ELSEVIER

Atherosclerosis 179 (2005) 127–132

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Association of CagA$^+$ *Helicobacter pylori* infection with aortic atheroma

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Received 26 March 2004; received in revised form 24 August 2004; accepted 17 September 2004
Available online 29 December 2004

Abstract

Background: To investigate possible association between infection with CagA$^+$ strains of *Helicobacter pylori* and aortic atheroma diagnosed by transesophageal echocardiography.

Methods and results: One hundred and eighty-eight consecutive subjects prospectively examined for presence of aortic atheroma (localized intimal thickening of $\geq$3 mm) by transesophageal echocardiography were tested for serum IgG antibodies against *H. pylori* (enzyme-linked immunosorbent assay) and CagA protein (Western blot assay). The association between infection with *H. pylori*, CagA status of the infecting *H. pylori* strains, and aortic atherosclerosis was evaluated after adjusting for coronary artery disease risk factors. There was a linear trend for presence of atheroma in subjects with CagA-positive *H. pylori* infection (51/81, 63%) compared to subjects with CagA-negative *H. pylori* infection (21/45, 46.7%) and uninfected subjects (18/62, 29%) ($p = 0.003$). *H. pylori* seropositivity was not associated with aortic atheroma (OR 2.9; 95% CI, 0.8–10.3; $p = 0.11$) when CagA status is not taken into account. On multivariate analysis, parameters associated with risk of aortic atheroma were CagA-positive *H. pylori* seropositivity (OR 4.4; 95% CI, 1.4–14.7; $p = 0.01$), older age (OR 1.2; 95% CI, 0.9–14.7; $p = 0.01$), having ever smoked cigarettes (OR 3.6; 95% CI, 1.3–10.0; $p < 0.001$), and elevated serum triglyceride level (OR 3.4; 95% CI, 1.3–9.4; $p = 0.02$).

Conclusions: After controlling for *H. pylori* infection and coronary artery disease risk factors, infection with a CagA-positive strain of *H. pylori* was independently associated with aortic atherosclerosis. This study suggests a gradient of atherosclerosis between uninfected individuals and patients with CagA-positive *H. pylori* infection and should prompt research into the role of CagA-positive *H. pylori* infection in the inflammatory atherosclerotic process.

Keywords: Atherosclerosis; Echocardiography; Infection; Aorta

1. Introduction

A 1998 meta-analysis of 18 epidemiological studies reported that previous claims of substantial correlations between *Helicobacter pylori* seropositivity and certain markers of atherosclerosis [1] were largely or wholly due to chance, the preferential publication of positive results, or both. Since then, several studies have examined the relationship between ischemic heart disease (IHD) and infection by the more virulent *H. pylori* strains expressing the cytotoxin-associated protein A (CagA). CagA is a high-molecular mass (120–128 kDa) *H. pylori* antigen, associated with enhanced virulence and cytotoxin production [2].

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0021-9150/$ – see front matter © 2004 Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2004.09.010

A clear association was reported between CagA-positive *Helicobacter* strains and severe forms of gastroduodenal diseases, including peptic ulcer and gastric cancer [3].

Recent prospective studies have reported an association between infection with CagA-positive strains of *H. pylori* and coronary heart disease, and provide some evidence that seropositivity for CagA strains is an independent risk marker for the development of IHD [4–7]. However, in other studies, CagA-positive strains appeared to be no more strongly related to IHD than other strains [8–11]. Lately, another possible association was found between ischemic stroke and CagA *H. pylori* infection, as well as an increased prevalence of infection with the virulent strains in patients with atherosclerotic stroke [12]. Moreover, in patients affected by atherosclerotic stroke, Gabrielli et al. [13] reported that CagA seropositivity was the only factor independently associated with carotid plaque irregularity.

Atherosclerosis of the thoracic aorta has been associated in several studies with autopsy determinations of atherosclerosis, with clinically significant coronary artery disease [14–16] and embolic risk in patients with embolic event [17]. Transesophageal echocardiography (TEE) is a sensitive and specific technique for the diagnosis and evaluation of aortic plaques, and provides quantitative information about early extracoronary atherosclerotic lesions and the risk of stroke [18].

We therefore conducted a prospective study in a cohort of consecutive patients undergoing TEE examinations, to assess the association of CagA-positive *H. pylori* strains with TEE confirmed aortic atheroma.

2. Methods

2.1. Patients

Between November 1, 1999 and December 31, 2000, our laboratory prospectively evaluated 188 consecutive patients referred for TEE. Indications for TEE were: atrial fibrillation prior to cardioversion in 39 patients, suspected infective endocarditis in 29, follow-up of valvular disease in 54, determination of a cardiac source of peripheral emboli in 57, and other indications in 9. None of the patients had received eradication treatment for *H. pylori* infection before entry to the study. Serologic testing for *H. pylori* was performed immediately before TEE, and the patients were divided into three groups on the basis of the findings: seronegative for *H. pylori* and CagA; seropositive for CagA-negative *H. pylori;* and seropositive for CagA-positive *H. pylori*.

2.2. Transesophageal echocardiography

TEE was performed with a commercially available 5 MHz multiplane transducer (Hewlett-Packard 21363 A); sonographs used were Hewlett-Packard Sonos 2000 and 5500. After cardiac examination, the transducer was rotated posteriorly, advanced to the distal esophagus (≈40 cm), and slowly withdrawn to obtain images from the distal thoracic aorta to the aortic arch. It was then rotated and advanced to image the ascending aorta. If abnormalities were detected, more detailed scanning at that level was performed. These procedures are part of our routine TEE examination.

The aortic intima was evaluated for changes in thickening, calcification, protrusion, mobility and ulceration. Aortic atheroma was defined as any localized intimal thickening of ≥3 mm in the ascending aorta, the aortic arch, or the descending aorta. All findings were recorded on super-VHS tape and evaluated independently by two echocardiography specialists. A diagnosis of aortic atheroma was made by a consensus reading of two echocardiography specialists who were blinded to patient *H. pylori* status.

2.3. Serology

Serum samples were obtained at the time of TEE and stored at −20 °C until assayed. IgG antibodies against *H. pylori* infection were tested by enzyme-linked immunosorbent assay (ELISA) (Orion Diagnostica; Espoo, Finland). Twelve samples from each patient were tested concurrently. The method was validated in our laboratory by a pilot study among patients undergoing endoscopy at our hospital, which yielded a sensitivity of 94%, specificity of 90%, and positive and negative predictive values of 100 and 90%, respectively. IgG antibodies against CagA protein were tested with immunoblot staining using a Western blot kit (Helicoblot 2.0; Genelabs Diagnostic, Singapore) [19,20].

2.4. Risk factors

The following risk factors for atherosclerosis were recorded: diabetes mellitus, hypertension, hypercholesterolemia, family history of coronary heart disease, and history of smoking. Diabetes was defined as a history of hyperglycemia requiring previous or current pharmacological therapy. Hypertension was defined as an elevation in systolic or diastolic blood pressure (>140/90 mmHg) or the need for ongoing antihypertensive medication. Hypercholesterolemia was defined as a total cholesterol level of >200 mg/dL. Hypertriglyceridemia was defined as triglyceride levels >200 mg/dL. Family history was positive if a first-degree relative had a coronary event before 55 years age. Ever smoked was defined as a self-report of 10 or more pack-years of cigarette use at some point in the patient's life.

Ultra sensitive C-reactive protein test was performed on a Roche Integra 400 analyzer, using a latex particle enhanced immunoturbidimetric kit [21]. The analytical sensitivity (lower detection limit) of the kit is 0.0085 mg/dL (reference range 0–0.5 mg/dL). Within-day precision (CV) obtained was 4.8% at a level of 0.12 mg/dL. Between-day CV was 3.0% at a level of 0.4 mg/dL.