Short Communication

Cancer Epidemiology, Biomarkers & Prevention

Seropositivity to *Helicobacter pylori* and Risk of Pancreatic Cancer

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Abstract

Helicobacter pylori (*H. pylori*) seropositivity has been inconsistently associated with pancreatic cancer. We, therefore, investigated the association between *H. pylori* seropositivity and pancreatic cancer in a case–control study nested within Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort of Finnish male smokers. Pancreatic cancer cases ($n = 353$) and control subjects ($n = 353$) were matched on date of baseline serum collection, age at randomization, and follow-up time (up to 23.9 years). We used a multiplex serology assay to determine the sero-status of antibodies against 15 *H. pylori*–specific antigens in fasting serum samples. Conditional logistic regression was used to calculate the odds ratio (OR) and 95% confidence intervals (CI). Neither targeted *H. pylori* antigens in serum nor the combination of all was associated with development of pancreatic cancer (combination of all: OR, 0.85; 95% CI, 0.49–1.49). Our results suggest that *H. pylori* is not a risk factor for pancreatic cancer. *Cancer Epidemiol Biomarkers Prev; 22(12); 2416–9. ©2013 AACR.*

Introduction

Exocrine pancreatic cancer is among the most fatal cancers worldwide and has few established risk factors for prevention (e.g., smoking, diabetes mellitus and obesity). Seropositivity to *Helicobacter pylori* has been hypothesized as risk factor for pancreatic cancer (1, 2). However, this association was not consistent across studies (3–8). Most previous studies included a small number of cases (35–121 cases; refs. 3–8). The largest study (373 cases and 390 controls) was a cross-sectional case–control design (7) which could have inherent methodological difficulties. Our previous study which had 121 cases from the Alpha-Tocopherol, Beta-Carotene Cancer (ATBC) Prevention Study cohort, showed evidence for association between *H. pylori* carriage, particularly the CagA strain, and pancreatic cancer (4). We conducted a nested case–control study in the same ATBC cohort, now with significantly longer follow-up (up to 23.9 years), to replicate previous findings with a larger number of cases (353 total cases). In addition, we applied new technology, *H. pylori* multiplex serology assay to test the association of multiple different *H. pylori* strains and pancreatic cancer. To the best of our knowledge, this is the first study to examine the *H. pylori* multiplex serology and risk of pancreatic cancer.

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Materials and Methods

Study population

The ATBC cohort, recruited between 1985 and 1988, includes 29,133 males aged 50 to 69 years in southwestern Finland who smoked at least 5 cigarettes per day (9). Participants completed questionnaires during their baseline visits. All cases of pancreatic cancer were identified through the Finnish Cancer Registry and death certificates. Cases diagnosed through April 1999 were also confirmed by one or two study physician(s) through reviewing the medical records. The study protocol was approved by the institutional review boards of both the National Public Health Institute in Finland and the National Cancer Institute in the United States We identified 353 exorine pancreatic cancer cases with serum collected at baseline during 23.9 years of follow-up (1985–2009). Controls were alive and cancer-free at the time of case diagnosis and matched to cases on age at randomization and month of baseline blood collection.

H. pylori multiplex serology assay

A multiplex serology assay was used to determine serostatus of antibodies against 15 *H. pylori* specific antigens (10). Four blinded replicate QC samples were randomly inserted in each plate to determine assay reliability. Among these quality control samples, six of the 15 antigens displayed 100% agreement/concordance (GroEL,

UreA, NapA, catalase, HcpC, Omp), 3 antigens displayed 99% concordance (CagA, VacA, Cad), and 6 antigens displayed between 90 and 97% (Cagδ, HpaA, HP0231, HyuA, CagM, HP0305). We created dichotomous variables for each antigen using cutoff points, as previously described and validated (10, 11). Assay validation used sera from the German National *H. pylori* Reference Center and four independent methods of *H. pylori* detection (10, 11). The antigen-specific cutoffs were calculated (three times the SD of the median fluorescence intensity for each antigen, excluding positive outliers) in 46 *H. pylori* negative sera run within the assay. We defined the overall *H. pylori* positivity as those seropositive to ≥4 antigens, as in previously published studies (10, 11).

Statistical analysis

The distributions of selected characteristics (Table 1) of cases and controls were compared using Wilcoxon rank sum test for continuous variables and χ^2test for categorical variables. We examined potential confounders (shown in table 1) and found none of them changed risk estimates by more than 10%. The trial interventions did not change or modify our results because the blood samples were collected at baseline before the trial intervention and the intervention did not affect the outcome of pancreatic cancer ($P_{\text{interaction}} > 0.05$; ref. 12). We present OR for pancreatic cancer and each studied antigen according to both crude and adjusted conditional regression model (adjusted for age, number of cigarettes per day, years smoked). A two-sided *P* value of less than 0.05 was considered statistically significant. We examined the interaction between *H. pylori* seropositivity and ABO blood type by stratified analyses using adjusted unconditional logistic regression models. The blood types of O and non-O were determined by SNP rs505922 as previously described (genotype TT as O type, others as non-O blood type; ref. 13). Only a subset was included for the analysis due to data availability for SNP rs505922 (37 cases and 54 controls with O blood type, 136 cases and 116 controls with non-O blood type).

Results

Table 1 presents the selected baseline characteristics for 353 cases and 353 matched controls. Cases and controls did not significantly differ by any of the selected baseline characteristics. The mean interval between baseline serum collection and diagnosis was 11.6 years (follow-up time up to 23.9 years), and the median age at pancreatic cancer diagnosis was 69 years old.

Table 2 shows that none of the examined antigens to *H. pylori*, nor the overall *H. pylori* seropositivity (defined as seropositive if the subject is seropositive to four or more antigens), were significantly associated with pancreatic cancer (overall seropositivity: OR, 0.85; 95% CI = 0.49–1.49).

We stratified our analyses by years of follow-up [median and interquartile for each tertile: 4.6(2.8–7.7);

Table 1. Characteristics of pancreatic cancer cases and control subjects (median and interquartile range or proportion)

Characteristic	Case subjects ($n = 353$)	Control subjects ($n = 353$)	Two-sided P[a]
Age, y (range)	57 (53–61)	57 (54–61)	0.90
Body mass index, kg/m^2	26.1 (23.8–28.3)	26.1 (23.8–28.8)	0.90
Primary school education or less, %	73%	80%	0.32[b]
Living in a city, %	65%	59%	0.29[b]
History of, %			
Peptic or duodenal ulcer	16.4%	15.6%	0.78[b]
Pancreatitis	1.98%	0.57%	0.10[b]
Gallstones	4.82%	5.38%	0.74[b]
Diabetes mellitus	5.38%	5.10%	0.87[b]
Family history of pancreatic cancer, %	3.68%	1.98%	0.18[b]
Smoking habits			
Years of smoking (range)	36 (32–42)	37 (32–42)	0.93
Total cigarettes smoked/day (range)	20 (15–25)	20 (15–25)	0.32
Dietary intake, per day			
Energy, kcal	2587 (2105–3074)	2606 (2138–3093)	0.70
Total fat intake[c]	45.6 (41.9–49.5)	45.8 (41.9–49.5)	0.89
Missing less than 10 teeth, %	33%	28%	0.22[b]

[a]Wilcoxon rank sum test.
[b]χ^2 tests.
[c]Energy adjusted using the residual method.

Table 2. Odds ratio and 95% confidence intervals for pancreatic cancer and *Helicobacter pylori* serology among all sampled subjects

	Case ($n = 353$)		Control ($n = 353$)		OR (95%CI)[b]	
Antibody	**No.**	**Positive**	**No.**	**Positive**	**Crude**	**Adjusted**
Overall[a]	325	92%	328	93%	0.85 (0.49–1.49)	0.86 (0.49–1.51)
GROEL	300	85%	300	85%	0.98 (0.65–1.48)	1.01 (0.67–1.52)
UREA	281	80%	266	75%	1.26 (0.88–1.80)	1.29 (0.89–1.86)
HP0231	230	65%	245	69%	0.82 (0.60–1.12)	0.80 (0.58–1.11)
NAPA	260	74%	266	75%	0.90 (0.64–1.27)	0.91 (0.65–1.29)
HP0305	238	67%	256	73%	0.78 (0.56–1.07)	0.79 (0.57–1.10)
HPAA	170	48%	171	48%	0.98 (0.73–1.32)	0.98 (0.73–1.33)
CAG_DELTA	195	55%	204	58%	0.90 (0.67–1.21)	0.90 (0.66–1.22)
CAGM	131	37%	126	36%	1.06 (0.78–1.44)	1.06 (0.78–1.44)
CAGA	258	73%	258	73%	0.99 (0.71–1.38)	1.00 (0.71–1.42)
HYUA	271	77%	277	78%	0.90 (0.63–1.28)	0.91 (0.64–1.30)
CATALASE	288	82%	271	77%	1.32 (0.92–1.90)	1.34 (0.93–1.92)
VACA	234	66%	243	69%	0.88 (0.64–1.21)	0.90 (0.66–1.23)
HCPC	226	64%	226	64%	0.99 (0.73–1.35)	1.01 (0.73–1.41)
CAD	116	33%	114	32%	1.02 (0.75–1.40)	1.04 (0.76–1.42)
OMP	288	82%	291	82%	0.93 (0.63–1.36)	0.96 (0.64–1.44)

[a]The subjects are considered as *H. pylori* seropositive if the subject is seropositive to four or more antigens.

[b]ORs and 95% CIs calculated using conditional logistic regression. The adjusted model was adjusted for age, number of cigarettes per day, and years smoked.

11.7(10.4–13.1); 18.3(16.5–20.1)] and observed no remarkable differences in risk estimates over time (data not shown). We stratified our analyses by O or non-O blood type and found no significant association between *H. pylori* seropositivity and pancreatic cancer risk among subjects with non-O blood type or among subjects with O–blood type (data not shown).

Discussion

Contrary to our previous study conducted in the same cohort (OR, 1.87; 95% CI, 1.05–3.34; ref. 4), we found no association between seropositivity to *H. pylori* and risk of pancreatic cancer. The disparity in results might be related to the extended follow-up or the different technologies used to measure *H. pylori*. Our previous study used ELISA for whole cell *H. pylori* and CagA using crude antigen preparations or individual denatured proteins while our current study used a multiplex assay that quantifies specific antibodies directed against conformational epitopes present on the soluble, affinity-purified GST fusion proteins representing 15 *H. pylori* antigens used in multiplex serology. Both assays measured CagA, however the multiplex assay is considered more sensitive than ELISA.

Six previous studies have evaluated the association between *H. pylori* carriage and pancreatic cancer by ELISA, of which three were case–control and three were prospective. The first, a case–control study conducted in Austria, included 92 pancreatic cancer cases and a control group consisting of 35 with colorectal cancer and 27 healthy volunteers and reported significant positive association between seropositivity to *H. pylori* and pancreatic cancer (OR, 2.1; 95% CI, 1.1–4.1; ref. 3). Four others (our previous study excluded) reported no association (4). One case–control study from Sweden included 45 pancreatic cases and 45 controls and showed a non-significant positive association (OR, 1.55; 95% CI, 0.62–3.88; ref. 8). The largest study was a case–control study that included 373 cases and 690 controls in USA (OR, 1.34; 95% CI, 0.94–1.92; ref. 7). Limitations of the case–control studies include their cross-sectional design with potential for survival and selection biases and the inability to establish temporal associations. Beyond our previous study conducted in the ATBC study population, one prospective study is the study of residents in Malmö, Sweden, which included cases and controls matched by birth-year cohorts (born 1921–1949) and showed a non-significant positive association (87 cases and 263 controls; OR, 1.25; 95% CI, 0.75–2.09; ref. 6). Another performed in adult subscribers to the Kaiser Permanente Medical Care Program enrolled for multiphasic health checkup from 1964 to 1969, and showed a non-significant inverse association (104 cases and 262 controls; OR, 0.85; 0.49–1.48; ref. 5).

The relationship between *H. pylori* infection and pancreatic cancer might be complex and influenced by multifactorial underlying genetic susceptibility, immunologic, or environmental exposures. For instance, the aforementioned large case–control study in the United States showed an association between *H. pylori* seropositivity and pancreatic cancer risk among individuals

with non-O blood type (OR, 1.37; 95% CI, 1.02–1.93), but not among those with O blood types (7). A similar pattern was not observed in our study, which might be due to the limited sample size of participants with both ABO genotyped and *H. pylori* data. In addition, the inconsistent results across studies might also be explained by unmeasured or poorly measured confounding factors or variation in measurement of *H. pylori*.

Strengths of our study include prospective study design with prediagnostic blood samples, relatively large number of pancreatic cancer cases, and long follow-up. The limitations of our study include its restriction to male smokers and limited sample size for the analyses of interaction between *H. pylori* seropositivity and ABO blood type. Our findings should be confirmed in populations that include non-smokers and women.

In conclusion, we found no association between seropositivity to *H. pylori* (defined by multiplex assay against 15 *H. pylori* antigens) and risk of subsequent pancreatic cancer in ATBC cohort.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: G. Yu, M. Pawlita, R.Z. Stolzenberg-Solomon
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G. Murphy, A. Michel, S. Männistö, D. Albanes, M. Pawlita, R.Z. Stolzenberg-Solomon
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