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Infection with virulent strains of *Helicobacter pylori* is not associated with ischaemic heart disease: evidence from a population-based case-control study of myocardial infarction

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Abstract

Background: Although the majority of evidence does not support association between *Helicobacter pylori* infection and ischaemic heart disease, the nature of this relationship may differ when virulence of the infecting strains are examined. **Methods and results:** The prevalence of IgG antibody evidence of infection with CagA positive strains of *H. pylori* was investigated in stored plasma samples from 259 cases of myocardial infarction (aged 25–70 years, 74 males) and 259 population based controls from the same area in Northern Ireland. Two-hundred and seventy (52.1%) subjects were seropositive for anti-CagA IgG. CagA seropositivity was more common in cases than in controls: 56.4 vs 47.9%, odds ratio for seropositivity in cases (95% CI) 1.41 (1.00, 1.99). Substantial attenuation of this relationship occurred on adjustment for age, sex, number of siblings, smoking and measures of socio-economic status: odds ratio (95% CI) 1.16 (0.79, 1.70). A similar pattern was seen for seropositivity for all *H. pylori* strains. **Conclusion:** Infection with the more virulent strains of *H. pylori*, as with all strains, is not associated with myocardial infarction. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *Helicobacter pylori*; Virulence; Myocardial infarction

1. Introduction

The relationship between *Helicobacter pylori* (*H. pylori*) infection and ischaemic heart disease (IHD) has been well investigated. Early reports of an association between infection and IHD [1,2] have not been substantiated in larger [3] and better designed studies [4,5] and current consensus opinion is that infection with *H. pylori* infection is not a risk factor for IHD. However, a recently published report [6] has shifted the focus

onto infection with virulent forms of the organism i.e. those possessing the cytotoxin-associated gene-A (CagA). These strains induce a stronger inflammatory response [7], are more ulcerogenic [8], and are more strongly associated with gastric cancer than CagA negative strains [9]. A strong association with IHD has been observed [6] which appears to be free from the confounding effects of socio-economic status in childhood. It is possible that previous studies of *H. pylori* and IHD have failed to observe positive associations because virulence factors were not examined. We examine the relationship between infection with virulent strains of *H. pylori* and IHD in a population based case-control study of myocardial infarction.

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2. Methods

2.1. The ECTIM Extension Study

This study utilised stored plasma from Northern Irish subjects recruited into the ECTIM Extension Study, a case control study of IHD, with recruitment centres in Belfast and Glasgow. In Northern Ireland, myocardial infarction patients, aged 25–70 at the time of infarct, and control subjects were recruited from Belfast and surrounding districts in the early 1990s. Both male and female cases were recruited (although the focus was on females) 3–9 months after an event which satisfied the WHO criteria for definite acute myocardial infarction [10]. Control subjects, resident in the same area, were randomly selected (with frequency matching for age and sex) from a centralised database of patients registered with general practitioners. Three hundred and one cases and 290 controls were recruited into the Belfast limb of the study. Cases and controls completed questionnaires and underwent a clinical examination according to a strict protocol. Data collected included age, sex, educational history, social class, sibship size, smoking status, alcohol intake, history of hypertension, hyperlipidaemia, diabetes, previous myocardial infarction or angina, other past medical history and the Rose angina and intermittent claudication questionnaires. The clinical examination included height, weight, blood pressure and waist and hip circumference. Fasting blood was taken for measurement of triglycerides, total-, HDL-, LDL- and VLDL-cholesterol, and apolipoproteins A1 and B, as previously described [11]. Aliquots of plasma used in this study had been stored at -70°C since collection.

2.2. Laboratory methods

Anti-CagA antibodies were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Invitech, UK). Optical densities were converted to semi-quantitative antibody levels using calibrator values according to the manufacturer's instructions. Specimens with an IgG level greater than 7.5 U were classified as positive with further categorisation of seropositive status into moderately (7.5–24.9 U), strongly (25–49.9 U) and very strongly positive (50 U or higher).

H. pylori specific IgG antibodies were measured by an in-house ELISA using an acid-glycine extract of *H. pylori* NCTC 11638. This assay and the derivation of the threshold for seropositivity has been described in detail elsewhere [12]. In addition to categorisation of subjects into *H. pylori* seronegative or seropositive, positive antibody responses were graded into weakly, moderately, strongly and very strongly positive.

2.3. Statistical/analytical methods

In all analyses, social class (coded according to the OPCS classification of occupations [13]) educational attainment, smoking status, alcohol intake and number of siblings were used as categorical variables (Table 1) while age, body mass index, systolic and diastolic blood pressure, triglycerides, apolipoproteins A1 and B, total-, HDL-, LDL- and VLDL-cholesterol were treated as continuous untransformed variables. The relationships between CagA and *H. pylori* seropositivity, socio-demographic and lifestyle variables and cardiovascular risk factors were examined in the controls using bi-variable analyses—the independent samples *t*-test and χ^2 -test. Observed associations between possession of anti-CagA or anti-*H. pylori* IgG and smoking status and number of siblings were adjusted, using multiple logistic regression, for age, sex and measures of socio-economic status. Logistic regression models were manually constructed with case-control status as the dependent variable, CagA or *H. pylori* seropositivity as predictor variables and age, sex, socio-economic status, number of siblings and smoking status as potential confounders.

3. Results

This report is based on 259 cases (86% of cases in the Belfast ECTIM Extension Study) and 259 controls (89% of controls) for whom stored plasma was available. Seventy-four cases and controls (28.6%) were male. Two hundred and seventy (52.1%) and 341 (65.8%) subjects were seropositive for anti-CagA and anti-*H. pylori* IgG respectively. Forty-six subjects (8.8%) possessed anti-CagA antibodies but not anti-*H. pylori* IgG antibodies and 117 (22.5%) were seropositive for anti-*H. pylori* but not anti-CagA IgG.

Table 1 shows the relationships, in the controls, between anti-CagA and anti-*H. pylori* antibody status and socio-demographic, lifestyle and cardiovascular risk factors. Seropositivity for anti-CagA IgG was not associated with age, sex, social class, or educational attainment but was associated with smoking status and number of siblings. Prevalence of anti-*H. pylori* IgG was associated with social class, smoking status and number of siblings and prevalence was higher in older age groups and in subjects with lower educational attainment but this relationship did not achieve statistical significance. CagA and *H. pylori* seropositivity were not associated with systolic or diastolic blood pressure, body mass index, or any of the lipid or lipoprotein variables.

Both CagA and *H. pylori* seropositivity remained associated with smoking status on adjustment for age, sex, social class and educational attainment; odds ratio

Table 1

CagA and *H. pylori* seropositivity in controls: relationships with sociodemographic, lifestyle and cardiovascular risk factors

		CagA seronegative	CagA seropositive			<i>H. pylori</i> seronegative	<i>H. pylori</i> seropositive		
		Number	Number (%)	χ^2 (for trend) value	<i>P</i> -value	Number	Number (%)	χ^2 for trend value	<i>P</i> -value
Age group <i>N</i> = 259	Less than 50	13	9 (41.0)			9	13 (59.1)		
	50–54	16	14 (46.7)			16	14 (46.7)		
	55–59	25	11 (30.6)			20	16 (44.4)		
	60–64	29	41 (58.6)			19	51 (72.8)		
	65–69	39	34 (46.6)			28	46 (62.2)		
Sex <i>N</i> = 259	70 or more	13	15 (53.6)	1.42	0.23	10	18 (64.3)	2.86	0.09
	Male	38	36 (48.6)			30	44 (59.5)		
Social class <i>N</i> = 255	Female	97	88 (47.6)	0.03	0.87	71	114 (61.6)	0.1	0.75
	Manual	59	59 (50.0)			30	88 (74.5)		
Educational attainment <i>N</i> = 259	Non-manual	75	62 (45.3)	0.57	0.49	68	69 (50.4)	15.6	<0.001
	University degree	13	12 (48.0)			12	13 (52.0)		
	Other post school qualification	21	25 (54.3)			20	26 (56.5)		
Smoking status <i>N</i> = 259	Secondary school	39	22 (36.1)			26	35 (57.4)		
	Primary school	62	65 (51.2)	0.02	0.88	43	84 (66.1)	2.66	0.1
	Never smoked	81	56 (40.8)			63	74 (54.0)		
	Ex-smoker	32	34 (51.5)			25	41 (62.1)		
Alcohol intake <i>N</i> = 259	Current smoker	21	32 (60.4)	6.32	0.01	13	40 (75.5)	7.30	0.007
	None	60	43 (41.7)			39	64 (62.1)		
	Occasional	30	22 (42.3)			21	31 (59.6)		
	1–3 days a week	35	50 (58.8)			34	51 (60.0)		
Number of siblings <i>N</i> = 259	4–7 days a week	10	9 (47.3)	3.6	0.06	7	12 (63.2)	0.023	0.88
	0,1	46	33 (41.7)			40	39 (49.4)		
	2,3	47	30 (38.9)			31	46 (59.7)		
	4,5	22	33 (60.0)			16	39 (70.9)		
	6 or more	20	28 (58.3)	6.05	0.01	14	34 (70.8)	7.93	0.005

Table 1 (Continued)

	CagA seronega- tive	CagA seropositive			<i>H. pylori</i> seronega- tive	<i>H. pylori</i> seropositive		
	Number	Number (%)	χ^2 (for trend) value	<i>P</i> -value	Number	Number (%)	χ^2 for trend value	<i>P</i> -value
Body mass index (kg/ m ²)	25.9	26.2	0.45	0.65	26.1	26.1	0.11	0.91
Systolic blood pressure (mmHg)	145.3	143.8	0.59	0.55	143.1	145.5	0.93	0.35
Diastolic blood pressure (mmHg)	81.9	80.6	1.05	0.29	81.4	81.2	0.18	0.35
Total cholesterol (mmol/l)	2.28	2.29	0.28	0.78	2.27	2.30	0.61	0.54
HDL (mmol/l)	0.51	0.51	0.02	0.98	0.51	0.51	0.24	0.81
LDL (mmol/l)	1.48	1.50	0.46	0.65	1.47	1.50	0.74	0.46
VLDL (mmol/l)	0.30	0.30	0.15	0.88	0.29	0.29	0.31	0.78
Triglycerides (mmol/l)	1.52	1.50	0.19	0.85	1.51	1.51	0.01	0.99
Apolipoprotein A1 (g/l)	1.57	1.53	0.9	0.36	1.57	1.54	0.87	0.38
Apolipoprotein B (g/l)	1.32	1.32	0.08	0.94	1.31	1.32	0.36	0.72

(95% CI) for anti-CagA and anti-*H. pylori* IgG in current smokers compared to never smokers 2.05 (1.04, 4.01) and 2.34 (1.09, 5.01) respectively. When the relationship between *H. pylori* seropositivity and number of sibs was adjusted for age, sex, social class and educational attainment having more than one sibling was associated with seropositivity. There was a gradient in the odds ratio with increasing number of sibs: the odds (95% CI) of having 2–3, 4–5 and 6 or more siblings in seropositives compared to seronegatives were 1.52 (0.77, 3.00), 2.32 (1.07, 5.05) and 2.66 (1.22, 5.78). A similar gradient was observed for CagA seropositivity but this did not achieve statistical significance: odds (95% CI) of having 2–3, 4–5 and 6 or more siblings in seropositives compared to seronegatives were 0.86 (0.44, 1.68), 2.01 (0.97, 4.16), and 2.06 (0.96, 4.44) respectively.

On bi-variable analysis, CagA seropositivity was more common in cases than in controls (56.4 vs 47.9%, χ^2 3.74, $P=0.05$) as was anti-*H. pylori* seropositivity (70.7 vs 61%, χ^2 5.36, $P=0.02$). This was reflected in the modestly elevated unadjusted odds ratio (95% CI) for CagA and *H. pylori* seropositivity in cases compared to controls: 1.41 (1.00, 1.99) and 1.54 (1.07, 2.22) respectively (Table 2). However, the ratios were decreased in magnitude and became statistically insignificant with adjustment for age, sex and measures of socio-economic status; odds ratios (95% CI), 1.27 (0.88, 1.83) and 1.23 (0.83, 1.81) respectively. These relationships were further attenuated after adjustment for number of siblings and smoking status; odds ratios (95% CI), 1.16 (0.79, 1.70) and 1.08 (0.72, 1.62) respectively. No relationship was seen between strength of CagA and *H. pylori* antibody responses and case-control status when included in the models.

In both final logistic regression models, number of siblings remained associated with case-control status despite inclusion of measures of socio-economic and smoking status. For example, in the CagA model (i.e. case control status as the dependent variable and CagA

seropositivity as the predictor variable) the odds (95% CI) of cases having more than one sibling when compared to controls were 2.10 (1.32, 3.35). This association held with further adjustment for body mass index, blood pressure, and lipid and lipoprotein levels.

4. Discussion

The high prevalence of IgG antibodies to *H. pylori* observed in the controls in this study is in keeping with previous observations in middle-aged adults from the same area [12] and is most likely the product of relatively high levels of deprivation prevailing in Northern Ireland when the subjects were children. Antibodies to CagA positive strains of the organism were not as prevalent as anti-*H. pylori* antibodies but in most respects the epidemiology of CagA positive strains mirrored that of all strains e.g. both CagA seropositivity and *H. pylori* seropositivity were strongly associated with smoking status and with having more than one sibling. Smoking has previously been shown to be a risk factor for *H. pylori* infection [12,14,15], although there have been discrepant findings [16–18]. The fact that the relationship between CagA seropositivity and smoking is no stronger than for *H. pylori* seropositivity suggests that smoking is not a particular risk factor for infection with the more virulent strains of *H. pylori*. Number of siblings may reflect poor living conditions during childhood which are known to be risk factors for *H. pylori* infection [19–21] possibly through providing the environment in which the spread of infection within the household is facilitated. The association between *H. pylori* infection and smoking may also reflect poor living conditions during childhood rather than direct effects of smoking on susceptibility to the infection.

Almost 9% of subjects were seropositive for CagA IgG but not for anti-*H. pylori* IgG. This indicates either a lack of sensitivity of the *H. pylori* ELISA or a lack of specificity of the CagA test. The former explanation may be more likely as the antigen utilised was prepared from only one strain of the organism although the test has been previously shown to have a sensitivity of 95% [12]. Whichever is the correct explanation, any misclassification of CagA or *H. pylori* antibody status will act to reduce, to some extent, the ability of the study to observe an association between infection and IHD, should it exist.

In this study the unadjusted odds ratio for CagA seropositivity in cases compared to controls was only modestly elevated and was attenuated on adjustment for age, sex and measures of socio-economic status. Further adjustment for smoking status, number of siblings and cardiovascular risk factors reduced the magnitude of the odds ratio almost to unity. This finding conflicts with that of Pasceri et al. [6] who observed a

Table 2
Unadjusted and adjusted odds ratios of the relationship between CagA and *H. pylori* seropositivity and IHD

Adjusted for	CagA seropositivity (95% CI)	<i>H. pylori</i> seropositivity (95% CI)
Unadjusted	1.41 (1.00, 1.99)	1.54 (1.07, 2.22)
Age	1.42 (1.00, 2.01)	1.55 (1.07, 2.24)
Age, sex, social class, educational attainment	1.27 (0.88, 1.83)	1.23 (0.83, 1.81)
Age, sex, social class, education level, number of sibs	1.22 (0.84, 1.77)	1.13 (0.76, 1.68)
Age, sex, social class, education level, number of sibs, smoking status	1.16 (0.79, 1.7)	1.08 (0.72, 1.62)

substantially elevated odds ratio of CagA seropositivity, 3.8 (95% CI 1.6–9.1), in a small study comparing subjects with angiographically demonstrated IHD to blood donor controls. These obviously discordant results, which may have their origin in differences in study size or design, impart a sense of déjà vu in that the initial positive findings of association between *H. pylori* and IHD were not replicated in subsequent studies.

It is interesting that in the final regression models, number of siblings was strongly associated with case-control status, a finding which has been previously observed [22]. Larger families are likely to result in more frequent transmission of infections between siblings. Should one or more of these infections impart an increased risk of IHD in later life, association between number of siblings and IHD would be expected. This finding may therefore support an infective origin for cardiovascular disease but the infection conferring the risk is unlikely to be *H. pylori* or its virulent strains. Alternatively, number of siblings may be a proxy for poor socio-economic circumstances during childhood which influence adult cardiovascular disease risk via mechanisms unrelated to infection.

In conclusion, the principal finding of this study is that infection with the more virulent strains of *H. pylori*, as with all strains, is not a risk factor for IHD. This conflicts with recently published findings and further study of the relationship between infection with virulent *H. pylori* strains and IHD is indicated.

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