Helicobacter pylori infection is related to atheroma in patients undergoing coronary angiography

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Abstract

Objectives: Helicobacter pylori infection has been related to an increased risk of ischaemic heart disease (IHD) possibly by raising plasma fibrinogen. The evidence for this association is conflicting. Furthermore, no attempt has been made to distinguish between an effect on atheroma and thrombosis. We have determined the association of H. pylori status with IHD assessed by coronary angiography. We have also evaluated the influence of H. pylori infection on haemostatic factors.

Methods: Caucasian patients undergoing coronary angiography for suspected IHD were recruited. H. pylori status was determined by serology Helico G. Plasma fibrinogen was measured by the Clauss assay. Coronary angiograms were assessed and significant atheroma defined as ≥ 50% stenosis. A history of myocardial infarction was ascertained by WHO criteria.

Results: 292 patients were recruited (median age 59, 95 female; 204 (70%) patients had IHD and 185 (64%) of all patients were H. pylori-positive; 68% patients with IHD were H. pylori-positive compared with 50% without IHD (P = 0.003). When adjusted for other risk factors by logistic regression, H. pylori remained significantly associated with IHD (odds ratio = 2.4, 95%CI = 1.2–5.1, P = 0.02). H. pylori status was not related to a history of myocardial infarction. Circulating levels of PAI-1, vWF, Factor VII and fibrinogen were not related to H. pylori status.

Conclusion: This study suggests that infection with H. pylori is associated with coronary atheroma, but that this relationship is unlikely to be mediated through raised plasma fibrinogen.

Keywords: Helicobacter pylori; Coronary atheroma; Thrombosis

1. Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in the developed world. Numerous risk factors have been demonstrated for CAD, but these explain the development of the disease in only a proportion of cases [1]. Helicobacter pylori (H. pylori) infection causes a long-term chronic inflammatory response in gastric mucosa [2] and it is postulated that this could lead to a chronic low-grade release of systemic inflammatory mediators resulting in an increased risk of CAD [3]. Initial studies suggested that H. pylori infection was associated with CAD and that the underlying mechanism might be an increase in plasma fibrinogen [4]. However, this association has not been confirmed in other studies with plasma fibrinogen being similar in infected and uninfected cases [5,6]. These conflicting results could be due to the different methods used to classify CAD. Trials have used the Rose angina questionnaire [5] and ECG abnormalities [4] which are less accurate than coronary angiography in diagnosing the presence of CAD. The only study to assess CAD cases by coronary angiography used general practice asymptomatic controls that were not investigated and may have
had subclinical disease [7]. This study suggested that *H. pylori* infection increased risk [3] but failed to reach statistical significance possibly due to the relatively small sample size or to the presence of asymptomatic CAD in the control group. The aims of this study were to investigate the association between *H. pylori* status and angiographically proven CAD in a large series of patients using as a control group patients without significant atheroma at coronary angiography. We have also attempted to distinguish between a possible association between *H. pylori* and atheroma, on the one hand, and thrombosis assessed by a history of myocardial infarction, on the other. In order to investigate a possible mechanism for this association, we have examined the relationship between *H. pylori* status, plasma fibrinogen, circulating levels of von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1) and Factor VII.

### 2. Methods

#### 2.1. Subjects

A total of 292 Caucasian patients admitted to Leeds General Infirmary for routine angiography to investigate chest pain or suspected CAD were recruited. Each subject gave informed consent. This was part of a larger investigation of haemostatic factors and genetic polymorphisms in relation to the risk of CAD which was approved by the United Leeds Teaching Hospitals (NHS) Trust Research Ethics Committee.

Smoking history was determined and classified as those who had ever smoked (to include present smokers and previous smokers up to 10 years prior to date of angiography), and non-smokers. Socio-economic status was evaluated using the Townsend Index of Deprivation [8] estimated from patients’ post-code. Blood pressure was measured with subjects lying and to the nearest 2 mmHg using the Dinamap automated sphygmomanometer (Critikon, 1846 SX/P Version 086). The diagnosis of myocardial infarction (MI) was ascertained from patients’ hospital records using the WHO criteria of at least 2 out of 3 from ST elevation of 1 mm in 2 or more successive leads, typical chest pain longer than 20 min duration, and creatinine kinase rise of more than twice the baseline value. Patients who were reported to have had a history of MI but did not meet the WHO criteria were excluded from the analysis. Body mass index was calculated from weight in kilograms divided by height in metres squared.

#### 2.2. Analytical methods

Patients were studied between 07.00 and 10.30 h after an overnight fast of 12 h. Free-flowing blood samples were taken using a 19G butterfly needle from an antecubital vein. Blood was taken into 0.9% citrate on ice for PAI-1 assay and centrifuged at 2560 × g and 4°C for 30 min and snap-frozen in liquid nitrogen prior to storage at −40°C. Plasma PAI-1 antigen was measured by ELISA (Imulys, Biopool, Umeå, Sweden). Fibrinogen was measured by the Clauss method, vWF was measured by an ELISA (with antibodies from Dako, Sweden), Factor VII was assayed with an ACL 3000 (instrumentation laboratory, Warrington, UK) using Factor VII deficient plasma rabbit thromboplastin as reagents. Samples were also collected for measurement of total cholesterol and triglyceride using a Hitachi 747 automatic analyser (Boehringer Mannheim, Mannheim, Germany). *H. pylori*-specific IgG titres were measured using a commercial enzyme-linked immunosorbent assay (Porton Cambridge Helico G test). Patients with titres >10 U/l were defined as *H. pylori*-positive, which has previously been shown to have an 88% sensitivity and 92% specificity in the Leeds population [9].

Selective coronary angiography was performed by the Judkins method. Analysis of the angiograms were done visually according to the clinical routine by cardiologists who were blinded to *H. pylori* status. Coronary angiography was classified as single, double or triple-vessel disease on the basis of ≥50% stenosis in each affected vessel in the 3 major coronary arteries or their branches. Patients with <50% stenosis were defined as not having significant atheroma.

#### 2.3. Statistics

Differences in frequencies of categorical variables (e.g., gender, diabetes mellitus) between groups were assessed by chi-squared test. Differences in means of continuous variables between groups were compared by Student’s *t*-test, and ANOVA if more than 2 groups (extent of CAD) were being assessed. Logistic and linear regression models were used to assess the independent association of various risk factors to CAD, and haemostatic factors (such as fibrinogen), respectively. Statistical analyses were performed using SPSS for windows (version 6.1).

### 3. Results

A total of 292 patients with a mean age of 58.5 ± 10.7 years (age range 33–83 years, 195 (69%) male) were studied; 204 (70%) had angiographically significant CAD, 84 (29%) had no significant CAD and angiography was unsuccessful in 4 (<1%) cases. Patients with evidence of significant CAD had a higher prevalence of established risk factors (Table 1). Similarly these patients had higher circulating levels of PAI-1 (21.3 ± 2.3 vs 16.4 ± 2.5 ng/ml) (*P* = 0.02). There was trend towards higher circulating levels of fibrinogen (3.4 ± 0.7 vs 3.2 ± 0.8 g/l) (*P* = 0.08), and vWF (1.2 ± 0.4 vs 1.1/ml ± 0.4 IU) (*P* = 0.08) in patients with significant atheroma. There was no difference, however, in circulating Factor VII levels (122.1 vs 121.7%).
There were 185 (63%) *H. pylori*-positive and 103 (36%) *H. pylori*-negative patients. There was a significant relationship between *H. pylori* infection and the presence of CAD; 68% of patients with CAD were *H. pylori*-positive compared with 50% of patients without significant CAD (odds ratio = 2.17, 95% CI = 1.29–3.65, χ² P = 0.003). *H. pylori* infection remained significantly associated with atheroma after adjusting for age, sex, smoking, cholesterol, serum triglyceride, diabetes mellitus, socio-economic status, systolic blood pressure, PAI-1, plasma fibrinogen, factor VII and vWF in a logistic regression model with no change in the odds ratio (OR = 2.43, 95% CI = 1.16–5.13, P = 0.02) (Table 2). There was no relationship, however, to the extent of coronary atheroma (Table 3). Of the 84 patients with non-significant coronary atheroma, 58 patients had ‘clean’ coronary arteries, and 26 patients had minimal atheroma (<50%) in one or more coronary arteries. Of the 58 subjects, 31 (53%) were *H. pylori*-negative and 27 (47%) *H. pylori*-positive. Of the 26 patients, 15 (58%) were *H. pylori*-positive and 11 subjects (42%) *H. pylori*-negative. All together 230 patients had some angiographic evidence of atheroma regardless of the degree of stenosis. Of these, 155 patients (67%) were *H. pylori*-positive. Hence, inclusion of the minimally diseased group in those with significant atheroma did not appear to change the overall results. Further analysis of subgroups within the proportion of patients with no significant atheroma could not be carried out because of the small numbers involved.

There was also no significant relationship between a history of MI and *H. pylori* status. 111/179 (62%) of patients without MI were *H. pylori*-positive compared with 70/106 (66%) of those fulfilling WHO criteria for MI (χ² = 0.5, P = 0.5).

There were no significant differences in the haemostatic factors for CAD between *H. pylori*-positive and -negative patients with the exception of plasma fibrinogen (*H. pylori*-negative 3.2 ± 06, *H. pylori*-positive 3.4 ± 0.8, P = 0.03). However, this positive relationship was not statistically significant after adjusting for age, sex, socio-economic status, body mass index, and smoking history (odds ratio = 1.1, 95% CI = 0.99–1.2, P = 0.1).  

### Table 1  
Clinical, biochemical and haemostatic characteristics in relation to the presence or absence of significant CAD

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt; 50% stenosis (n = 84)</th>
<th>&gt; 50% stenosis (n = 204)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.9 ± 9.0</td>
<td>60.0 ± 8.8</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>40/44</td>
<td>152.52</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.2 ± 4.0</td>
<td>27.1 ± 3.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Townsend index</td>
<td>2.9 ± 1.4</td>
<td>3.0 ± 1.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>139 ± 22.9</td>
<td>150 ± 25.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (2%)</td>
<td>20 (10%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>32 (39%)</td>
<td>98 (49%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.0 ± 1.2</td>
<td>6.4 ± 1.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.9 ± 1.4</td>
<td>2.3 ± 1.4</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values shown are mean (standard deviation), and antilogged where appropriate.  
Student’s t-test, bχ²-test. *Mann-Whitney U*-test.

### Table 2  
Risk factors which were significant in a logistic regression model with atheroma as dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95%CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> +ve</td>
<td>2.43</td>
<td>1.16–5.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Male gender</td>
<td>8.86</td>
<td>2.89–27.04</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8.16</td>
<td>0.75–89.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.96</td>
<td>0.86–4.45</td>
<td>0.11</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.44</td>
<td>1.01–2.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Age</td>
<td>1.09</td>
<td>1.04–1.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1.01</td>
<td>0.99–1.03</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Townsend index, vWF, plasma fibrinogen, serum triglyceride, PAI-1, Factor VII and body mass index were also included in the model.

### Table 3  
*H. pylori* status in relation to the presence and extent of atheroma

<table>
<thead>
<tr>
<th>HP-negative</th>
<th>HP-positive</th>
<th>χ², df, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No atheroma</td>
<td>42 (50%)</td>
<td>42 (50%)</td>
</tr>
<tr>
<td>Atheroma</td>
<td>64 (51%)</td>
<td>140 (69%)</td>
</tr>
<tr>
<td></td>
<td>χ² = 8.7, df = 1, P = 0.003</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>42 (50%)</td>
<td>42 (50%)</td>
</tr>
<tr>
<td>Single</td>
<td>18 (30%)</td>
<td>43 (70%)</td>
</tr>
<tr>
<td>Double</td>
<td>16 (30%)</td>
<td>37 (70%)</td>
</tr>
<tr>
<td>Triple</td>
<td>30 (33%)</td>
<td>60 (67%)</td>
</tr>
<tr>
<td></td>
<td>χ² = 8.9, df = 3, P = 0.03</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

Coronary artery disease involves the processes of atheroma formation, plaque rupture and superimposed thrombus formation. Since these are distinct processes, it is possible that the risk factors that relate to atheroma may not necessarily be related to thrombosis. In this study we have assessed patients with atheroma by angiography, and thrombosis by a history of myocardial infarction. Our results suggest that *H. pylori* infection is associated with the presence of atheroma but not with thrombosis. The
association between \textit{H. pylori} infection and atheroma was of a similar magnitude to more 'conventional' risk factors such as smoking, serum cholesterol and systolic blood pressure. This conflicts with the results of a much larger study using the Rose angina questionnaire to diagnose angina pectoris [5]. As the authors acknowledged, this method of defining CAD is not as accurate as coronary angiography and may have resulted in significant case misclassification which can bias any true associations towards zero. Our data support previous studies using ECG abnormalities or angiography to diagnose CAD [3,4] though these studies were not designed to make a distinction between atheroma and thrombosis.

A report that \textit{H. pylori} was associated with increased plasma fibrinogen suggested a mechanism by which this infection could cause CAD [4]. Fibrinogen has emerged as a potent predictor of CAD and future coronary events as shown in a number of large prospective studies including the Framingham [10], Northwick Park Heart [11], and PROCAM [12] studies. FVII:C independently predicted fatal coronary events in the Northwick Park Study [11]. In the European Concerted Action on Thrombosis and Disabilities (ECAT) study, vWF as well as fibrinogen levels were identified as independent and significant predictors of MI or sudden death in patients who presented with angina [13]. Raised levels of PAI-1 resulting in suppression of fibrinolysis have been found in a number of studies to relate to the presence of CAD (assessed by angiography) [13,14]. We have found a trend towards an increase in plasma fibrinogen in \textit{H. pylori}-positive patients, but this did not achieve statistical significance. We have previously reported that \textit{H. pylori} was not associated with plasma fibrinogen [6] and this is consistent with a large cross-sectional population survey [5]. Patel et al. [4] investigated males between the ages of 50 and 69 years whilst the negative studies have investigated both sexes over a wider age range. The significance of an increase in plasma fibrinogen in a selected group of individuals that is not confirmed by other much larger population studies is doubtful. We have also demonstrated that \textit{H. pylori} is not associated with circulating levels of Factor VII, vWF and PAI-1.

An association between \textit{H. pylori} and atheroma remains biologically plausible. \textit{H. pylori} causes an increase in the production of interleukin-6 and tumour necrosis factor in gastric mucosa [15] and this could induce a low-grade systemic acute-phase response increasing the risk of developing CAD [16]. Atherosclerosis is increasingly thought to be a chronic inflammatory disease, characterized by foci of macrophages and T-lymphocytes in the arterial wall as well as proliferation and migration of vascular smooth-muscle cells.

\textit{H. pylori} may also activate other systemic immune mediators to facilitate the development of CAD. For example, antibodies to bacterial heat-shock proteins have been reported to exhibit cross-reactivity with heat-shock proteins expressed in atherosclerotic lesions [17]. The effects of \textit{H. pylori} on immune mediators needs investigation to provide evidence for a causal association with CAD. Circulating concentrations of lipid peroxides which have been implicated in the pathogenesis of an atheromatous plaque were found to be raised in patients with gastritis associated with \textit{H. pylori}, providing a further possible mechanism for the association between the infection and atheroma.

These results may reflect selection bias as patients attending for coronary angiography with atheroma may have differed in factors such as social class compared with those who did not have atheroma. The association between \textit{H. pylori} infection and atheroma remained, however, after controlling for a large number of risk factors for CAD including social class. Furthermore, there was no reduction in the odds ratio, suggesting that residual confounding is unlikely. Childhood deprivation has been linked with CAD [18,19] and \textit{H. pylori} infection [20,21], and it is possible that \textit{H. pylori} may be acting as a marker for socio-economic status in early life. This study did not assess childhood deprivation as it is difficult to accurately establish this retrospectively. The effects of childhood poverty on CAD in adult life remain controversial and are usually not as strong as the effect of \textit{H. pylori} on CAD reported in this trial [22]. Previous studies attempting to assess childhood deprivation suggested only minor confounding of the effect of \textit{H. pylori} on CAD by this variable [3,4]. This indicates that \textit{H. pylori} is unlikely to be acting as a surrogate marker for early life experiences, but interventional studies are needed to resolve this issue.

This study provides further evidence for an association between chronic bacterial infections and CAD. Simple, effective antibiotic regimens against \textit{H. pylori} are available [23] and if the association between \textit{H. pylori} and CAD is shown to be causal in prospective interventional studies, this will have major public health implications.

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**References**


