) | 1.08 (.48–2.44) | 0.98 (.42–2.27) |
| Q4 | 22/49 | 0.82 (.37–1.83) | 0.77 (.33–1.76) | 0.73 (.31–1.70) | 0.69 (.29–1.66) |
| $P_{trend}$ | … | .950 | .989 | .775 | .806 |

Abbreviations: CI, confidence interval; OR, odds ratio; PGE-M, prostaglandin E2 metabolite; Q, quartile.

[a]Conditional logistic regression with cases and controls matched on sex, age, and date of biological collection.

[b]Conditional logistic regression with cases and controls matched on sex, age, and date of biological collection, adjusted for education, body mass index (BMI), and smoking.

[c]Conditional logistic regression with cases and controls matched on sex, age, and date of biological collection, adjusted for education, BMI, smoking, and *Helicobacter pylori* infection (positive or negative).

[d]Conditional logistic regression with cases and controls matched on sex, age and date of biological collection, adjusted for education, BMI, smoking, and Omp and HP 0305 status ($Omp^-$ and $HP\ 0305^-$, $OMP^+$ or $HP\ 0305^+$, and $Omp^+$ and $HP\ 0305^+$).

No effect modification of the PGE-M and gastric cancer association was found by *H. pylori* infection, combined Omp and HP 0305 status, sex, or follow-up time (data not shown). Secondary analyses that examined the PGE-M association with gastric cancer, excluding current smokers, ever drinkers, or cases included in our previously published article, did not find differing results (data not shown) [12]. Additionally, when stratifying by site, a trend of increasing risk with increasing PGE-M level was observed for both cardia and noncardia gastric cancer, although the association was weaker and did not reach statistical significance for noncardia gastric cancer (highest vs lowest quartile of PGE-M levels: OR, 1.44 [95% CI, .94–2.22], $P_{trend}$ = .057).

## DISCUSSION

Utilizing prospectively ascertained data in a region with high gastric cancer incidence, we found that after adjustment for *H. pylori* infection, high urinary levels of PGE-M, a stable end metabolite of $PGE_2$, were associated with the subsequent risk of gastric cancer. Consistent with prior studies, the timing of sample collection in relation to cancer development was suggested to be a factor affecting the association, which was strongest for individuals diagnosed within 5 or 10 years of blood draw, leading to a significant over 2-fold increased risk of gastric cancer for individuals with PGE-M levels in the highest quartile [12, 16]. Combined with harboring antibodies to *H. pylori*, an individual with above-median levels of PGE-M was at a 5-fold increase in odds of gastric cancer diagnosis, and this rose to a >6-fold increase in odds for individuals also seropositive to the virulent *H. pylori* proteins Omp and HP 0305. These results suggest an additive predictive value to *H. pylori* status of PGE-M measurement. Previous studies have found enhanced expression of COX-2 in tumor tissues and excessive prostaglandin production in gastric carcinogenesis [23, 24]. In addition, overexpression of COX-2 in metaplastic and adenomatous cells suggests that COX-2 may contribute to the early event in the formation of gastric cancer [23, 24].

Previously a study within SWHS found a strong association of PGE-M levels and colorectal cancer risk, with relative risks of 2.5, 4.5, and 5.6 with increasing quartiles of PGE-M [16]. For breast cancer overall, no association with PGE-M in the SWHS

**Table 4. Association of Gastric Cancer Risk According to Urinary PGE-M Levels, *Helicobacter pylori*, and Omp and HP 0305 in the *H. pylori* Biomarker Cohort Consortium**

| *Helicobacter pylori* Status | PGE-M Levels (ng/mg Creatinine) | Cases/ Controls, No. | OR (95% CI) |
|---|---|---|---|
| *H. pylori*$^{-}$ | ≤50th percentile | 9/69 | 1.00 (reference) |
| | >50th percentile | 9/45 | 1.61 (.59–4.44) |
| *H. pylori*$^{+}$ | ≤50th percentile | 135/281 | 3.52 (1.70–7.29) |
| | >50th percentile | 206/305 | 5.08 (2.47–10.43) |
| Omp$^{-}$ and HP 0305$^{-}$ | ≤50th percentile | 12/92 | 1.00 (reference) |
| | >50th percentile | 14/76 | 1.38 (.59–3.24) |
| Omp$^{+}$ or HP 0305$^{+}$ | ≤50th percentile | 45/111 | 3.09 (1.55–6.17) |
| | >50th percentile | 59/110 | 4.41 (2.20–8.81) |
| Omp$^{+}$ and HP 0305$^{+}$ | ≤50th percentile | 87/147 | 4.33 (2.25–8.34) |
| | >50th percentile | 142/164 | 6.54 (3.40–12.60) |

Conditional logistic regression with cases and controls matched on sex, age, and date of biological collection, adjusted for education, body mass index, and smoking.

Abbreviations: –, negative; +, positive; CI, confidence interval; OR, odds ratio; PGE-M, prostaglandin E2 metabolite.

was found, although among normal-weight postmenopausal women only, there was a linear trend of increasing PGE-M quartile and increasing risk ($P$ = .005) [25]. A different study in Shanghai found a positive association with pancreatic cancer risk comparing the third tertile of PGE-M levels to the first (OR, 1.63 [95% CI, 1.01–2.63]) [26]. However, only 1 publication has examined the association between PGE-M and gastric cancer risk [12]. This study from our group observed a significant linear association, but was hampered by small numbers and the inability to consider the heterogeneity of *H. pylori* infection.

Several studies have shown that *H. pylori* induces COX-2 expression in human gastric mucosa, and COX-2 expression is decreased after anti–*H. pylori* treatment [24, 27, 28]. Hence, in *H. pylori*–associated gastric carcinogenesis, COX-2 overexpression might be an important step [29]. Consistent with previous findings, our study found that more virulent *H. pylori* strains were associated with higher PGE-M levels in all groups of the population. And the highest gastric cancer risk was observed in the more virulent *H. pylori* strains together with higher quartiles of PGE-M. Therefore, urinary PGE-M may be a promising noninvasive biomarker that, taken together with *H. pylori* infection, could aid in the risk assessment of gastric cancer.

Epidemiological studies suggest that the long-term use of NSAIDs decreases the incidence of and mortality from gastrointestinal cancers [30, 31]. The principal pharmacological effects of NSAIDs are due to their ability to inhibit COX-2 and prostaglandin synthesis [32, 33]. However, in our study population, only 4.1% participants took NSAIDs regularly. And COX-dependent mechanisms may not completely explain the chemopreventive effect of aspirin on cancer prevention [34].

Consistent with the prior literature, we observed increasing levels of urinary PGE-M were related to several lifestyle factors, including age, smoking, and obesity. Elevated levels of multiple proinflammatory mediators, known inducers of COX-2 and $PGE_2$ synthesis, occur during aging and may contribute to this finding [35]. It has also been suggested that reduced levels of estrogen, with known anti-inflammatory properties, may contribute to aging-related increases in urinary PGE-M levels [36]. Alternatively, the age-related increase in PGE-M may simply reflect the presence of atherosclerosis or other age- related inflammatory conditions [37]. In the current study, we similarly observed a direct association between age and higher PGE-M levels. Smoking was also an independent determinant of elevated urinary PGE-M. Previously, smokers were shown to have high urinary PGE-M levels due to increased COX-2 activity, ultimately leading to increased $PGE_2$ production [20, 38]. In our study, we also found current smokers to be overrepresented in the higher PGE-M level groups. Additionally, obesity is recognized as an inflammatory condition [39]. Increasing adiposity leads to the recruitment of macrophages that produce and release proinflammatory cytokines, thus upregulating COX-2 expression, and elevated $PGE_2$ levels have been found in inflamed white adipose tissue [39]. Consistent with a previous study, we found that obesity was suggested to be associated with elevated levels of urinary PGE-M [25, 36].

Certain limitations of the present study should be considered when interpreting the results. While individuals who self-reported a history of cancer at baseline were excluded from these analyses, both the SMHS and SWHS participants were not screened for undiagnosed cancer, which is typical in large prospective cohort studies. Thus, it is possible that undiagnosed asymptomatic cancer could explain part of the association observed in the early years of follow-up. However the association persisted, even after excluding cases diagnosed in the first 2 years of follow-up in our study, suggesting that this finding could not be entirely explained by cancer-related PGE-M production [12, 16]. Also, premalignant gastric lesions were not accessible, and further exploration is warranted to explain the role of PGE-M in the progress of gastric cancer at differing stages.

Our study's strengths include its prospective design, large study size, urine samples provided by most participants, long follow-up period, and adjustment for most gastric cancer risk factors, especially *H. pylori* infection. To our knowledge, it comprises the largest number of prospectively ascertained gastric cancer cases to examine the association of PGE-M with gastric cancer risk with adjustment of *H. pylori* in the high-risk region of East Asia.

In conclusion, this large prospective study in China provides the insight that, independent of *H. pylori*, the major causal factor for gastric cancer, increased PGE-M in urine is associated with gastric cancer in this population at high baseline risk. Also, higher PGE-M levels may indicate additional risk for gastric cancer even among individuals infected with the more virulent strains of *H. pylori* in East Asia. Hence, urinary PGE-M, together with *H. pylori* protein–specific antibodies, may be

promising building blocks for a model to assess risk of gastric cancer in East Asia.

## Notes

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