

Association between infection with *Helicobacter pylori* and cerebral noncardioembolic ischemic stroke

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Background: *Helicobacter pylori* infection has been associated epidemiologically and pathogenetically with atherosclerosis of coronary arteries but data regarding chronic infection with this organism and cerebral noncardioembolic ischemia are not clear. **Aims and Design:** In this study we have investigated the association of this pathogen and noncardioembolic ischemic stroke under a case-control study. **Methods and Material:** Samples are taken among patients who were admitted in our hospital due to their first ischemic stroke during 2003–04. Patients with a known cardiac origin for cerebral emboli and those with major risk factors of ischemic strokes were excluded. Controls were selected from the study population and matched for age, sex, and area of residence. IgG and IgA antibodies to *H. pylori* were measured by enzyme immunoassay. **Statistical analysis:** The *t* and *c*-tests and Odds ratio were applied to examine variables differences. **Results:** A total of 91 cases (43 women, 48 men) and 80 controls (40 women, 40 men) were included for analysis ($P = 0.8$). The mean age of cases was 64.3 ± 10 years and of controls was 61.73 ± 10.3 years ($P = 0.1$, $CI = 95\%$). There was seropositivity for *H. pylori* (IgG or IgA) in 66 patients (72.5%) but they were positive only in 45 controls (56.3%) ($P = 0.04$). Mean of serum IgG levels was significantly high in stroke group ($P < 0.005$) but the IgA antibody elevation against *H. pylori* did not show any risk. **Conclusions:** Our case-control study provides evidence of an association between the immune response to *H. pylori*, a marker of prior infection with this organism and noncardioembolic ischemic stroke.

Key words: Atherosclerosis; helicobacter pylori; ischemic stroke.

Helicobacter pylori is notorious for causing chronic infections and has been seroepidemiologically linked to the coronary heart disease and atherosclerosis.^{[1],[2]} Patel et al. found ischemic 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 ischemic heart disease.^[3] Murray et al. found that *H. pylori* seropositive people had 1.51 times more risk of evidence of coronary heart disease according to the Rose angina questionnaire after being controlled for social class and conventional cardiovascular risk factors.^[4]

Data on chronic infection in cerebrovascular disease are limited. In a pilot study Hugh and coworkers found a significant relation between *H. pylori* infection and stroke.^[5] On the other hand Heuschmann et al. found that chronic *H. pylori* infection was associated with a higher risk of stroke caused by small-artery occlusion and a lower risk of cardioembolic stroke but totally elevated *H. pylori* antibodies were not associated with ischemic stroke.^[6]

Because direct detection methods for *H. pylori* depend on cerebrovascular wall samples retrieved in vivo, which are not available obviously, so antibody levels must be used to describe the association between stroke and previous infection in an indirect manner. Therefore, we have investigated, in a case-control study, whether serological evidence of *H. pylori* infection is an independent risk factor in noncardioembolic ischemic stroke. In contrast to other studies, we excluded cases with other known risk factors in order to evaluate an independent role of this organism.

Patients and methods

During a period of one year (2003–2004) all Ischemic stroke patients with following criteria were enrolled in this retrospective analysis study: (a) first ischemic stroke, (b) stroke treatment in a hospital, and (c) admission to a hospital within the first 72 h after stroke onset. Stroke was defined according to World Health Organization criteria. Patients with a known cardiac source for cerebral embolism (Atrial fibrillation, valvular heart disease in echocardiography and prosthetic heart valves), those with major risk factors for atherosclerosis: hypertension, referred to a blood pressure of 140/90 mmHg

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or greater in repetitive controls; diabetes mellitus, based on American Diabetes Association criteria and any symptoms related to peptic ulcer disease (known peptic ulcer disorder using endoscopy, use of antiulcer treatments, suspected cases according to history and gastrointestinal symptoms), were excluded.

Cerebral infarction was diagnosed on the basis of clinical setting and results of the neuroimaging study. Brain CT was done on all patients but, MRI only on the posterior circulation strokes. All patients had abnormal neuroimaging compatible with cerebral ischemic stroke in the certain arterial territory. According to the above inclusion criteria, 91 patients were selected for study. Because patients with lacunar strokes are not hospitalized routinely in our center, only a few cases were lacunar infarctions. Controls selected randomly from general population (hospital workers, individuals who referred our hospital for periodic check up examinations). Controls did not have any history of cerebrovascular and coronary accidents and suspected peptic ulcer disease and matched for age, sex, and area of residence so a total amount of 80 controls could be included in the study.

Physical and neurological examination was done for all cases and blood samples were taken within the first week after admission. Information was collected directly from the patient and in case of disability of participant to communicate, from a close relative. Control subjects were interviewed and evaluated in the same manner as cases.

Blood samples of either cases or controls were labeled immediately after taking blood. Thus laboratory investigators were blind to case or control status during the laboratory tests. Sera were stored at 2–8°C for 1 week and at 20°C for longer. IgG antibodies to *H. pylori* were detected using a rapid enzyme linked immunosorbent assay (ELIA WELL; REF K5HPG kit; RADIM, Roma, Italia). assay procedure was performed according to the manufacturer's order and recommended measures were chosen to indicate IgG *H. pylori* seropositivity. Samples with IgG values less than 15 UR/ml were considered nonreactive for anti-*H. pylori* IgG antibodies. Values higher than 30 UR/ml were considered reactive and values between 15 and 30 UR/ml considered questionable. According to the manufacturer's statement, the sensitivity and specificity of the method were 95.8 and 96.2%, respectively. (for more information see: <http://www.radim.it>)

We used Trinity Biotech Captia™ *H. Pylori* IgA Enzyme-Linked Immunosorbent Assay kit (USA, ISO 9001 certified, P/N 6430-29 Rev C), for the detection and qualitative determination of IgA antibodies. based on the manufacture's guide, an Immune Status Ratio (ISR) was calculated for each specimen and ISR values of ≤ 0.9 was interpreted as negative, 0.91–1.09 as equivocal and retested and ISR values of ≥ 1.10 analyzed as positive. Relative sensitivity of test with 95% CI is 74.1–100% and specificity of 93.2–100%. (For more information contact: Trinity Biotech USA, Jamestown, NY, Fax (353) 488-1990)

Also, the mean levels of each antibody compared between groups. Statistical analyses were performed with the SPSS 11.0 software packet. The *t* test was used to test differences in quantitative variables, and the χ^2 -test was used to test differences in proportions of qualitative variables. For variables relative risk evaluation we used Odds ratio with a confidence interval (CI) of 95%.

Results

Total of 91 cases (43 women, 48 men) and 80 controls (40 women, 40 men) were included for analysis (CI 95%, *df*=1, Fisher Exact Test=0.76). The mean age of patients was 64.3 ± 10 years and of controls was 61.73 ± 10.3 years (*t*=1.652, *df*=169, CI 95%, *P*=0.1). Seropositivity for antihelicobacter IgG antibody was found in 36 controls and in 59 patients. about IgA type antihelicobacter antibodies, seropositivity was observed in 29 controls (36.3%) and 33 patients (36%) in stroke groups. There was seropositivity for *H. pylori* (IgG or IgA) in 66 patients (72.5%) but they were positive only in 45 controls (56.3%). Table 1 represents a summary of demographic characteristics of cases and controls and prevalence of elevated IgA and IgG antibodies to chronic infection with *H. pylori* among cases and controls.

The mean serum levels of antibodies were analyzed between groups. Mean serum levels of antihelicobacter IgA antibody was 0.92 ± 0.4 in control group and 0.98 ± 0.76 in patients and there was not significant differences between groups (*t*=0.824, *df*=169; CI 95%, *P*=0.411). Mean of IgG levels was 33.55 ± 17.20 in control group and 47.25 ± 26.02 in noncardioembolic stroke group (*t*=4.105, *df*=169; CI 95%, *P*<0.001). [Figure 1] shows our findings.

Elevated serum antibodies to *H. pylori* were significantly associated with a higher risk of noncardioembolic stroke. According to immunoglobulin type, this risk was remarkable only for IgG type seropositivity (Odds ratio=2.053; 95% CI, 1.085 to 3.886), but the IgA antibody elevation against *H. pylori* did not show risk for noncardioembolic ischemic stroke (adjusted Odds ratio=1.001; 95% CI, 0.54 to 1.87). This risk was equal for both sexes.

Discussion

In our case-control study, IgG antibody response for *H.*

Table 1: Prevalence of elevated antibodies to infection with *H. pylori* among groups

	Stroke cases 91 (100%)	Controls 80 (100%)	<i>P</i>
Serum anti- <i>H. pylori</i> IgG Abs	47.25±26.02	33.55±17.20	<0.001 *
Serum anti- <i>H. pylori</i> IgA Abs	0.98±0.60	0.92±0.4	0.41
Seropositive (IgG)	59 (64.8%)	36 (45%)	<0.015 *
Seropositive (IgA)	33 (36%)	29 (36.3%)	NS
Seropositive (IgG or IgA)	66 (72.5%)	45 (56.3%)	<0.04 *

*Statistically significant.

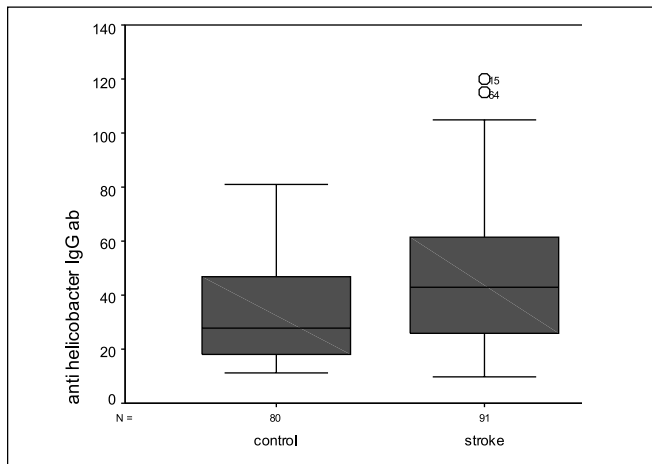


Figure 1: Mean serum level of IgG antibodies against *H. pylori* between study groups

pylori was associated with an increased risk of noncardioembolic stroke. However, no evidence found for any strong association between elevated IgA antibodies to *H. pylori* and the overall risk of stroke. Because of small sample size, we could not analyze the data according to stroke subgroup. It was the limitation of our study. But in contrast to some previous studies we evaluated definite cases of noncardioembolic strokes. We used numerous exclusion criteria that resulted to a small sample size but we think this relates to robustness strength of our study and results are more definite for evaluation of *H. pylori* role in noncardioembolic ischemic strokes.

Our findings were consistent with the results of the Markus and Mendall study that addressed the relation between chronic *H. pylori* infection and ischemic stroke.^[7] They reported elevated IgG antibodies to *H. pylori* in the both subgroups of lacunar strokes [Odds ratio 2.21 (95% CI 1.12–4.38), $P=0.02$] and large vessels strokes with an adjusted OR of 2.58 (95% CI 1.44–4.63, $P=0.001$), which is compatible with our finding (Odds ratio=2.053; 95% CI, 1.085 to 3.886). Because we excluded cardioembolic ischemic strokes from study, therefore, our findings are consistent with a positive association of chronic *H. pylori* infection and those ischemic strokes which mainly occurred by an atherosclerotic mechanism of cerebral arteries or other types like lacunar infarction and infarction of unusual cause.

Moayyedi et al. in a study on 467 in-patients with clinical evidence of acute ischemic stroke and 388 healthy controls with no evidence of cerebrovascular disease, found a significantly more *H. pylori* positive individuals in the cases compared to the controls. They concluded (that) infection with *H. pylori* was associated with an increased risk of stroke and increased fibrinogen levels.^[8]

Ponzetto and colleague in a similar study evaluated the prevalence of *H. pylori* infection in 80 consecutive patients with acute ischemic stroke. *H. Pylori* infection was diagnosed by means of both ¹³C urea breath test and IgG antibodies to *H.*

pylori also. They evaluated *H. pylori* infection in a group of 320 blood donors matched for sex and age served as controls. The presence of *H. pylori* infection was higher in patients than in controls: 64/80 (80%) vs 190/320 (59.4%) ($P<0.001$).^[9] Also Grau et al. found *H. pylori* seropositivity as a possible independent risk factor for stroke of atherothrombotic origin.^[10]

One study in Argentina demonstrated the presence of the microorganism in carotid lesions. However, they were unable to speculate on the role of *H. pylori* in the initiation, progression, and/or complication of the atherosclerotic process.^[11]

Some mechanisms may play a role in atherogenesis of chronic *H. pylori* infection. These are: a low-grade acute phase response, free radical formation, and immune mediated mechanisms. Blood markers of an acute phase response are raised in chronic *H. pylori* infection. Free radical formation and immune mediated mechanisms may also be important. Antioxidants have been shown to be decreased in subjects with *H. pylori*.^[12] Another possible mechanism linking *H. pylori* and atherogenesis is lipid peroxidation due to antioxidants loss.^[13] Another explanation about relation between cardiovascular disease and *H. pylori* is via hyperhomocysteinaemia.^[14] There is a potential that chronic bacterial infection may aggravate pre-existing plaque by enhancing T-cell activation as well as other inflammatory responses that may participate in the destabilization of the intimal cap resulting in plaque rupture, progression to acute ischemic syndromes, and ultimate enlargement of the atherosclerotic plaque.^[15] Further studies are required to evaluate whether antibiotic treatment may result in clinical benefit to the patients or stroke primary prevention.

According to some recent studies, infection with more virulent *H. pylori* strains, such as cytotoxin-associated gene-A (CagA) bearing strains, may be of particular relevance for ischemic diseases. Preusch investigated whether *H. pylori* and CagA seropositivity are independent risk factors for cerebral ischemia or its etiologic subtypes. They determined IgG antibodies against *H. pylori* and CagA protein in 190 patients with acute cerebral ischemia and in 229 age and sex matched control subjects. CagA seropositivity was more common in patients (114/190; 60.0%) than in control subjects (99/229; 43.2%; Odds ratio, 1.97; 95% CI, 1.33 to 2.91; $P<0.001$), which remained significant after adjustment for age, sex, vascular risk factors and diseases, and childhood and adult social status. *H. Pylori* seropositivity in general was not associated with increased risk of stroke or its etiologic subtypes.^[16] Another recent study has shown infection with CagA-positive *H. pylori* strain in atherosclerotic stroke patients is associated with greater intima-media thickness and poorer short-term outcome compared with CagA-negative patients.^[17]

Conclusion

In summary, our study shows an association between anti-

body response to *H. pylori* and first noncardioembolic ischemic stroke. This finding is in accordance with recently published epidemiological studies and meta-analyses on coronary artery and cerebrovascular diseases.^{[18],[19],[20]} According to Cremonini et al., association between *H. pylori* positivity, anti-CagA positivity and stroke is modest and seems higher with stroke due to large vessel disease.^[20] Further studies that are presently ongoing and planned for the near future are expected not only the elucidation of the pathophysiology related to the association between chronic bacterial infection and atherosclerosis but evaluating whether antibiotic treatment may result in clinical benefit of the patient.

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Invited Comments

Well documented risk factors for acute ischemic stroke, including elevated blood pressure, diabetes mellitus, hyperlipidemia, heavy smoking, and ischemic heart disease do not fully account for the whole risk of stroke and it is frequently observed that stroke patients often do not present any of these predisposing conditions.

Recent studies have demonstrated that inflammatory parameters and chronic and acute infectious diseases have been considered to modify stroke risk independent of conventional risk factors. Increasing evidence has linked infections (especially respiratory and dental infections) to atherosclerosis, myocardial infarction, and stroke.^[1,2] On the basis of current knowledge, infection/inflammation, specific genetic predispositions, and traditional risk factors interact with each other and may cooperatively enhance the risk of stroke^[1]. Therefore, a genetically determined strong response to inflamma-

tory stimuli (e.g., acute or chronic infection) may be associated with an increased risk for stroke.

Infections may induce thrombosis and brain infarction by several mechanisms. Some mechanisms indicate that infections are related to large-artery atherogenesis. Other mechanisms suggest a prothrombotic state. Chronic infection may indirectly influence the risk of atherosclerosis and thrombosis by other pathways, such as: 1) changes in lipid metabolism, 2) immune-mediated mechanisms; 3) recurrent bacteremia which may induce platelet activation and a procoagulant state, 4) alterations in endothelial function, 5) spasm in vascular smooth muscle, and 5) atheroma instability and subsequent plaque rupture.

Infection with *Helicobacter pylori* is among the infectious disease discussed in this respect. Recent results regarding *H. pylori* antibody titers and stroke reported an association be-

tween atherothrombotic and lacunar stroke (but not with other stroke subtypes) and *H. pylori* seropositivity, as shown by the study published in the current issue of the Journal.^[3] However, in a recent study, only increased antibody titers against cytotoxin-associated gene-A bearing strains of *H. pylori* were independently associated with cerebral ischemia, whereas *H. pylori* seropositivity in general was not.^[4]

The link between *H. pylori* infection and ischemic stroke requires further studies. *H. pylori* infection is a potentially curable disease and for this reason, the identification of this condition as a stroke risk factor may have important implications for the prevention of acute cerebrovascular accidents. If the potential role of *H. pylori* infection as a risk factor for stroke will be confirmed, antimicrobial treatment would have a role in the prophylactic strategies of cerebral ischemia, together with effective drug therapies based on antiplatelet

agents, therapeutic anticoagulation or treatment with statins.

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