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Influence of chronic Helicobacter pylori infection on ischemic cerebral stroke risk factors

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Infection by *Helicobacter pylori* (Hp) has been linked to extradigestive pathologies including ischemic cerebral disease. The aim of our study was to assess the relationship between chronic Hp infection and ischemic stroke risk factors.

Material/Methods: 80 patients (pts) aged 60–75 years with ischemic stroke confirmed by CT scans (group I) and 80 age- and gender-matched healthy controls (group II) were included into trial. Atherosclerotic plaques from 20 Hp positive pts were obtained at carotid endarterectomy for Hp DNA assessment by PCR. In all groups following parameters were determined; 1) the prevalence of Hp infection using ^{13}C-Urea Breath Test (UBT), 2) plasma anti-Hp and anti-CagA IgG and interleukin-8 (IL-8), and 3) plasma lipids and fibrinogen. Hp positive pts and controls received one-week anti-Hp therapy and after six months total cholesterol, low-density lipoprotein (LDL)-cholesterol, fibrinogen and IL-8 levels were re-examined.

Results: Hp infection was detected by UBT in 83.75% of stroke pts but only in 65% of controls. CagA seropositivity was also significantly higher in stroke pts (57.5%) than in controls (33.75%). Plasma levels of cholesterol, LDL-cholesterol and fibrinogen as well as IL-8 were significantly higher in Hp positive subjects, especially in pts with ischemic stroke. Six months following successful anti-Hp therapy, the plasma levels of total cholesterol, LDL-cholesterol, fibrinogen and IL-8 were significantly lower than those in Hp positive stroke pts and controls.

Conclusions: Hp infection represents risk factor of ischemic stoke via an interaction of Hp cytotoxins or cytokines with atherosclerotic plaques in carotic arteries.

key words: **Helicobacter pylori • inflammation • atherosclerosis • stroke**

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BACKGROUND

Helicobacter pylori (Hp) is a Gram-negative microaerophilic *bacterium* that colonizes gastric mucosa and that is considered as the main etiological factor in chronic active gastritis and a risk factor for peptic ulcers and gastric cancer [1,2]. Seroprevalence of *Hp* infection was found positive in about 50% of the world population and the results showed higher *Hp* infection in developing than in developed countries. *Hp* infection was also increased with the age of population [3].

Recent evidence indicates that local and systemic immunological response elicited by *Hp* infection is an important factor not only for the gastric mucosal damage but also for the extradigestive pathologies [4–6]. Clinical pathology of *Hp*-infected gastric mucosa depends upon the expression of bacterial CagA and VacA cytotoxins and immunological responses, particularly the release of proinflammatory cytokines in infected subjects [7–9]. Production of excessive amounts of proinflammatory factors and cross mimicry between bacterial and host antigens may contribute to the development of gastric mucosal damage and extradigestive manifestations associated with this infection [10–13]. *Hp* infection has been epidemiologically linked to some extradigestive conditions such as idiopathic thyreoiditis and some skin diseases [4,6,14,15]. It has been also associated with ischemic coronary heart and cerebral vascular diseases, but the number of supporting publications is relatively small and the results obtained are often contradictory and poorly controlled [16–21].

Diabetes, hyperlipemia, alternations in clotting factors, hypertention, smoking, obesity and life style are well recognized modifying factors and age is the major unmodifying factor for the development of atherosclerosis and ischemic cerebral disease [22]. Recently, several studies performed *in vitro* on serum and vascular tissue specimens provided evidences for participation of infectious, bacterial and viral, factors in the pathogenesis of atherosclerosis [23–27]. Studies in humans and animals also emphasized the importance of infectious factors in atherogenesis [10,11,28,29].

Hp infection is one of the most widely spread infections in humans and its prevalence is positively correlated with age of population [3]. Acute ischemic stroke in Poland remains the fourth major cause of mortality and its prevalence also increases with the age of population [30]. Higher levels of proinflammatory and procoagulant factors such as: C-reactive protein (CRP), increased leukocyte blood count, enhanced fibrinogen concentration and altered plasma lipid profile were observed in subjects infected with *Hp* [31-34]. There are discrepancies regarding the influence of *Hp* infection on major plasma biochemical risk factors of atherogenesis. According to some authors the *Hp* infection enhances atherosclerosis by altering the plasma concentrations of biochemical indices of atherogenesis [35-38], but others believe that bacterium directly contributes to the atherogenesis by induction of chronic inflammatory response in vascular wall without major alterations in biochemical atherosclerotic risk factors [39–42]. It is well-known that chronic *Hp* infection enhances plasma levels of proinflammatory interleukins (IL), including IL-1, IL-6, IL-8 and tumor necrosis factor α (TNF α) [10]. IL-8 may play an important role in recruitment and activation of inflammatory cells, which are the key factors in initiation and progression of atheromathic processes [43-45]. Increased concentrations of IL-8 were detected mainly in cerebrospinal fluid (CSF) and also in serum of patients with ischemic stroke during the first month after ischemic brain injury [46].

Earlier studies have reported an association between chronic gastric mucosa *Hp* infection and ischemic heart or cerebral diseases [16-18,34], however, the possible mechanisms by which *Hp*-induced gastric inflammation could cause atherogenesis remains unknown.

The main purpose of this study was to assess the relationship between chronic *Hp* infection and acute ischemic stroke in elderly persons with special reference to the influence of *Hp* strain cytotoxicity on some blood chemicals implicated in atherogenesis.

MATERIAL AND METHODS

Eighty non-diabetic, non-smokers of similar social class patients (pts), 60–75 years old, with first-ever signs of acute ischemic cerebral stroke due to large vessel disease admitted consecutively to a Neurological Cerebrovascular Department of Neurological Clinic in 1999 and 2000 year were enrolled into the study (group I). Stroke pts due to cardioembolism or of unknown etiology were not included to the study. Ischemic cerebral stroke in supratentorial area in our pts were confirmed by computed tomography (CT). CT scans were performed at first and fourth days of clinical manifestation of the disease.

Control group was recruited from 80 age-, gender- and socioeconomic status-matched persons without any neurological symptoms (group II). In addition, twenty *Hp* positive pts with critical stenosis of common carotid artery, confirmed ultrasonographically by duplex ultrasound and angiographically, were included into the study (group III). Each patient from each study group was examined using CT, carotid duplex ultrasound and electrocardiography (ECG).

Determination of *Hp* infection status in stomach

The active *Hp* infection in stomach was estimated using ^{13}C-Urea Breath Test (UBT) as described previously [47]. After overnight fast two baseline (prior to urea administration) breath samples were collected into testing vials (Labco exetainer) from each subject. This was followed by ingestion of gelatine capsule containing 38 mg of ^{13}C-urea swallowed with 25 ml of water. After 3 min, each subject drank additional 25 ml of water and breath samples were again collected after 10 and 20 min upon the ^{13}C-urea administration. The final results of $^{13}CO_2/^{12}CO_2$ ratios were measured with the use of isotope-ratio mass-spectrometry (IRMS, Heliview, Medichems Seoul, Korea) and were expressed as $\delta^{13}CO_2$ (per mil) values. A change of mean $\delta^{13}CO_2$ value over baseline (DOB) after urea capsule ingestion, of more than 2.5 was considered as positive result.

In all tested subjects, the samples of venous blood were withdrawn under basal conditions after overnight fast and the plasma was separated and stored at –70°C until it was used for further examinations.

Examination of the IgG antibodies against *Hp* and CagA protein by enzyme-linked immunosorbent assay (ELISA)

The *Hp* infection status was assessed by determining IgG antibodies against *Hp* using commercial rapid enzyme linked immunosorbent assay kit (ELISA, BioSource, Europe S.A.). As recommended by producer, titers higher than 15 AU/ml were considered positive. The IgG antibodies to CagA cytotoxins were detected by 'in house' ELISA test using recombinant CagA (gift from Ora Vax Cambridge, USA) as antigen. The optimal antigen concentration was 0.5 μg/well and such aliquots were loaded into wells in 96-well microtiterplate. The optimal dilution of human serum was 1:100 and horseradish peroxidase-conjugated anti-human IgG was used at a dilution of 1:4000. Titers higher than 0.3 OD were considered as CagA positive.

Determination of plasma IL-8 concentration

Plasma IL-8 levels were measured by ELISA using commercially available kit (BioSource, Europe S.A.) and assay was performed according to the manufacture's instructions.

Determination of plasma fibrinogen, cholesterol and LDL-cholesterol

Plasma obtained from *Hp*-positive and *Hp*-negative patients and control subjects was also examined for concentrations of total plasma cholesterol, LDL cholosterol and fibrinogen (Clauss assay) using standard enzymatic laboratory methods.

PCR detection of *Hp* DNA in atherosclerotic plaque

Sample of carotid plaques obtained at carotid artery endartherectomy were stored at –70°C before processing. DNA extraction was performed using the Trizol Reagent according to the manufactures instructions (Gibco BRL/Life Technology, Eggenstein, Germany). The polimerase chain reaction (PCR) was used to identify bacterial DNA with a pair of primers that amplify a specific DNA region codifying for the 16 S ribosomal RNA of Hp (sense: 5'-TCA GCC TAT GTC CTA TCA GC-3'; anti sense: 5'-CAG TAA TGT TCC AGC AGG TC-3'). The amplified 499 bp product was analysed by gel electroforesis on 1.5% agarose gel stained with ethidium bromide. As a positive control for *Hp* the DNA extracted from pure *Hp* culture was also amplified. As a negative control for *Hp*, the autopsy material from carotid arteries without atherosclerotic changes were used. The reaction was considered positive when migrating in the band of molecular weight of the positive controls. Sequence analysis of the PCR-products confirmed, that the amplified gene products were specific for *Hp*.

All *Hp* CagA(+)/CagA(–) stroke pts and controls received standard one week anti-*Hp* triple therapy (Clarithromycin 500 mg bd, Amoxycillin 1000 mg bd and Omeprazole 20 mg bd). Six months after successful therapy confirmed by ^{13}C UBT, serum IL-8 and plasma total cholesterol, LDL-cholesterol and fibrinogen levels were again determined in 10 *Hp* CagA(+)/CagA(–) stroke pts and 10 control subjects.

Statistical analysis

Statistical analyses were made by using Mann-Whitney test to calculate frequency of CagA(+) or CagA(–) *Hp* infection in studied groups. Kruskal-Wallis and Tuckey's repeated-measures tests, Student's t-test and Odds Ratio (OR) were used to compare plasma biochemical parameters and risk of stroke in *Hp* infected subjects.

RESULTS

The prevalence of *Hp* infection, detected by UBT and confirmed by anty-*Hp* IgG in studied stroke pts and healthy controls, shows that the *Hp* infection rate was significantly higher in stroke pts than in healthy controls (83.75% vs 65% for UBT and 86.25% vs 67.5% for IgG – Table 1). The prevalence of CagA positive *Hp* infection was also significantly higher in stroke pts than in con-

Table 1. The prevalence of Hp infection in controls and stroke pts groups.

Test	Control	Stroke pts
Hp 13C-UBT	65.00%	83.75%*
Anti-*Hp* IgG	67.50%	86.25%*
Anti-*CagA* IgG	33.75%	57.50%*

*p<0.05 indicates the statistical difference between studied groups

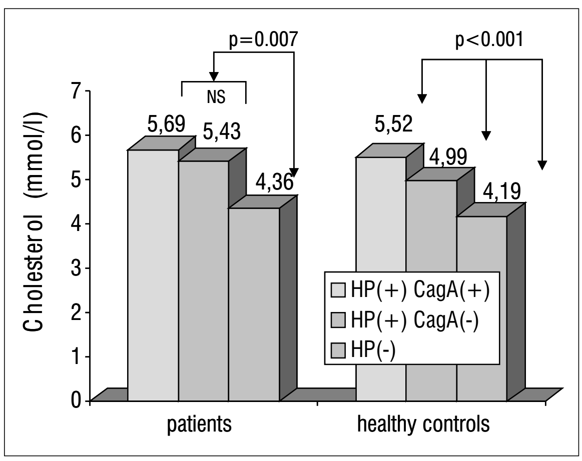

Figure 1. Mean total plasma cholesterol concentrations in Hp CagA(+), Hp CagA(–), Hp (–) stroke pts and controls

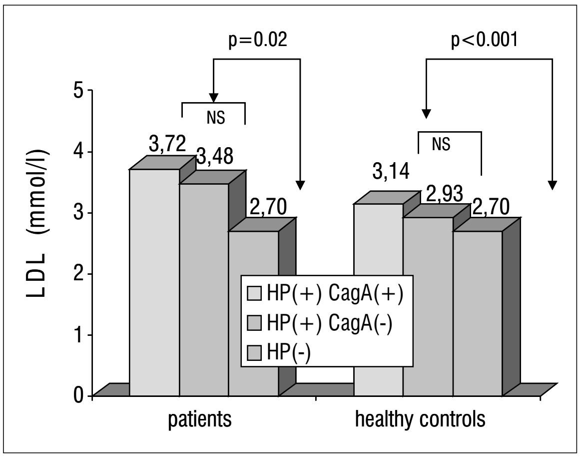

Figure 2. Mean total plasma LDL-cholesterol concentrations in Hp CagA(+), Hp CagA(–), Hp (–) stroke pts and controls

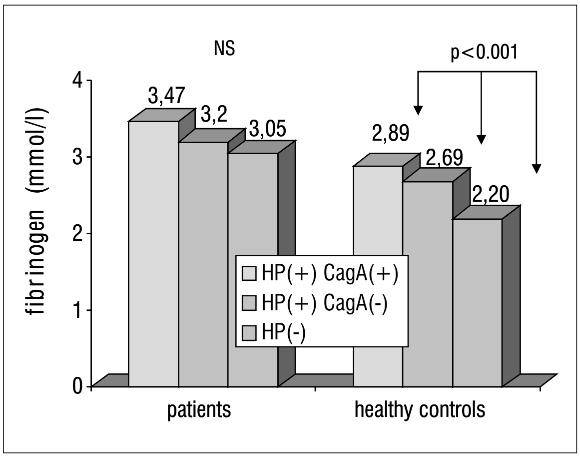

Figure 3. Mean total plasma fibrinogen concentrations in Hp CagA(+), Hp CagA(–), Hp (–) stroke pts and controls

trols (57.5% vs 33.75%, Table 1). The OR calculated for *Hp* infected stroke pts was 2.35 (95% CI; 1.08–6.98).

Figure 1 shows the mean total plasma cholesterol concentrations in stroke pts infected with *Hp* CagA(+) or CagA(–) and uninfected stroke pts in comparison to control subjects infected with *Hp* CagA(+) or *Hp* CagA(–) and without *Hp* infection. In *Hp* infected CagA(+) and CagA(–) stroke pts, total plasma cholesterol concentrations were significantly higher than in *Hp* negative stroke patients (5.69 and 5.43 vs 4.36 mmol/L, respectively, p=0.007). In stroke pts infected with CagA(+)*Hp* strain, mean total cholesterol concentration tended to reach higher value than that in *Hp* CagA(–) pts, but the difference did not reach statistical significance.

Healthy controls infected with *Hp* CagA(+) or CagA(–) strains had higher mean total plasma cholesterol concentrations 5.52 and 4.99 mmol/L, respectively, than uninfected subjects (4.19 mmol/L). The highest value of total plasma cholesterol concentration was observed in *Hp* CagA(+) and this was significantly higher than that recorded in controls infected with *Hp* CagA(+), the va-

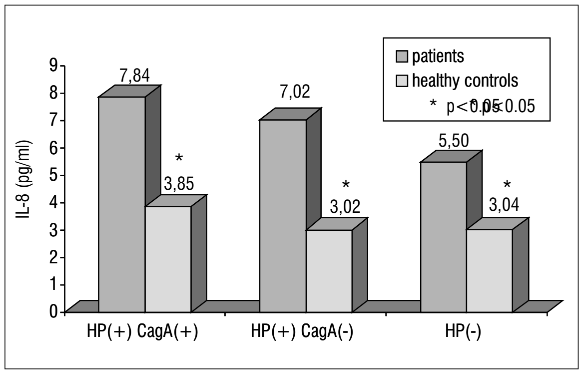

Figure 4. Mean total plasma IL-8 levels in controls and stroke pts respectively in subgroups Hp CagA(+), Hp CagA(–), Hp (–).

lue in latter group being significantly higher than in *Hp* negative controls. The differences in mean total plasma concentrations reached statistical significance between each studied subgroup (p<0.001).

Influence of *Hp* infection on mean plasma concentration of LDL-cholesterol, another well-known atherogenic factor, in stroke pts and controls is shown on Figure 2. Stroke pts infected with *Hp* CagA(+) or CagA(–) showed significantly higher plasma LDL concentration than *Hp*-negative stroke pts (p=0.02). The highest value of LDL-cholesterol concentration i.e. 3.72 mmol/L was observed in stroke patients infected with *Hp* CagA(+).

Controls infected with *Hp* CagA(+) or *Hp* CagA(–) had also higher LDL-cholesterol concentration in comparison to that in uninfected subjects (3.14; 2.93 and 2.70 mmol/L), respectively). There was no statistically significant difference in LDL-cholesterol concentrations between *Hp* CagA(+) and CagA(–) stroke subjects or control subgroups, but it was observed between *Hp* CagA(+) and *Hp* negative individuals (p<0.001).

Figure 3 shows the plasma fibrinogen concentrations in *Hp* CagA(+) or CagA(–) infected and *Hp* uninfected stroke pts and control subgroups. In stroke pts infected with *Hp* CagA(+) or CagA(–) and *Hp* negative mean plasma fibrinogen concentrations were similar and no significant difference between these subgroups was observed. Such differences were observed however between controls infected with *Hp* CagA(+) and *Hp* CagA(–) or those not infected with *Hp* (p<0.001). The highest value of plasma fibrinogen concentration in controls was found in *Hp* CagA(+) subgroup (2.89 mmol/L). Plasma fibrinogen concentrations detected in all stroke pts subgroups (*Hp* CagA(+), *Hp* CagA(–) or *Hp*-negative) were higher than those obtained in respective control subgroups.

Plasma IL-8 levels in stroke pts infected with *Hp* CagA(+) or CagA(–) and in uninfected were significantly higher than those in respective control subgroups (Figure 4). The mean highest value of plasma IL-8 concentration was 7.84 pg/ml and it was reached in stroke pts infected with *Hp* CagA(+) subgroup but this value was not significantly different from that in CagA(–) or *Hp* nega-

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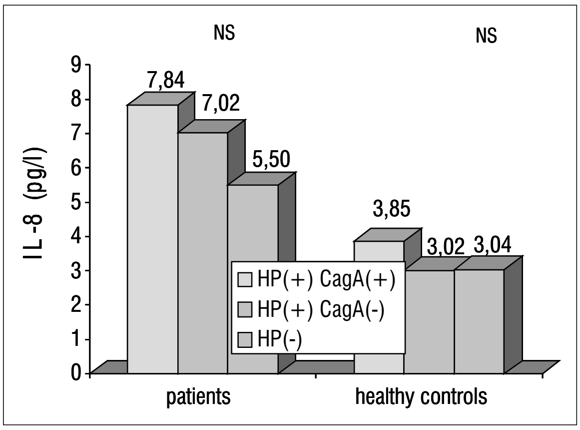

Figure 5. Mean serum IL-8 levels in Hp CagA(+), Hp CagA(–), Hp (–) stroke pts and controls

tive stroke pts (Figure 5). No statistically significant difference in IL-8 was also found between subgroups of *Hp* positive CagA(+) or CagA(–) and *Hp* negative healthy controls (Figure 5).

Figure 6 shows the ethidium bromide-stained 1.5% agarose gel electrophoresis. DNA product for *Hp*, 16S rRNA, was not detected in five *Hp* negative controls taken at autopsy from subjects without carotid atherosclerotic plaques. DNA for *Hp* 16S rRNA was found in atherosclerotic plaques of 5 out of 20 (25%) tested stroke patients with carotid endarterectomy.

Table 2 shows the plasma concentrations of total cholesterol, LDL-cholesterol, fibrinogen and serum IL-8 levels in 10 *Hp* CagA(–) and 10 *Hp* CagA(+) control subjects before and 6 months after successful (based on negative UBT) standard anti-*Hp* therapy. In *Hp* CagA(–) subgroup there was no statistical difference in plasma concentrations of total cholesterol, LDL-cholesterol and fibrinogen before and after therapy, but mean plasma IL-8 level was statistically diminished in those subjects after therapy. In control *Hp* CagA(+) subjects total plasma cholesterol and IL-8 levels were statistically reduced 6 months after anti-*Hp* therapy, but reduction in plasma LDL-cholesterol and fibrinogen concentrations tended to decrease but this failed to reach statistical significance.

Table 3 shows the plasma concentrations of total cholesterol, LDL-cholesterol and fibrinogen and plasma IL-8 level in 10 *Hp* Cag(–) and 10 *Hp* Cag(+) stroke patients before and 6 months after successful standard anti-*Hp* triple therapy. In both subgroups plasma concentrations of total cholesterol, LDL-cholesterol and serum IL-8 were significantly decreased 6 months after anti-*Hp* therapy. Only plasma fibrinogen concentrations was not significantly affected 6 months after anti-*Hp* therapy in these stroke patients.

Discussion

Our study provides an evidence for the possible implication of *Hp* infection in cerebrovascular stroke via enhancing some risk factors of atherogenesis.

Table 2. Mean (±SD) of plasma and serum levels of total cholesterol, LDL-cholesterol, fibrinogen [mmol/l] and IL-8 [pg/ml] in the same 10 *Hp* Cag A(–) and 10 *Hp* Cag A(+) control subjects before and 6 months after the *Hp* eradication.

	Parameter	Before treatment X±SD	After treatment	p
Hp Cag A(–)	Total cholesterol	4.80±0.66	4.89±0.83	NS
	LDL cholesterol	2.99±0.61	2.83±0.67	NS
	Fibrinogen	2.73±0.19	2.50±0.53	NS
	IL-8	6.11±2.48	4.25±1.58	0.017
Hp Cag A(+)	Total cholesterol	5.46±1.14	4.99±1.07	0.003
	LDL cholesterol	3.16±0.78	3.04±0.61	NS
	Fibrinogen	2.65±0.72	2.50±0.53	NS
	IL-8	5.77±1.36	5.00±1.07	0.03

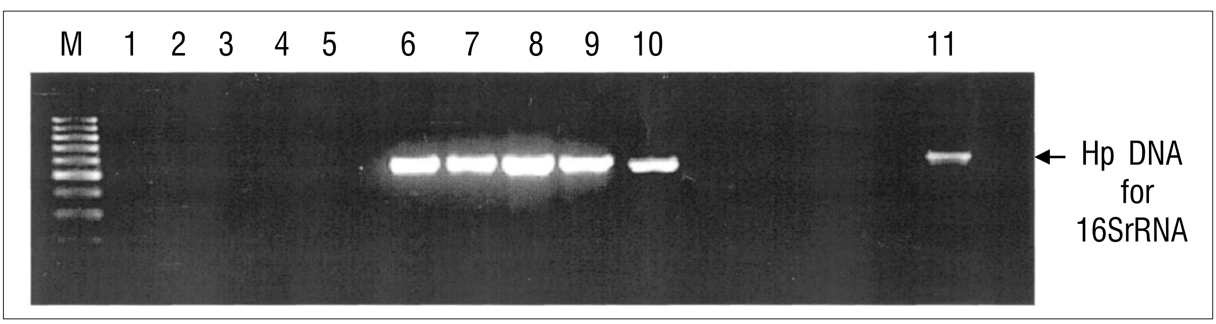

Figure 6. Analysis of the PCR products for Hp 16S rRNA. Molecular marker (line M), five Hp negative subjects (lines 1–5), five Hp positive patients (lines 6–10) and Hp DNA positive control (line 11). Electrophoresis in ethidium bromide stained 1, 5% agarose gel. Molecular weight of product generated with primers (sequence given in Methods section) is 499 bp.

Table 3. Mean (±SD) of plasma and serum levels of total cholesterol, LDL-cholesterol, fibrinogen [mmol/l] and IL-8 [pg/ml] in the same 10 *Hp* Cag A(–) and 10 *Hp* Cag A(+) patients before and 6 months after the successful *Hp* eradication.

	Parameter	Before treatment X±SD	After treatment X±SD	p
Hp Cag A(–)	Total cholesterol	6.22±1.12	4.71±0.36	0.007
	LDL cholesterol	3.67±0.81	2.75±0.45	0.04
	Fibrinogen	3.01±0.88	3.29±0.49	NS
	IL-8	7.84±1.60	5.75±1.58	0.04
Hp Cag A(+)	Total cholesterol	6.61±0.73	5.23±1.07	0.008
	LDL cholesterol	3.65±0.82	2.86±0.78	0.06
	Fibrinogen	3.43±0.44	3.00±0.82	NS
	IL-8	8.75±4.29	6.38±3.34	0.016

Several previous studies were carried out to investigate the relationship between *Hp* seropositivity associated with *Hp* infection and both, coronary heart and cerebrovascular diseases risk factors. The results obtained favored an association of chronic gastric *Hp* infection, especially expressing CagA cytotoxins, with ischemic heart disease and ischemic stroke [9,16,34,48]. In our present study we confirmed a higher prevalence of *Hp* infection in patients with ischemic cerebral disease as compared to age- and gender-matched controls. Moreover, we found a significantly higher prevalence of more virulent *Hp* CagA positive strains in ischemic stroke pts as compared to healthy controls.

Recently, new approach has been attempted to explain the pathomechanism of the heart and cerebral vessel atherosclerosis in relation to extradigestive manifestation of chronic infection with *Hp*. Various mechanisms by which *Hp* could increase the risk of arterial plaque formation have been proposed. At present, the data support both indirect and direct effects of bacterial infection on atherogenesis [29,49]. *Hp* infection could result in a low grade chronic inflammatory process in vascular endothelium and this could promote atherogenic changes by altering some major vascular risk factors such as fibrinogen and plasma lipid fractions [16,50,51]. In our study, the *Hp* seropositivity correlated with increased total plasma cholesterol concentration not only in stroke pts but also in control subjects. Similar association was also observed with respect to plasma LDL-cholesterol concentrations which were significantly elevated in *Hp* infected stroke pts and control groups, especially in *Hp* subjects infected with more virulent CagA positive strains, being in agreement with the data published elsewhere [9,31,52]. Our finding supports the hypothesis that chronic *Hp* infection may modify the plasma lipid profile in a way that increases the risk of atherosclerosis.

Underlying processes that might explain the association between infectious agents and atherosclerosis remain still unclear and they are the subject of debate. Feingold et al [35] postulated that chronic infection may alter lipid profile in an atherogenic direction *via* the action of proinflammatory cytokines such as IL-1 and IL-6, interferon α and TNFα that are capable to affect lipid metabolism in different ways. They suggested that cytokines may activate adipose tissue lipoprotein lipase, stimulate hepatic fatty acid synthesis and influence lipolysis. Basso and his colleagues [53] observed significantly higher polymorphonuclear leukocyte oxidative burst in *Hp* infected patients than in *Hp* negative or healthy controls. This indicates that free radical formation could also play an important role in atherogenesis. It has also been shown that *Hp* infected subjects exhibit the decreased level of antioxidants [54]. These events may be associated with elevated lipid peroxydation, especially LDL fraction, which could be an important mechanism linking *Hp* infection and various phases of atherosclerotic plaque formation *via* elevated oxidized LDL levels [55].

In the present study the plasma fibrinogen concentration was not influenced by *Hp* infection in stroke pts or healthy controls, but the plasma fibrinogen was significantly higher in *Hp* positive stroke patients than in *Hp* negative controls. Our study is in keeping with results obtained by other authors [16,56–58] that the higher value of plasma fibrinogen concentration was observed in *Hp* infected CagA positive subjects than in uninfected controls. *Hp* may be a life-long bacterial infection of the gastric mucosa, that is mainly acquired in childhood [3]. Fibrinogen is an acute phase protein and its level strongly corresponds to the process of atherogenesis. Fibrinogen seems to participate directly in early phases of atherosclerotic plaque formation and arterial thrombosis [59,60]. Moreover, fibrinogen is one of the more important components of the acute and chronic inflammatory responses [61]. Our finding suggests that fibrinogen level may slowly and gradually increase during prolonged chronic *Hp* infection and may be related to the length of disease. Zito et al [56] and Patel et al [57] showed increased levels of plasma fibrinogen in *Hp* infected individuals even after controlling for possible confounding factors related to either infection or fibrinogen. Bierti and colleagues [62] also found a significantly higher mean plasma fibrinogen level in *Hp* infected than uninfected individuals. Moreover, they found higher levels of factor VII and protrombin cleavage fragment in *Hp* positive subjects. There are, however, conflicting results whether treatment of *Hp* gastric mucosa infection decreases plasma fibrinogen concentrations. Treiber [63] and Torgano et al [64] observed beneficial effect of *Hp* eradication in patients with ischemic heart disease. Oderda et al [65] failed to detect the increased plasma fibrinogen concentration in *Hp* infected children when compared to *Hp* negative controls, but significant decrease in plasma fibrinogen levels were observed after *Hp* eradication and no change, but a mild increase when infection persisted. Higher plasma fibrinogen concentrations were, however, seen in older *Hp* positive children suggesting that this observation may be related to the length of disease. The importance of this finding is highlighted by the possibility of an effective pharmacological intervention against *Hp* to decrease the levels not only plasma fibrinogen concentration but also some others acute and chronic mediators of inflammation as well as atherogenesis [65]. Other authors failed to observe the significant association between *Hp* infection and the elevated plasma fibrinogen level [66–68]. However, in some of *Hp* positive subjects a spontaneous rise in fibrinogen plasma concentration was observed and this could influence the stability of ischemic heart disease [63].

In other studies the association between chronic *Hp* infection and hemostatic system has been examined. Increased levels of circulating activated platelets and platelet aggregates were found in pts who were *Hp* seropositive and also platelet P-selectin expression was enhanced in *Hp* infected human and mice [69]. Moreover, chronic infection of mice with *Hp* lead to increased thrombi formation resulting in embolism after damage to arterioles [70]. Therefore, platelet activation and aggregation observed in humans and experimental animals may contribute to the microvascular dysfunction associated with *Hp* infection. This phenomenon as well as plasma fibrinogen alterations could be, at least in part, explanatory to the pathogenesis of *Hp* infection in atherogenesis.

Over the past few years, a growing body of evidence has stressed the role of inflammation in the pathophysiology of atherosclerosis and acute ischemic stroke [71–75]. Most inflammatory reactions have been attributed to cytokines, such as interleukins, that are responsible for up regulation of adhesion molecules, recruitment and activation of leucocytes, promotion of leukocyte-endothelium interaction and conversion of local endothelium to a prothrombotic state [72]. These mediators are also able to change the hemostatic system by increasing the expression of procoagulant substances (fibrinogen, plasminogen activator inhibitor-1) [76], down regulating the fibrinolitic system [77] as well as they could cause a prolonged endothelial dysfunction [78]. Cytokines may also affect lipid metabolism by liver stimulation of fatty acid synthesis and lipolysis in adipocytes resulting in proatherogenic alteration in plasma lipid profile [35, 37,38].

Increased synthesis of IL-8 was observed in acute ischemic stroke up to one month after onset of symptoms, however this, has not been restricted to the ischemia of central nervous system (CSN), but also could be detected systemically [46,79–81]. IL-8 is a well-known chemokine that promotes invasion of leucocytes into the brain. The activation by IL-8 or proteases and free radicals formation due to infiltration of neutrophils into the cerebral tissue might augment the production of oxygen or nitrogen reactive species as well as lipid peroxydation and subsequently neuronal damage [80]. This remains in agreement with the observation of increased IL-8 concentration in cerebrospinal fluid (CSF) compared with plasma in pts with ischemic stroke indicating that IL-8 in this pts is predominantly of CNS origin, probably at the site of the neuronal tissue damage. Furthermore, following the CSN injury, the blood-brain barrier may become leaky, and this could facilitate the entry of activated circulating immune cells into the CSN to generate reactive oxygen species [82].

A strong systemic response as reflected by increased serum levels of IL-8, IL-1β, IL-6, TNF-α in stroke pts may indicate a possible role for systemic cells regarding the production of cytokines within CSN [73,83]. An inflammatory process in peripheral tissues distal from the CSN that occurs parallelly to the ischemic event e.g. concomitant infection may result in increased levels of cytokines derived by activated peripheral blood mononuclear cells (PMBC). Numbers of activated PMBC in the CSN strongly correlate with the severity of the ischemic event [46]. Systemic up-regulation of cytokine expression may contribute to the pathogenesis of ischemic stroke through a potentiation of the secondary inflammatory process [46].

In *Hp* infected individuals increased levels of IL-8 are regularly detected. The major source of IL-8 in *Hp* infected gastric mucosa are neutrophils and epithelial cells. IL-8 and many others released cytokines, especially TNFα, probably contribute to the enhanced gastrin release and gastric acid secretion [84-86]. Most of immunological responses are strongly enhanced by *Hp* strain expressing cagA and vacA encoded cytokines [9,87,88].

Our study failed to show any statistical significant differences in mean plasma IL-8 concentration between *Hp* CagA(+) and *Hp* CagA(–) stroke pts or controls and in *Hp* negative pts and controls. However, in all these subgroups the overall levels of IL-8 were significantly higher in stroke pts as compared to healthy subjects. Thus, after ischemic stroke plasma IL-8 concentrations rise about twofold in each studied *Hp* infected or uninfected subgroup. The highest values of IL-8 concentration were reached in *Hp* CagA(+) subgroup. These results indicate that acute local neural tissue necrosis and inflammation is accompanied by enhanced systemic expression of IL-8 that in turn may play an important role in ischemic brain injury. The higher concentration of IL-8 in *Hp* positive pts, predominantly in those infected with *Hp* strains expressing CagA are more prone to develop ischemic stroke and preexisting *Hp* infection may correlate with severity and long term clinical outcome of stroke.

It has been also proposed that cytokines such as IL-8 and TNFα and acute phase inflammatory response reactants are significantly higher in *Hp* infected coronary artery disease (CAD) pts than in control subjects [89]. This correlation is enhanced in *Hp* CagA(+) infected CAD pts. Pasceri et al [9] and Kowalski et al [89] have proposed that long term persistent infection with cytotoxic *Hp* strain enhances atherosclerotic process through synthesis of acute phase reactants.

In the present study the *Hp* positive control subjects and stroke patients received standard anti-*Hp* therapy and after 6 months we again determined plasma concentrations of total cholesterol, LDL-cholesterol, fibrinogen and serum level of IL-8. In control subjects we failed to observe any alterations in plasma concentrations of atherogenic risk factors, but in stroke patients lipid plasma parameters were significantly reduced. After therapy there was also significant reduction in serum IL-8 level in both *Hp* Cag(–) and Cag(+) control and stroke pts subgroups, respectively. These results indicate the possible beneficial influence of anti-*Hp* therapy on the plasma levels of atherogenic and inflammatory stroke risk factors. The traditional risk factors explain the origin of about half of atherosclerotic plaques [90] and new approaches to the discovery of additional unexplained causes of atheromatic and thrombotic events should be elucidated. Bacteria such as *Chlamydia pneumoniae* and *Hp* and also hereditary predisposition to these infections are considered as independent factors of carotid plaque formation [91-92]. The question remains whether chronic or repeated treatment of bacterial infections could successfully eradicate chronic infection or prevent bacterial reinfections and in consequence reduce their influence on systemic atherosclerotic plasma risk factors and acute and chronic inflammatory reactions, which are key events in atherosclerotic plaque formations. This seems to be unlikely that treatment of such infections would be the sole or main way to prevent atherosclerosis but could be considered in the future as one of the important factors in ischemic stroke and in primary or secondary prevention of this disease.

In our study we investigated the presence of *Hp* in 20 atherosclerotic plaques obtained at carotid endartherectomy using highly sensitive polimerase chain reaction method. The DNA for *Hp* 16S rRNA was found in 5 of 20 (25%) atherosclerotic plaques. This finding indicates that bacterial infection occurring within the vessel wall may be directly implicated in the pathophysiological cascade leading to atherosclerosis through the processes such as initiation, progression and/or destabilization of atherosclerotic plaque and also *via* the initiation of athero-thrombotic events around the destroyed arterial wall.

Recently, further studies involve the direct implication of *Hp* infection in atherogenesis. *Hp* produces 60 kDa heat shock proteins which have a high degree of sequence homology with human 60 kDa heat shock proteins expressed in atherosclerotic lesions [93-94]. Cross reacting antibodies to heat shock proteins are a risk factor for carotid atherosclerosis and may be relevant for the pathogenesis of the vessel damage [95]. The existence of putative antigenic mimicry between atherosclerotic plaques and *Hp* antigens existed in serum of infected individuals was also shown by Cammarota [96]. *Hp* anti-CagA antibodies cross-reacting specifically with two high molecular weight vascular antigens were discovered by Franceschi [97]. Binding of anti-CagA antibodies to those antigens in injured arteries could influence the progression of atherosclerosis in CagA positive *Hp* infected pts. There are now also growing evidences for the presence of specific *Hp* DNA in atherosclerotic plaques detected by PCR [98-100].

In summary we found that chronic, especially *Hp* CagA(+) infection, seems to be more prevalent in stroke pts than in healthy population. Chronic *Hp* infection may raise total plasma cholesterol and LDL-cholesterol levels which are considered as a risk factors of atherosclerosis. Chronic *Hp* infection enhances plasma fibrinogen concentration and, therefore, may increase blood viscosity and promote clot formation in *Hp* positive persons. Deleterious effect of chronic *Hp* infection and stroke may be attributed to the higher generation of IL-8, because this cytokine levels are positively correlated with the severity of stroke and in consequence with clinical outcome. *Hp* infection with CagA(+) strain exhibit the highest modulating influence on studied levels of plasma risk factors of atherogenesis. Anti-*Hp* therapy may have beneficial influence on atherogenic and inflammatory plasma biochemical stroke risk factors. *Hp* infection occurring within the arterial wall may be directly implicated in the atherosclerotic processes.

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