**Helicobacter pylori** infection
An independent risk factor for ischemic cerebrovascular disease?

**OBJECTIVE** To evaluate the association between chronic *Helicobacter pylori* (Hp) infection and cerebrovascular ischemic disease, since, recently, many investigators have correlated chronic infection and low grade inflammation with vascular inflammatory response and atherogenesis.

**METHOD** Evaluation was made in 102 patients (54 female, mean age=75.44±19 years) with transient cerebral ischemia or stroke and 76 sex and age matched controls (39 female, mean age=71.2±17 years) for Hp IgG and CagA Hp antibodies. Inflammation markers implicated in the atherosclerosis procedure (CRP, fibrinogen plasma levels and ESR) were studied. A multivariate regression analysis statistical model was applied to evaluate possible associations with dyslipidemia, diabetes mellitus, smoking, atrial fibrillation and blood pressure.

**RESULTS** Patients and control subjects were evenly matched with respect to socioeconomic status, diabetes mellitus, and smoking status (P>0.1, respectively). Hp seropositivity was detected in 54.9% of patients and in 48.68% of control subjects (P=0.439). CagA seropositivity was detected in 12 of 102 patients and in 30 of 76 controls (P<0.05). In the patient group, 47/57 seropositive and 34/45 seronegative subjects had elevated CRP levels (P<0.01). In the control group, 30/37 (81.08%) seropositive and 22/39 (56.41%) seronegative subjects had elevated CRP levels (P<0.03). Forty-four of 57 (77.19%) seropositive and 31 of 45 (68.8%) seronegative patients had elevated fibrinogen levels (P<0.01). Twenty-four of 37 (64.86%) seropositive and 20 of 39 (51.28%) seronegative controls had elevated fibrinogen levels (P<0.03).

**CONCLUSIONS** *Helicobacter pylori* infection was not found to be an independent risk factor for ischemic cerebrovascular disease.

Since 1983, when *Helicobacter pylori* (Hp) was discovered in the stomach of patients with chronic gastritis by Warren and Marshal, the bacterium has been considered to be the most important aggressive pathogenic factor in peptic ulcer disease. An increasing body of literature has suggested a relationship of Hp chronic infection with the development of mucosa associated lymphoid tissue (MALT) lymphoma, gastric adenocarcinoma and a number of extra-gastrointestinal disorders, such as ischemic heart disease, atherosclerosis, Raynaud's phenomenon and various skin diseases.\(^1\)

The mechanisms by which Hp might influence cardiovascular risk are unknown. In recent years, investigations have established an association between infective and inflammatory processes and the occurrence of vascular disease.\(^2,4\) Infective processes may act through both systemic effects and direct arterial invasion.\(^3\) *Chlamydia pneumoniae*, cytomegalovirus, HSV1, HSV2, hepatitis A virus and Hp have all been implicated in the pathogenesis of systemic atherosclerosis.\(^2,5,10\) Raised serum fibrinogen levels, and increased procoagulant activity from mononuclear cells have been proposed as the underlying mechanism to account for the role of Hp in coronary heart disease (CHD).\(^2,5,10\) Data regarding Hp association with ischemic heart disease are contradictory, with studies suggesting a strong positive relationship,\(^10\) mild association\(^17\) or even negative findings.\(^12\)

Cytotoxin associated gene A (CagA) positive Hp strains have a well recognized role in the pathogenesis of peptic ulcer disease and gastric cancer, by inducing a significantly greater inflammatory response in gastric mucosa\(^13,14\) than CagA negative strains. Thus, it is likely that infection by
more virulent strains may provoke a more intense systemic inflammatory response and in this way alter the procoagulant pathway. In the study of Pasceri et al, an association between Hp CagA strain seropositivity and ischemic heart disease was demonstrated, but not a causal relationship.  

The purpose of the present study was to investigate chronic Hp infection as a risk factor for atherosclerosis manifestation in a vascular bed other than coronary vessels by a case-control study of cerebral ischemia.

**MATERIAL AND METHOD**

The institutional ethics committee approved the study. All subjects (patients and controls, or their family in cases where they could not give an informed consent) signed an informed consent before their inclusion in the study. Investigation of 102 consecutive patients with acute ischemic stroke or transient ischemic attack and 76 hospitalized control subjects was conducted.

The medical history of vascular risk factors (atherothrombotic stroke, cardioembolic stroke and stroke of mixed origin) and previous vascular diseases was assessed in a personal interview using a standardized questionnaire in all patients and control subjects. The next of kin was interviewed when information was not available from the patient. Patients and controls were considered to suffer from CHD when a history of angina or myocardial infarct and/or electrocardiographic findings was present. The presence of cerebrovascular disease (i.e., ischemic infarct) was documented by central nervous system computed tomography (CT). The final diagnosis was reviewed by an experienced neurologist independent of imaging findings. All patients and controls were submitted to echo cardiogram and carotid investigation by echo Doppler performance.

The socioeconomic status was recorded and subjects were distributed based on the longest held occupation or, when they did not work, on their highest school qualification. Subjects were distributed into three classes: classification in the highest class typically required an academic degree or similar training; in the middle class subjects were skilled employees and specialized workers and the lowest class they consisted mostly of non specialized workers. The first potential control subject admitted after a respective patient of the same sex and comparable age was invited to participate.

Control patients suffered from brain tumors, non-vascular non-inflammatory neurological diseases, weight loss, epigastric discomfort and gastrointestinal bleeding. Patients with infections, systemic inflammatory or systemic vascular diseases or known conditions affecting the measured inflammatory markers were not eligible for the control group.

Fasting blood samples were drawn for total leucocytes, platelets, erythrocyte sedimentation rate (ESR), plasma fibrinogen levels (Organon Technica analyser) and C-reactive protein (CRP). Routine biology along with serum glucose, urea, creatinine and lipid plasma levels was performed for all subjects on admission and in the morning of the first hospitalization day. CRP was estimated by nephelometry quantitative assay. Serum cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay. HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran-sulphate magnesium (Roche Hitachi 717 analyser). LDL cholesterol was calculated using the Friedwald formula. Patients were considered dyslipidemic when fasting serum cholesterol levels were detected to be >220 mg/dL or serum triglyceride levels were >200 mg/dL or HDL cholesterol levels were <45 mg/dL or there was any combination of the above. High glucose levels within the first three days after cerebral ischemia were not considered for the diagnosis of diabetes mellitus.

Hp IgG antibodies were detected using enzyme linked immunosorbent assay (ELISA, Pharmacia & Upjohn, normal range <10 U/mL). Anti-CagA Hp antibodies were also determined with ELISA (RADIM, normal range <10 U/mL).

Mean and standard deviations (SD) were calculated for continuous variables. Chi-square test was used to analyze categorical variables and the Mann-Whitney U-test for analysis of continuous variables. Relative risk odds ratios (OR) were obtained using logistic regression. Multiple logistic regression analysis was used to adjust for potentially confounding factors. Factors in the model were predefined and included hypertension, diabetes mellitus, atrial fibrillation, ischemic heart disease and dyslipidemia. The statistical software package SPSS was used for the statistical analyses.

**RESULTS**

The study included 102 consecutive patients (48 male, 54 female, mean age=75.44±19 years) with acute cerebral ischemia or ischemic stroke and 76 (37 male, 39 female, age=71.2±17 years) age- and sex-matched control subjects. Patients and control subjects were matched with respect to socioeconomic status, diabetes mellitus, and smoking status (P>0.1, respectively). Regarding smoking status, the distribution of non-smokers, current smokers and ex-smokers was similar (P>0.1, respectively). Patient and control subject sub-populations were not comparable for hypertension and dyslipidemia (P<0.01). The patient subgroups (Hp positive and Hp negative) were comparable for the total series of cerebral ischemia. The demographic and clinical characteristics of the study population are shown in table 1.

The presence of Hp infection was detected in 57 of 102 patients (54.9%) and in 37 of 76 control subjects (48.68%). This was not found to be an independent risk factor for cerebral ischemia (P=0.439). The difference of Hp seropositivity between groups was further attenuated after adjustment for hypertension, dyslipidemia, diabetes
mellitus and smoking. Hypertension and dyslipidemia significantly increased and diabetes mellitus, atrial fibrillation and ischemic heart disease tended to increase the odds for cerebral ischemia whereas smoking was not found to be associated with cerebral ischemia as an independent risk factor (tab. 2).

CagA seropositivity was detected in 12 of 102 (11.76%) patients and in 30 of 76 (40%) of controls (P<0.05). Patients with positive serology for Hp were older (78.5±16 vs 72.4±20 years) and tended to suffer more often from hypertension (55% vs 48%) than seronegative patients. There was significant association of Hp infection with total cholesterol levels, since 20/57 (35.08%) seropositive patients had elevated cholesterol levels (>220 mg/dL) whereas only 10/45 seronegative patients (22.2%) had elevated cholesterol levels (P<0.01).

Mean values of blood parameters (HDL and total cholesterol, triglycerides, fibrinogen, ESR, CRP, leucocyte and platelet count) in the subpopulations of Hp positive/negative patients and controls are shown in table 3.

In the patient group, 47 of 57 (82.5%) seropositive and 34 of 45 (75.45%) seronegative subjects had elevated CRP levels (P<0.01). In the control group, 30 of 37 (81.08%) seropositive and 22 of 39 (56.41%) seronegative subjects had elevated fibrinogen levels (P<0.03). Among patients 44 of 57 (77.19%) seropositive and 31 of 45 (68.8%) seronegative patients had elevated fibrinogen levels (P<0.01). Twenty four of 37 (64.86%) seropositive and 20 of 39 (51.28%) seronegative subjects had elevated fibrinogen levels (P<0.03). Finally, 23 of 57 (40.35%) seropositive and 17 of 45 (37.7%) seronegative patients had elevated CRP levels (P<0.01). Twenty four of 37 (64.86%) seropositive and 20 of 39 (51.28%) seronegative subjects had elevated fibrinogen levels (P<0.03). Among patients 44 of 57 (77.19%) seropositive and 31 of 45 (68.8%) seronegative patients had elevated fibrinogen levels (P<0.01). Twenty four of 37 (64.86%) seropositive and 20 of 39 (51.28%) seronegative subjects had elevated fibrinogen levels (P<0.03). Finally, 23 of 57 (40.35%) seropositive and 17 of 45 (37.7%) seronegative patients had elevated CRP levels (P<0.01). Twenty four of 37 (64.86%) seropositive and 20 of 39 (51.28%) seronegative subjects had elevated fibrinogen levels (P<0.03).

**DISCUSSION**

In this study, slightly more patients with ischemic cerebrovascular disease were found to have elevated antibody
titers against Hp than control subjects but this difference was not statistically significant. Studies reported in the literature show strong positive, mild or no association at all between Hp seropositivity and ischemic heart disease, and studies that have tried to establish an association between Hp and ischemic cerebrovascular disease have also given conflicting results. In a recent study, similar to the present study association between Hp seropositivity and cerebrovascular disease was demonstrated but this trend was revealed to be statistically non-significant after adjustment with univariate analysis for socioeconomic status. Furthermore, when a second analysis for Hp association with stroke caused by large artery atherosclerosis of brain supplying arteries was made, the results were based on rather small numbers and conclusions regarding causality could not be drawn. In this study, adjustment for socioeconomic status of subjects was made before their inclusion in the control group. In the large study of Markus and Mendall, adjustment for socioeconomic status was also performed before inclusion, and association was established only with cerebrovascular disease caused by large vessel stenosis and with the degree of carotid stenosis. Ameriso et al succeeded detecting Hp DNA in 53% of atherosclerotic plaques of human carotids using a supersensitive PCR method, but their findings disagreed with most earlier work which failed to find Hp in vascular lesions. The findings of the present study are thus in accordance with data from current studies.

Careful matching for socioeconomic status of control subjects was made before inclusion in the study. The study was designed in this way in order to avoid bias errors, since it is well known that Hp seropositivity is strongly correlated with low socioeconomic status. Serological testing to assess Hp infection was used as in all previous studies, since it has been shown that serology provides high sensitivity (93–96%) and specificity (89–96%). A positive culture from a gastroduodenal biopsy is considered as the gold standard to indicate positivity for Hp infection but upper gastrointestinal endoscopy could not be performed without other indications, for ethical reasons.

Analysis of the data demonstrated that established risk factors, such as hypertension and dyslipidemia, significantly increased, and diabetes mellitus and ischemic heart disease tended to increase the odds for cerebral ischemia in the patient population of the study. Dyslipidemia (plasma total cholesterol but not HDL cholesterol and triglyceride levels) was also found to be significantly correlated with Hp infection although this has not been shown in previous studies. Smoking as an independent risk factor was not found to be associated with cerebrovascular disease or Hp infection although some earlier studies, but not all, correlate tobacco consumption with Hp infection.

One of the pathogenetic mechanisms proposed to link chronic infections to ischemic stroke is continuous low-grade inflammation that could stimulate procoagulant pathways and promote atherogenesis, e.g. by activating mononuclear cells, free radical formation, lipid peroxidation, and immune-mediated mechanisms. Antioxidants have been shown to be decreased in subjects with Hp, fibrinogen, leucocyte count and CRP, but all risk factors for cardiovascular disease, have been reported to be raised in those who are seropositive for Hp and these point to a low-grade inflammatory response. In this study, fibrinogen and CRP, but not ESR, well-known inflammation markers, were found to be correlated to Hp infection in both patients and control subjects. Therefore it can be considered that the data demonstrate that even though a strong relationship was not found between Hp and cerebrovascular disease, chronic Hp infection may be implicated in atherogenesis via a low-grade inflammatory response.

In the present study CagA Hp serum antibodies were also detected. CagA is known to be a high molecular mass Hp antigen, associated with enhanced virulence and cytotoxin production. Since inflammatory diseases are implicated in atherosclerosis, it can be hypothesized that the stronger persistent immunoinflammatory burden evoked by virulent CagA positive Hp strains could play a role in atherogenesis. A cross mimicry between CagA protein and endothelial wall antigens has been demonstrated and postulated to promote the atherosclerotic process. Pasceri et al, in their original study on CagA association with ischemic heart disease, demonstrated an association but not a causal relationship but their age and sex matched control group of similar socioeconomic status may not have been representative, since it came from a pool of blood donors. In a more recent study a correlation between CagA positivity and ischemic stroke was demonstrated while an association with a stroke subtype was not established. This study found a positive correlation between CagA seropositivity and ischemic stroke both in the large vessel and small vessel occlusion subgroups and the authors believe that these findings support the theory that Hp strains of strong virulence are implicated in the atherosclerotic process.

In conclusion, the study data provide evidence that Hp seropositivity is correlated with higher mean CRP and fibrinogen plasma levels in both patients and control subjects. Although Hp infection was not found to be independently associated with ischemic cerebrovascular disease, it appears that there is an implicating role for some Hp strains in creating a low-grade chronic inflammatory vascular response.
Λοίμωξη από Helicobacter pylori: Ανεξάρτητο παράγοντας κινδύνου για τα ισχαιμικά αγγειακά εγκεφαλικά επεισόδια;

Γ.Ι. ΛΑΖΑΡΑΚΗ, Α.Ι. ΧΑΤΖΗΤΟΛΙΟΣ, Σ.Α. ΜΕΤΑΛΛΙΔΗΣ, Ν. ΕΛΕΥΘΕΡΙΑΔΗΣ, Μ. ΧΑΤΖΗΔΗΜΗΤΡΙΟΥ, Ε. ΔΗΜΗΤΡΑΚΟΥΔΗ, Γ.Ν. ΖΙΑΚΑΣ

1Α΄ Προπαιδευτική Παθολογική Κλινική, 2Μικροβιολογικό Τμήμα, 3Α΄ Νευρολογική Κλινική, Πανεπιστημιακό Γενικό Νοσοκομείο «ΑΧΕΠΑ», Αριστοτελείο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη


ΣΚΟΠΟΣ
Μελέτη της πιθανής σχέσης της χρονίας λοίμωξης από Helicobacter pylori (Hp) με τα ισχαιμικά αγγειακά εγκεφαλικά επεισόδια (ΙΑΕΕ), αφού πρόσφατα πολλοί ερευνητές έχουν συσχετίσει την παρουσία χρονίας λοίμωξης με την αγγειακή φλεγμονώδη απόκριση και την αθηρογένεση.

ΥΛΙΚΟ-ΜΕΘΟΔΟΣ
Μελετήθηκαν 102 ασθενείς (54 γυναίκες, μέσος όρος ηλικίας 75,44±19 έτη) με ΙΑΕΕ (εμβολικό, θρομβωτικό ή παροδική ισχαιμία) και 76 ασθενείς ελέγχου παρόμοιας ηλικίας και φύλου (39 γυναίκες, μέσος όρος ηλικίας 71,2±17 έτη) για αντισώματα Hp IgG και CagA Hp. Για τη στατιστική ανάλυση χρησιμοποιήθηκε μοντέλο πολυπαραγοντικής ανάλυσης που αξιολόγησε τις πιθανές συσχετίσεις με δυσλιπιδαιμία, σακχαρώδη διαβήτη, κάπνισμα, κολπική μαρμαρυγή και αρτηριακή υπέρταση. Μελετήθηκαν οι δείκτες φλεγμονής που συσχετίζονται με την αθηροσκλήρυνση (C-αντιδρώσα πρωτεΐνη, ινωδογόνο και ταχύτητα καθίζησης ερυθρών, ΤΚΕ).

ΑΠΟΤΕΛΕΣΜΑΤΑ
Οι ασθενείς και τα άτομα της ομάδας ελέγχου ήταν συγκρίσιμα ως προς το κοινωνιοοικονομικό επίπεδο, το σακχαρώδη διαβήτη και το κάπνισμα (Ρ>0,1). Αντισώματα Hp IgG ανιχνεύτηκαν στο 54,9% των ασθενών και στο 48,68% της ομάδας ελέγχου (Ρ=0,439). Αντισώματα έναντι CagA Hp ανιχνεύτηκαν σε 12 από 102 ασθενείς και σε 30 από 76 άτομα της ομάδας ελέγχου (Ρ<0,05). Στην ομάδα των ασθενών, 47/57 οροθετικοί για Hp και 34/45 οροαρνητικοί είχαν υψηλά επίπεδα CRP (Ρ<0,01). Στην ομάδα ελέγχου, 30/37 (81,08%) οροθετικοί και 22/39 (56,41%) οροαρνητικοί είχαν υψηλά επίπεδα CRP (Ρ<0,03). Σαράντα τέσσερις από 57 (77,19%) οροθετικούς και 31 από 45 (68,8%) οροαρνητικούς ασθενείς παρουσίαζαν υψηλά επίπεδα ινωδογόνου (Ρ<0,01). Είκοσι τέσσερις από 37 (64,86%) οροθετικούς και 20 από 39 (51,28%) οροαρνητικούς συμμετέχοντες στην ομάδα ελέγχου παρουσίαζαν υψηλά επίπεδα ινωδογόνου (Ρ<0,03).

ΣΥΜΠΕΡΑΣΜΑΤΑ
Η λοίμωξη από Ηp δεν βρέθηκε να αποτελεί ανεξάρτητο παράγοντα κινδύνου για ισχαιμική αγγειακή νόσο.

Λέξεις ευρετηρίου: Αγγειακό εγκεφαλικό επεισόδιο, Αντισώματα CagA, Αρτηριοσκλήρωση, Ελικοβακτηρίδιο πυλωρού

References


30. GLYNN J. Helicobacter pylori and the heart. Lancet 1994, 344:146


Συγγραφείς υπεύθυνος Αλληλογραφίας:
G.I. Lazaraki, 1st Propeudeutic Department of Internal Medicine, ‘ΑHEPA’ General Hospital, Thessaloniki, GR-542 48 Thessaloniki, Greece

e-mail: lazarakg@yahoo.com