Helicobacter pylori infection: a risk factor for ischaemic cerebrovascular disease and carotid atheroma

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Abstract

***Objectives*—Chronic *Helicobacter pylori* infection has been associated with ischaemic heart disease although the mechanism by which it mediates this effect remains unclear.The objective was to determine whether it is also a risk factor for ischaemic cerebrovascular disease**

***Methods*—A total of 238 patients and 119 controls were studied. Patients were characterised into stroke subtypes based on pathogenic mechanisms and carotid atheroma load was estimated using duplex ultrasound. *H pylori* seropositivity was determined on serum samples.**

***Results*—*H pylori* seropositivity was more common in cases (58.8% *v* 44.5%, p=0.01). The odds ratio for cerebrovascular disease associated with seropositivity was 1.78 (95% confidence interval (95% CI) 1.14–2.77), and this remained significant after controlling for other risk factors including socioeconomic status (1.63 (95% CI 1.02–2.60). *H pylori* seropositivity was associated with large vessel disease (odds ratio 2.58 (95% CI 1.44–4.63), p=0.001) and lacunar stroke (odds ratio 2.21 (95% CI 1.12–4.38), p=0.02) but not stroke due to cardioembolism or unknown aetiology (odds ratio 1.16 (95% CI 0.66–2.02), p=0.5). Mean (SD) carotid stenosis was greater in patients seropositive for *H pylori* (37.3 (29.7) *v* 27.9 (26.2)%, p=0.01). There was no difference in the prevalence of seropositivity between patients with stroke and transient ischaemic attack (59.6% *v* 58.6%, p=0.9)**

***Conclusion*—Chronic *H pylori* infection is an independent risk factor for ischaemic cerebrovascular disease and may act, at least in part, by increasing atherosclerosis.**

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Keywords: cerebral ischaemia; *Helicobacter pylori*; homocysteine; folate

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Helicobacter pylori is a recently discovered gram negative organism which causes chronic active gastric inflammation. The chronic active gastritis seems to be lifelong, unless eradicated by antibiotics.[1] Infection is strongly associated with duodenal ulceration, gastric ulceration, and gastric cancer. In the United Kingdom about 30% of 30 year olds are infected while the proportion increases to 60% in those aged over 45 years.[1 2]

Recently some small case-control studies have reported an association between *H pylori* seropositivity and ischaemic heart disease independent of conventional risk factors.[3–5] Two larger cross sectional population studies have examined the relation. Patel *et al* found ischaemic ECG to be more common in men with *H pylori* seropositivity with an odds ratio of 3.82 after adjusting for a range of socioeconomic indicators and risk factors for ischaemic heart disease.[6] Murray *et al* found that *H pylori* seropositive people had a 1.51 times risk of evidence of coronary heart disease according to the Rose angina questionnaire after controlling for social class and conventional cardiovascular risk factors.[7] A recent analysis of data from the British Regional Heart Study in 40–59 year old men found those developing myocardial infarction were more likely to be *Helicobacte*r seropositive (70% *v* 57%) although the odds ratio of 1.77 and 2.41 for all and fatal myocardial infarction were much reduced to 1.31 and 1.56 respectively after controlling for manual social class and other cardiovascular risk factors.[8]

By contrast with ischaemic heart disease there has been little data on the relation between *H pylori* seropositivity and stroke. In a pilot study we found a significant relation between *H pylori* infection and stroke.[8] In addition to the relation with myocardial infarction, the British Regional Heart Study also examined this relation; 137 cases of stroke occurred and *H pylori* seropositivity was more common in this group (68% *v* 57% in controls); however, the association was no longer significant after controlling for social class and other risk factors. In this study there were relatively few strokes and the diagnosis of stroke was made from death certificates and general practice records without any prospective clinical examination or brain imaging. Therefore cerebral haemorrhage and cerebral infarction were not distinguished. It is unlikely that *H pylori* is a risk factor for the first. The mechanism underlying an association between *H pylori* and vascular disease is uncertain. *H pylori* results in a low grade chronic inflammatory response and it has been suggested that this may promote atherogenesis by altering some cardiovascular risk factors including haemostatic factors and lipids.[6 10] In this study we extended our previous study and examined the relation between cerebrovascular disease and *H pylori* seropositivity in a larger population of

patients with well characterised ischaemic cerebrovascular disease. We hypothesised that it might be associated with large vessel atheroma and therefore stroke caused by large vessel disease, and therefore examined the relation between seropositivity and different stroke subtypes, as well as the association between seropositivity and mean internal carotid artery stenosis determined on high resolution carotid duplex ultrasound.

Methods

Two hundred and thirty eight consecutive white patients presenting with non-haemorrhagic stroke or transient ischaemic attack presenting to a neurological cerebrovascular disease service in south London, UK, were studied. Patients with asymptomatic disease or venous thrombosis were not included. A white control population was recruited from spouses of the same patients with cerebrovascular disease. We used spouse controls to improve controlling for socioeconomic status; there has been concern that the relation between *H pylori* and cardiovascular disease may be explained by socioeconomic factors which themselves are related to cardiovascular risk.[11] It has been shown that spouses tend to have similar childhood and parental socioeconomic status.[12] A ratio of two patients to each control was studied for two reasons; firstly, the larger group of patients allowed sufficient sample sizes for subgroup analysis of the relation between seropositivity and different stroke subtypes. Secondly, not all spouses were alive and therefore one to one matching was not possible. Therefore the first 72 male and 47 female controls presenting were enrolled. Controls were excluded if they had clinical cerebrovascular disease (two cases) but were included if they had ischaemic heart disease or vascular risk factors. In both patient and control groups hypertension was defined as either a systolic blood pressure>160 mm Hg, or a diastolic pressure>95 mm Hg, or current treatment with antihypertensive drugs. A smoker was defined as a current or ex-smoker. A diabetic patient was defined as non-insulin or insulin dependent. Social class was recorded and subjects were divided into class 1, 2, 3a 3b, 4, 5, or undefinable.

Duplex ultrasound was performed using an Acuson XP colour flow imager. Internal carotid artery stenosis was calculated from a combination of Doppler data (ICA systolic/CCA diastolic ratio) for stenoses greater than 50%, and using the B mode modality to measure the ratio of maximum plaque thickness to luminal diameter for lesser degrees of stenosis. Mean carotid stenosis was calculated from the mean of left and right common/internal carotid artery stenosis.

Computed tomography, carotid ultrasound, and ECG were performed in all patients and echocardiography in about 40%. Magnetic resonance imaging and magnetic resonance angiography of both the extracranial and intracranial vertebrobasilar systems was performed in most patients with posterior circulation symptoms. On the basis of clinical features and the investigation results, patients were divided into four subtypes :

(1) Large vessel disease: internal carotid or vertebral artery stenosis of≥50% with symptoms in that arterial territory. Carotid stenoses were detected on duplex ultrasound. Vertebral stenoses were detected on duplex ultrasound or magnetic resonance angiography.

(2) Lacunar stroke: a clinical lacunar syndrome with an appropriate CT infarct or a typical clinical syndrome[13] and a normal CT.

(3) Uncertain or probable cardiac embolic source: these two categories were included together as not all patients had echocardiography and transthoracic echocardiography does not detect many cardioembolic sources.

(4) Tandem pathology: more than one cause of stroke.

Serum was stored at −70°C and IgG antibodies to *H pylori* were detected using a rapid enzyme linked immunosorbent assay (ELISA; Helico-G kit, Porton, Cambridge, UK). Serology was performed blinded to case-control status. An antibody concentration of 10 units/ml was taken as indicating positive serology; the sensitivity and specificity when validated against diagnosis on samples taken at gastroscopy were 94% and 93% respectively.[14]

During the study it was suggested that low *Helicobacter* seropositivity might predispose to cardiovascular disease by raising serum homocysteine concentrations[15] and therefore in a subgroup of controls (57) and cases (132) we measured homocysteine concentrations. In addition in 54 controls and 112 cases we measured serum folate. Homocysteine was assayed by reverse phase high performance liquid chromatography (HPLC) with precolumn derivatisation and fluorimetric detection based on the method of Fiskerstrand *et al*.[16] The coefficient of variation was<8%. Folate was measured by an ion capture assay using the Abbott IMX analyser; the coefficient of variation was 6%.

Statistical analysis was performed using SPSS. Following this the relation between *Helicobacter* seropositivity and cardiovascular risk factors in the control population was determined using logistic regression analysis. The relation between risk factors and cerebrovascular disease was determined using a *t* test or χ^2 tests as appropriate, and subsequently with logistic regression analysis to control for social class and other cardiovascular risk factors. Social class was treated as a categorical variable. The relation between *H pylori* and degree of carotid stenosis was examined using student's *t* test and subsequently multiple regression to control for other risk factors

Results

H PYLORI SEROPOSITIVITY AND CARDIOVASCULAR RISK FACTORS

Within the control population age was slightly but not significantly higher in *H pylori* positive patients (mean (SD) 66.5 (10.3) years *v* 64.4 (8.8) years, p=0.16). There was no relation between *H pylori* seropositivity and hypertension (p=0.14), smoking history (p=0.85),

Table 1 Characteristics of the cerebrovascular disease (CVD) and control populations

	CVD patients (n=238)	*Controls (n=119)*	*Odds ratio (95% CI), p Value*
Age (y)	65.9 (9.8)	64.5 (9.6)	0.92
Male sex	144 (60.5)	72 (60.5)	
Current or ex-smoker	164 (68.9)	55 (46.2)	2.58 (1.64-4.06) 0.00003
Hypertension	147 (61.8)	47 (39.5)	2.47 (1.58-3.89) 0.00001
Diabetes mellitus	34 (14.3)	10 (8.4)	1.82 (0.86-3.82) 0.11
History of myocardial infarction	26 (10.9)	5 (4.2)	2.8 (1.05-7.48) 0.03
H. pylori seropositive	140 (58.8)	53 (44.5)	1.78 (1.14-2.77) 0.01

Values given indicate mean (SD) for age and proportion (%) for other data. Odds ratios are uncorrected for other risk factors.

Table 2 Effect on the relation between H pylori and seropositivity of controlling for potential confounding risk factors

Adjustment	*Odds ratio*	*95% CI*	*p Value*
None	1.78	1.14–2.77	0.01
Age and sex	1.77	1.14–2.77	0.01
and social class	1.79	1.15–2.82	0.01
and diabetes	1.80	1.15–2.83	0.01
and smoking status	1.73	1.09–2.75	0.02
and hypertension	1.63	1.02–2.60	0.04

diabetes (p=0.3), male sex (p=0.69), or social class (p=0.72).

PATIENT STUDY

Table 1 shows the characteristics of the patient and control populations. The two populations were well matched for age. *H pylori* was significantly more common in cases than controls (58.8% *v* 44.5%, odds ratio 1.78, 95% CI 1.14–2.77, p=0.01), The association was independent of age, sex, diabetes, smoking, hypertension, and social class (table 2) with an adjusted odds ratio of 1.63 (95% CI, 1.02–2.60, p=0.04). There was no difference in the prevalence of *H pylori* seropositivity between patients with stroke (109/186, 59.6%) and those with transient ischaemic attack without an infarct on CT (31/52, 58.6%, p=0.9). Hypertension and smoking history were also independent risk factors for cerebrovascular disease. Adjusted odds ratios (95% CI) were: hypertension 2.25(1.40–3.62), p=0.0008; smoking history 2.52(1.57–4.08), p=0.0001.

Mean (SD) percentage carotid stenosis was significantly higher in patients seropositive for *H pylori* (37.3 (29.7) *v* 27.9 (26.2) %, p=0.01). This relation remained significant after controlling for other risk factors (p=0.048). Mean carotid stenosis was also significantly independently related to hypertension (p=0.006) and smoking history (p=0.0003).

The prevalence of *H pylori* seropositivity in the different subgroups is shown in table 3. The association between *H pylori* seropositivity and cerebrovascular disease was significant for both large vessel disease and lacunar groups but not for cardioembolic/unknown. Mean degree of carotid stenosis in the different subgroups was large vessel 62.2 (9.0)%, lacunar 12.5 (13.9)%, cardioembolic/unknown 12.5 (13.8)%, tandem 52.0 (20.5)%.

There was no relation between *H pylori* seropositivity and either serum homocysteine or serum folate concentrations. Serum homocysteine was mean (SD): controls seropositive 19.2 (7.2) nmol/l *v* seronegative 20.7 (11.2) nmol/l, p=0.58; cases seropositive 22.16 (8.61) nmol/l *v* seronegative 26.19 (15.44) nmol/l, p=0.08. Mean (SD) serum folate was mean (SD) controls, seropositive 6.37 (4.42) µg/l *v* seronegative 6.90 (3.79) µg/l, p=0.63, cases seropositive 5.77 (4.03) µg/l *v* seronegative 5.97 (2.89) µg/l, p=0.77.

Discussion

This study shows that *H pylori* seropositivity is a risk factor for symptomatic cerebrovascular disease, independent of the other conventional risk factors measured. Consistent with our a priori hypothesis we found that there was a stronger independent association with both cerebrovascular disease caused by large vessel disease and with the degree of carotid stenosis. However, there was also a strong independent association with lacunar stroke indicating that the association is not totally explained by a proatherogenic effect. A concern in interpreting the relation between *H pylori* seropositivity and vascular disease has been that *H pylori* may merely be a marker for poor socioeconomic conditions which are themselves the causal risk factor for vascular disease.[11] However, the relation was independent of social class in this study. In addition we did not find an association between *H pylori* seropositivity and social class, an association which has been reported in previous studies.[1 2] This may be due to the use of spouse controls which were designed to reduce any confounding by social class. The degree of association we found with cerebrovascular disease (a corrected odds ratio of 1.63) is of a similar degree to that seen in some previous studies in patients with ischaemic heart disease. In the control population we found no relation between *H pylori* seropositivity and hypertension, smoking history, cholesterol, or diabetes, in agreement with previous studies.[3 4]

In our study *H pylori* seropositivity was associated with increased mean internal carotid artery stenosis. Furthermore the association between cerebrovascular disease and *H pylori* seropositivity was stronger when the analysis was confined to patients with large vessel

Table 3 H pylori seropositivity in the different cerebrovascular disease subtypes

Subgroup	*n*	*H pylori positive (%)*	*Odds ratio (95% CI) (uncontrolled)*	*Adjusted odds ratio (95% CI)*
Large vessel	83	56 (67.5%)	2.58 (1.44–4.63) p=0.001	2.17 (1.11– 4.21) p=0.02
Lacunar	50	32 (64.0%)	2.21 (1.12–4.38) p=0.02	2.51(1.19–5.28) p=0.01
Cardiac / unknown	85	41 (48.2%)	1.16 (0.66–2.02) p=0.5	1.21 (0.68–2.14) p=0.5
Tandem	20	11 (55.0%)	1.52 (0.59–3.94) p=0.38	0.97 (0.29–3.31) p=0.97

Odds ratios compared to controls are shown both before and after controlling for age, sex, hypertension, diabetes, smoking history, and social class.

disease and this relation was independent of other risk factors. Some mechanisms may link chronic *H pylori* infection with atherogenesis including a low grade acute phase response, free radical formation, and immune mediated mechanisms. Serum markers of an acute phase response are raised in chronic *H pylori* infection. Fibrinogen, leucocyte count, and C-reactive protein, all risk factors for cardiovascular disease,[17] are raised in those seropositive for *H pylori* and point to a low grade inflammatory response.[6 18] Free radical formation and immune mediated mechanisms may also be important. Antioxidants have been shown to be decreased in subjects with *H pylori*.[19] This could result in lipid peroxidation, another possible mechanism linking *H pylori* and atherogenesis.[20] Cross reacting antibodies to heat shock proteins are a risk factor for carotid atherosclerosis.[21] *H pylori* produces 60 kDa heat shock proteins, which have a high degree of sequence homology with human 60 kDa heat shock proteins.[22] An alternative explanation for the relation between cardiovascular disease and *H pylori* is via hyperhomocysteinaemia.[15] Homocysteine concentrations are raised in the presence of low vitamin B12 and folate, and chronic gastric inflammation could result in malabsorption particularly of vitamin B12 and secondary hyperhomocysteinaemia. However, we found no association between *H pylori* seropositivity and either serum homocysteine or folate concentrations .

The association between *H pylori* and stroke cannot be accounted for solely by promotion of large artery atherogenesis. We found a similar independent association with lacunar stroke. The major risk factor for lacunar stroke both in our study and in many other studies is hypertension but the relation between *H pylori* and lacunar stroke was still significant after controlling for hypertension. Some studies have found an association between acute infections and stroke and its had been suggested that these induce a prothrombotic state.[23] It is possible that chronic *H pylori* infection could have a similar effect. Alternatively it may also promote atherosclerosis in the small perforating vessels or at the point that they branch from the intracerebral arteries.

Identifying the importance of different pathogenic mechanisms in patients with stroke is complex as many patients may have more than one pathogenic process caused by shared risk factors. For example, hypertension is a risk factor for cardioembolic stroke via atrial fibrillation or myocardial infarction, carotid disease, or lacunar stroke. Furthermore, current diagnosis of the stroke subtype is subject to error. Therefore, further studies are required in patients with the underlying disease processes (for instance, large vessel atheroma) but without stroke—for example, the relation between *H pylori* and atheroma could be determined in asymptomatic people with atheroma load quantified by duplex ultrasound as in our study.

If further studies confirm that *H pylori* is a risk factor for cerebrovascular disease this has important clinical implications. *H pylori* infection can be eradicated by a short course of combination antibiotic therapy. If the association with stroke is causal its eradication may reduce the risk of subsequent stroke and other vascular events.

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