

# Cytotoxin-Associated Gene A Strains of *Helicobacter Pylori* Represent a Risk Factor for the Development of Microalbuminuria in Type 2 Diabetes

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The reasons why diabetic patients with microalbuminuria have marked endothelial damage (1,2) and an increased risk of atherosclerosis (3,4) are unclear. Infection with *Helicobacter pylori*, especially with strains carrying the cytotoxin-associated gene A (CagA), might represent a causal factor, since anti-CagA antibodies elicited by infection may cross-react with endothelial antigens (5), increasing the vascular damage associated with diabetes itself and consequent albumin leakage. Furthermore, infection might induce a persistent systemic inflammatory response, which is a recognized risk factor for atherosclerosis (6). In this study, we investigated the possible pathogenetic role of CagA-positive *H. pylori* strains in type 2 diabetic patients with microalbuminuria.

## RESEARCH DESIGN AND METHODS

Consecutive ambulatory patients with a known history of type 2 diabetes who were  $\geq 40$  years of age were considered. Data on age, sex, BMI, smoking habit, history of hypertension or hyperlipidemia, disease duration, previous myocardial infarction, previous stroke, and HbA<sub>1c</sub> (A1C) were collected. Serum level of C-reactive protein (CRP), a marker of systemic inflammation, was measured only in patients without obvious inflammatory conditions. Exclusion criteria were presence of renal failure (cre-

atinine  $>133 \mu\text{mol/l}$ ), previous *H. pylori* eradication, and evidence of connective tissue, neoplastic, or hematological disease. Since virtually all peptic ulcers are due to *H. pylori* (7), and most of them to CagA-positive strains (8), a history of peptic ulcer would have implied in practice "a priori" knowledge of infection, probably with CagA-positive strains. Thus, to avoid any bias in the selection of patients, we also decided to exclude patients with a history of peptic ulcer.

The investigation was performed according to the principles of the Declaration of Helsinki, written informed consent was obtained from all subjects, and the study was approved by the ethical committees of our institutions.

Urinary albumin excretion was measured with the use of nephelometry (Beckman Array System). Patients were classified as having normoalbuminuria if albumin excretion was  $<30 \text{ mg/24 h}$  and as having microalbuminuria if it was  $30\text{--}299 \text{ mg/24 h}$ . Patients with macroalbuminuria ( $300 \text{ mg/24 h}$ ) were excluded. Serum IgG antibodies to *H. pylori* and to CagA protein were measured in duplicate by enzyme-linked immunosorbent assay (Helori and CTX, respectively; Eurospital) according to the manufacturer's instructions. The range of interassay variations for IgG to *H. pylori* and to CagA was 0–10% (median 7%). CRP was assessed by rate nephelometry (Behring NA

latex CRP; Behring Institute) and, in samples with  $<0.25 \text{ mg/dl}$ , by enzyme immunoassay (Imx Abbott Laboratories).

Data are presented as means  $\pm$  SD. A two-tailed *P* value of  $<0.05$  was considered statistically significant.

Univariate analysis and a multiple logistic regression, including factors possibly affecting the risk of microalbuminuria or the risk of *H. pylori* infection, were used to estimate the odds ratios (ORs) and 95% CIs for microalbuminuria associated with infection by *H. pylori* or by CagA-positive strains. Mann-Whitney *U* test was used when comparing CRP values. All analyses were performed with SPSS release 13 software.

**RESULTS**— Overall, 687 patients were screened for the study. However, 500 patients were actually included, since 187 did not meet inclusion criteria or refused participation. A total of 112 patients (22.4%) had microalbuminuria. They were older ( $59.9 \pm 8.2$  vs.  $56.5 \pm 11.8$  years;  $P = 0.0006$ ) and had a more frequent history of stroke (9.8 vs. 2.8%; OR 4.50 [95% CI 1.95–10.35]) or myocardial infarction (18.7 vs. 8.5%; 2.48 [1.37–4.49]) compared with normoalbuminuric patients. No significant difference was observed in other characteristics, such as male sex (62.5 vs. 57.7%), BMI ( $29. \pm 3.0$  vs.  $28.7 \pm 3.1 \text{ kg/m}^2$ ), smoking (38.3 vs. 39.4%), father's manual job (53.3 vs. 53.6%), hypertension (60.7 vs. 51.0%), hyperlipidemia (10.7 vs. 9.3%), disease duration ( $7.8 \pm 6.5$  vs.  $6.9 \pm 6.4$  years), and A1C ( $5.9 \pm 1.2$  vs.  $5.8 \pm 1.1\%$ ).

Anti-*H. pylori* IgGs were found in 73 (68%) patients with microalbuminuria and 224 (58%) normoalbuminuric patients ( $P = \text{NS}$ ), whereas anti-CagA antibodies were detected in 44 (39%) patients with microalbuminuria and 56 (14%) patients with normoalbuminuria (OR 3.83 [95% CI 2.38–6.15],  $P < 0.0001$ ). This significant difference persisted in a multivariate analysis (Table 1).

CRP values were  $2.07 \pm 1.81 \text{ mg/dl}$  in 157 patients with microalbuminuria and  $0.88 \pm 1.22 \text{ mg/dl}$  in 66 normoalbuminuric patients ( $P < 0.0001$ ). No signif-

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**Abbreviations:** CagA, cytotoxin-associated gene A; CRP, C-reactive protein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—ORs for CagA-positive H. pylori strains in microalbuminuric patients compared with normoalbuminuric patients after adjusting for possible confounding factors

|                                | OR   | 95% CI    | P       |
|--------------------------------|------|-----------|---------|
| Age                            | 1.38 | 1.07–1.69 | 0.01    |
| Male sex                       | 1.38 | 0.86–2.21 | 0.17    |
| BMI                            | 1.03 | 0.96–1.11 | 0.38    |
| Smoking                        | 1.06 | 0.67–1.69 | 0.78    |
| Father's manual job            | 1.19 | 0.75–1.88 | 0.45    |
| Hypertension                   | 1.40 | 0.88–2.22 | 0.15    |
| Hyperlipidemia                 | 1.12 | 0.91–1.55 | 0.44    |
| Previous stroke                | 3.24 | 1.20–8.75 | 0.02    |
| Previous myocardial infarction | 2.61 | 1.27–5.36 | 0.009   |
| Diabetes duration              | 1.04 | 0.99–1.10 | 0.07    |
| A1C                            | 1.10 | 0.90–1.33 | 0.32    |
| H. pylori infection            | 1.17 | 0.65–1.52 | 0.36    |
| CagA seropositivity            | 4.71 | 2.64–8.40 | <0.0001 |

icant difference in CRP values was detected between 20 CagA-positive subjects and 46 CagA-negative patients with microalbuminuria:  $2.02 \pm 1.89$  vs.  $2.1 \pm 1.61$  mg/dl.

**CONCLUSIONS**— This study shows that virulent strains of *H. pylori* are associated with microalbuminuria in type 2 diabetic patients. A cause-and-effect relationship may be plausible, since these strains have antigenic sequences common to endothelial cells (5), and the systemic immune response to the infection may cause immunomediated injury at the level of the endothelium, causing albumin leakage. Interestingly, these endothelial antigenic sequences are not exposed under normal conditions (5); therefore, endothelial damage associated with diabetes itself may cause their exposure, determining the vicious cycle of additional damage due to anti-CagA antibodies. The persistence of this mechanism may trigger the cascade of events leading to atherosclerosis, which has been found to be associated with CagA infection in several studies (9–18).

As shown in previous reports (19–21), we found an increase of CRP, a sensitive marker of systemic inflammation, in patients with microalbuminuria. We did not detect, however, an association between CRP and CagA infection. Therefore, although systemic inflammation probably plays a role in inducing atherosclerosis in patients with microalbuminuria, sources other than *H. pylori* infection are probably involved.

Our findings may be important for the prevention of microalbuminuria in type 2 diabetes. In fact, all strains of *H.*

*pylori* may be eradicated by a short course of specific antibiotic therapy (22), and antibody response tends to disappear with time after eradication (23). Prospective studies are needed to confirm this hypothesis.

In conclusion, we found a strong association between infection with CagA-positive strains of *H. pylori* and microalbuminuria in type 2 diabetes. This association may explain the widespread endothelial damage and the high risk of atherosclerosis reported in these patients. This finding may open new perspectives for the prevention of this complication.

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