Helicobacter Pylori Infection and Risk of Death From Cardiovascular Disease Among the Japanese Population: a Nested Case-Control Study within the JACC Study

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Aim: An increasing number of studies have linked Helicobacter pylori (H. pylori) infection to extra-gastric diseases; however, the role of H. pylori in the pathogenesis of cardiovascular disease (CVD) remains controversial. We examined the association between H. pylori infection and risk of death from coronary heart disease (CHD) and stroke in a nested case-control study within a large prospective cohort study of Japanese subjects.

Methods: The cases were 627 subjects who died from CHD and stroke during the follow-up period until December 31, 2003, and 627 control subjects were selected and matched to cases on sex, age, and area. Commercial immunoassay IgG enzyme-linked immunosorbent assay kits were used for the determination of the seropositivity for H. pylori. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using a conditional logistic regression model.

Results: Overall, H. pylori infection was not associated with CVD (CHD and stroke) mortality risk. The multivariable OR was 0.96 (0.76–1.21) for the H. pylori positive subjects in comparison with H. pylori negative subjects. As for the subtype of CVD, H. pylori appears to be inversely associated with the risk of death from CHD, with an OR of 0.79 (0.50–1.25), but this was not statistically significant. No significant association was observed between H. pylori infection and stroke, with an OR of 1.02 (0.78–1.33).

Conclusion: The results of this nested case-control study suggest that there is no association between H. pylori infection and CHD and stroke mortality risk in otherwise healthy, elderly Japanese individuals.


Key words: Helicobacter pylori, Cardiovascular disease, Nested case-control study

Introduction

Helicobacter pylori (H. pylori) colonizes the human stomach, often causing gastritis and peptic ulcers¹. There is also convincing evidence that H. pylori infection is causally related to gastric cancer². An increasing number of studies have linked H. pylori infection to extra-gastric diseases, including iron deficiency anemia, idiopathic thrombocytopenic purpura, and cardiovascular disease (CVD)³. The positive association of H. pylori infection with CVD was first reported in a case-control study⁴ in which individuals with H. pylori seropositivity had an increased risk, approximately two-fold, of developing CVD. However, not all subsequent studies have supported these results⁵-13. For example, a 1998 meta-analysis of 18 studies found no significant association between H. pylori and risk of coronary heart disease (CHD), a major component of CVD¹⁴. Furthermore, neither of the two recent cohort studies showed a significant, positive associa-
tion between these outcome measures. Although the role of *H. pylori* in the pathogenesis of CVD remains controversial, the proposed mechanism of action, i.e., the changes in inflammatory markers in *H. pylori* positive individuals may be associated with increased risk of CVD, persists.

The prevalence of *H. pylori* infection is approximately 50% among Japanese adults aged over 50 years, though a decreasing trend has been observed in various age groups. To our knowledge, only one prospective, nested case-control study has examined the relationship between *H. pylori* infection and risk of myocardial infarction and stroke in Japanese subjects, which found no significant associations. Given that CVD is the second leading cause of death in Japan, it would be highly beneficial to identify any novel, significant risk factors whereby avoidance may help to reduce overall CVD risk. Thus, we have prospectively examined the association between *H. pylori* infection and risk of death from CVD in an attempt to identify such a risk factor.

## Methods

### Study Population

We conducted a nested case-control study within the Japanese Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study), a large, prospective study that investigated potential associations between various lifestyle factors and various outcome measures including cancer and CVD mortality. From 1988 to 1990, we recruited 110,585 people aged 40–79 years from 45 locations throughout Japan. At baseline, participants completed a self-administered questionnaire covering demographic characteristics, medical history, and lifestyle factors, and approximately 35% of the participants provided a blood sample. Information on hypertension and diabetes was based on self-report. For alcohol drinking, the cohort participants were asked to describe the drinking status, frequency, and amount. No significant differences were noted in characteristics, such as age, body mass index (BMI), location, education level, and medical history, between those who donated the blood sample and those who did not. The sera were separated from these samples as quickly as possible after blood withdrawal and then stored at −80°C until analysis.

The causes of death were noted from the death certificates and classified according to the International Classification of Diseases, tenth revision (ICD-10). The codes were I20-I25 for CHD and I60-69 for stroke. Of the 27,410 deaths from all the causes documented during the follow-up period of the JACC Study, 4287 men and 4043 women died from CVD (18). For our nested case-control study, the cases that were selected met the criteria of having provided a baseline blood sample and having died from CHD and stroke, as determined by the JACC Study, during the follow-up until December 31, 2003. Those who were identified in the JACC Study as being disease-free at the time the study ended were then selected from the remaining participants as controls. The controls were matched with the cases in terms of sex, age, and area at a ratio of 1:1. The cohort participants were excluded if they reported a history of stroke or myocardial infarction at baseline. As a result, 627 cases and 627 control subjects were included in the present study.

### Measurement of Metabolic Profiles

Blood pressure was measured using a mercury sphygmomanometer. Serum total and high-density lipoprotein (HDL) cholesterol was measured using the enzymatic method with an automatic analyzer (Hitachi 7600-210, Hitachi Medical Corp., Tokyo, Japan). Two commercial immunoassay IgG enzyme-linked immunosorbent assay (ELISA) kits were used for determining the seropositivity for *H. pylori*. J-HM-CAP (Kyowa Medex, Japan) was used for cases who died before 1998, as well as their matched controls, whereas for cases who died between 1998 and 2003, and their controls, E-Plate (Eiken Inc., Tokyo) was used because of the termination of J-HM-CAP production. Assays were performed according to the manufacturer’s instructions and seropositivity was defined using recommended cutoff values. Both ELISA kits used antigens derived from Japanese *H. pylori* strains and have been shown to perform better than other commercial ELISA kits. All laboratory tests were conducted at the laboratory of the Department of Public Health, Aichi Medical University School of Medicine, with staff blinded to the case-control status.

### Statistical Analysis

The sample size was estimated based on the following assumptions: odds ratio = 2, *H. pylori* seropositivity among control subjects = 75%, alpha = 5%, power = 80%, and case/control ratio = 1. Under the above assumptions, 212 cases and 212 controls are needed. Our study had a sufficient sample size to detect statistically significant associations. Characteristics between cases and controls were compared using the Mann–Whitney tests for continuous variables and chi-square tests for discrete variables. Conditional logistic regression models were used to estimate crude and multivariable-adjusted odds ratios (OR) and 95% confidence intervals (CI) for CHD or stroke death in rela-
Table 1. Characteristics of cases and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=627)</th>
<th>Controls (n=627)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>307</td>
<td>307</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>320</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>66.5 ± 8.7</td>
<td>67.2 ± 9.4</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.8 ± 3.0</td>
<td>22.7 ± 3.2</td>
<td>0.72</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>29.2</td>
<td>19.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>4.2</td>
<td>9.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>24.6</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>15.5</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>53.1</td>
<td>46.9</td>
<td></td>
</tr>
<tr>
<td>Drinking status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinkers</td>
<td>39.6</td>
<td>40.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Ex-drinkers</td>
<td>5.3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Current drinkers</td>
<td>48.9</td>
<td>48.3</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>136.2 ± 17.9</td>
<td>142.2 ± 19.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mgHg</td>
<td>78.3 ± 10.9</td>
<td>81.8 ± 11.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>203.5 ± 40.2</td>
<td>199.0 ± 41.3</td>
<td>0.03</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol, mg/dL</td>
<td>44.9 ± 15.2</td>
<td>43.7 ± 14.9</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> seropositivity, %</td>
<td>75.9</td>
<td>76.4</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Plus minus values are mean ± standard deviation
The percentages do not add up to 100% due to missing values.

Results

Table 1 outlines the characteristics of cases and controls. The cases were more likely to have a history of hypertension and to be a current smoker, in comparison with controls. The cases were less likely to have a history of diabetes when compared with controls. Mean SBP and DBP was lower in cases than that in controls, and the mean total cholesterol was higher in cases than that in controls. Importantly, the prevalence of *H. pylori* seropositivity was comparable between cases and controls, 75.9% and 76.4%, respectively.

Table 2 summarizes the associations between *H. pylori* infection and risk of death from CHD and stroke. Overall, the multivariable OR was 0.96 (0.76–1.21) for *H. pylori*-positive subjects compared with *H. pylori*-negative subjects, after adjustment for potential confounding factors. *H. pylori* was inversely associated with the risk of death from CHD (OR, 0.79; 95% CI, 0.50–1.25). However, the association was not statistically significant. Similarly, no significant association was observed between *H. pylori* infection and stroke (OR, 1.02; 95% CI, 0.78–1.33).

Discussion

To date, there is insufficient evidence on the association between *H. pylori* infection and CVD risk in Asian countries. Our results suggest that *H. pylori* is not significantly associated with the risk of death from CHD or stroke within a Japanese population group.

The majority of previous research on the above association was conducted in Western countries and findings have been inconsistent and inconclusive. It is possible, however, that variations in the study...
methodology and the adjustment for potential confounders has contributed to inconsistent results in the literature. For example, the majority of studies showing a positive association are case-control or cross-sectional studies. However, these studies may have been limited by a small sample size and lack appropriate adjustment for potential confounders. In contrast, the findings from several prospective studies involving a large sample size showed no or only modest association between *H. pylori* infection and overall CVD mortality.

Although a review of evidence till date does not support the major role of *H. pylori* in the development of CVD, it is possible that *H. pylori*, especially specific CagA positive *H. pylori* strains, is associated with the risk of certain subtypes of stroke or CHD. A prospective analysis of data from the National Health and Nutrition Examination Survey III showed an inverse association between *H. pylori* and stroke mortality, with a hazard ratio of 0.69 (0.44–1.08) for individuals infected with *H. pylori*). A case-control study found that chronic *H. pylori* infection was significantly associated with increased risk of ischemic stroke in Japanese, with an OR of 2.57 (1.09–6.08) for all subtypes combined. In contrast, our nested case-control study did not show any significant associations between *H. pylori* and subtypes of stroke, including subarachnoid hemorrhage, cerebral hemorrhage, and cerebral infarction (data not shown). Further studies are required to clarify the underlying mechanisms of varied associations amongst differing populations. In addition to sample size and adjustment for confounders, another possible contributing factor is differing measuring equipment across studies. The effect of measurement kits on the results should be considered because the validity of serologic tests may vary depending on the type of antigens used in ELISA kits. Although both ELISA kits in this study used the antigen derived from Japanese subjects and have been shown to perform better when compared with other commercial ELISA kits, data were lacking on the direct comparison of performance for the ELISA kits used. Because separate analyses for subjects measured using either J-HM-CAP or E-Plate ELISA kits showed similar results, we chose to combine data sets derived from the two ELISA tests.

Table 2. The association between *H. pylori* infection and CVD mortality risk

<table>
<thead>
<tr>
<th></th>
<th>Seropositivity</th>
<th></th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>CVD (CHD + stroke)</td>
<td>151/148</td>
<td>476/479</td>
<td></td>
</tr>
<tr>
<td>Case/Control</td>
<td>1.00</td>
<td>0.99 (0.82–1.19)</td>
<td>1.00</td>
</tr>
<tr>
<td>Multivariate OR (95%CI)</td>
<td>1.00</td>
<td>0.96 (0.76–1.21)</td>
<td>1.00</td>
</tr>
<tr>
<td>CHD (I20–25)</td>
<td>40/34</td>
<td>139/145</td>
<td></td>
</tr>
<tr>
<td>Case/Control</td>
<td>1.00</td>
<td>0.91 (0.64–1.29)</td>
<td>1.00</td>
</tr>
<tr>
<td>Multivariate OR (95%CI)</td>
<td>1.00</td>
<td>0.79 (0.50–1.25)</td>
<td>1.00</td>
</tr>
<tr>
<td>Stroke (I60–69)</td>
<td>111/114</td>
<td>337/334</td>
<td></td>
</tr>
<tr>
<td>Case/Control</td>
<td>1.00</td>
<td>1.02 (0.82–1.26)</td>
<td>1.00</td>
</tr>
<tr>
<td>Multivariate OR (95%CI)</td>
<td>1.00</td>
<td>1.02 (0.78–1.33)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval; CVD: cardiovascular disease; CHD: coronary heart disease
OR and CI were estimated from conditional logistic regression models
Multivariate OR: adjusted for body mass index, cigarette smoking, alcohol drinking, and systolic blood pressure.
The outcome was CHD and stroke mortality.
jects, and found no significant differences in blood pressure, history of diabetes, serum total, or HDL cholesterol levels between the two groups (data not shown). The differences in sample size and measurement methods may partly account for the inconsistent findings on the metabolic profiles related to H. pylori infection.

Strengths of this study include its prospective design, a relatively large sample size, and the ability to adjust for potential confounders such as smoking, alcohol consumption, and risk factors for CVD that had already been identified. In contrast, there were several limitations to the study. Firstly, one concern is that using death certificates may lead to the misclassification in the causes of death. Unfortunately, incidence data on CVD were unavailable for the present analysis. Secondly, we did not have data on CagA status, which is an important virulence factor for H. pylori. This is relevant because previous studies involving populations of European ancestry have suggested a positive association among individuals with CagA-positive-H. pylori strains and atherosclerosis. However, we believe that this issue may not be a concern because the majority of H. pylori strains were CagA-positive in East Asian populations, including those of Japan. Thirdly, the data were lacking on the eradication therapy for H. pylori. The risk estimates might have been biased if case and control subjects had a different proportion of eradication therapy. Finally, although we adjusted for known CVD risk factors, we could not rule out the possibility that other confounders such as exercise and socioeconomic status may have influenced these estimates. Exercise data were unavailable for the present analysis. For socioeconomic status, prior research has consistently demonstrated that a lower socio-economic status and/or a low level of education is associated with an increased prevalence of H. pylori infection.

In conclusion, the results of this study indicate no association between H. pylori infection and CVD (CHD and stroke) mortality risk in otherwise healthy, elderly Japanese individuals. Given the complex role of H. pylori in health and disease, this association needs to be further investigated through additional prospective studies in Asian countries that have a high prevalence of H. pylori infection.

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Conflicts of Interest

The authors have no financial relationship to disclose.

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