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Occurrence of Serum Class G Immunoglobulins Interacting with Specific Antigens of *Helicobacter pylori* in Patients with Unstable Coronary Artery Disease and in Symptomless Individuals

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A b s t r a c t

An impact of *Helicobacter pylori* on the process of atherogenesis may be related to the intensity of humoral response against selected specific antigens of this bacteria. We performed serological studies in which the recognition of 7 selected antigens was possible. The investigated group consisted of 56 patients hospitalized due to unstable angina pectoris. The control group consisted of 29 symptomless volunteers. The levels of class G serum immunoglobulins interacting with glycine extract (GE) of *H. pylori* antigens were assessed by ELISA test in both groups. The same sera were tested by the Milenia blot *H. pylori* IgG system. In this assessment the presence of IgG antibodies interacting with antigens of molecular weight of 120, 87, 64, 35, 30, 26, and 20 kDa was estimated separately for every listed antigen. The results revealed significant differences between investigated groups in the prevalence of anti-GE IgG (unstable angina – 100% vs. controls – 60%) and in the level of such antibodies expressed as total optical density units – OD450 (6.1 ± 3.0 vs. 3.4 ± 3.0 respectively, $p<0.05$). However, anti-GE IgG detected in the sera of patients as well as controls reacted with similar frequency with selected *H. pylori* antigens: highly specific (120, 87, 64, 30 kDa) and specific (35, 26, and 20 kDa). We conclude, that although *H. pylori* infection is so common and mainly associated with gastroduodenal symptoms, it is also recognized by serological methods with high prevalence in patients with coronary artery disease, and less frequently in symptomless individuals. The humoral response against *H. pylori* in class G immunoglobulins in patients with unstable angina is characterized by higher levels of anti-*H. pylori* IgG but not by the higher prevalence of serum IgG interactions with the highly specific and specific *H. pylori* antigens. Such infection could be considered as a cofactor for atherogenesis by inducing strong humoral response against surface antigens of this bacteria.

K e y w o r d s: immunity, *H. pylori*, atherosclerosis, unstable angina

Introduction

Helicobacter pylori is one of the most common pathogens of humans causing a lifelong infection of gastric mucosa in >50% of the global population. This infection has been proved to be an important pathogenic factor for some gastro-duodenal diseases like type B gastritis, ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma (Rosenstock *et al.*, 1997; Howden *et al.*, 1996). Recent studies point to a possible role of *H. pylori* in extradigesive diseases, like Sjörgrene syndrome, thyroid diseases or Schönlein-Henoch purpura (Gasbarini *et al.*, 1998). Also vascular diseases like Raynaud phenomenon or headaches are considered to be linked with this infection. Although human atherosclerosis is a multifactoral disease, some possible links between this chronic process, infections and inflammation have been emphasized (Watanabe *et al.*,1996). A relationship between *H. pylori* seropositivity and atherosclerosis in general and coronary heart disease in particular has been reported in mid 1990s (Mendall *et al.*, 1994; Patel *et al.*, 1995); however the role of this infection in atherogenesis is still controversial – in some large studies, there was no correlation shown between *H.pylori*, cardiovascular risk factors and ischaemic heart

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disease (Murray *et al*., 1995; Ridker *et al.*, 2001). Since then, the methods of serological detection of *H. pylori* have become more sophisticated and accurate. This is a problem of great importance, as this bacteria shares a number of antigens with other microorganisms, giving false-positive cross-reactions in serological studies (Johansen *et al.*, 1995; Paziak-Domańska *et al.*, 2000). In the recent studies it was found that patients with unstable angina demonstrated enhanced humoral response to a glycine acid extract of *H. pylori* (Rechciński *et al.*, 2002). Glycine extract is a mixture of *H. pylori* surface antigens whose molecular weight ranges from 14 to 120 kDa. It is to be emphasized that in previous studies, the composition of circulating IgGs in respect to different specific and highly specific *H. pylori* antigens in patients with angina pectoris was not estimated or compared with symptomless individuals. In our present study, we intended to find out whether the enhancement of humoral response was generalized or rather limited to the immunoglobulins of a strictly limited idiotype (antigenic specificity). The aim of this study was also to find out how prevalent the patients with unstable angina are when infected by more virulent strain of *H. pylori*, characterized by the presence of cytotoxin-associated protein A (CagA), highly specific for this bacteria.

Experimental

Material and Methods

Fifty-six males (age 30–65 years) hospitalized due to chest pains in the Cardiology Department were included in the study after an unstable angina pectoris according to Braunwald definition was diagnosed. Coronary angiography confirmed the atheromatic background of symptoms (Braunwald, 1989). In this group, the prevalence of previous myocardial infarction was 52%, arterial hypertension – 72%, diabetes mellitus – 18%, hyperlipidemia – 62%, nicotinism – 18%, rate of the revascularisation (percutaneous transluminal coronary angioplasty or coronary artery by-pass graft) – 58%. The control group consisted of 29 symptomless age-matched males. All controls had negative history of ischaemic heart disease or gastric symptoms. The comparison of prevalence of risk factors of atherosclerosis in investigated group and controls is presented in Table I. Table II shows the pharmacological treatment obtained by patients and controls prior to blood sampling.

ELISA. Blood from antecubital vein was obtained from all study participants for serological tests. The sera were stored at a temperature of –70°C. The level of anti-*H.pylori* IgG was estimated using ELISA method with GE of *H. pylori* CCUG 17874 as the coating antigen and with rabbit anti-human IgG antibodies labeled with horseradish peroxidase HRP (Dako, Glostrup, Denmark). The serum samples for anti-GE IgG ELISA were diluted from 1:500 to 1:128000. The results were expressed as total optical density

Table I
The comparison of risk factors of atherosclerosis in investigated group and controls

Risk factor	Unstable angina pectoris	Controls
Diabetes mellitus	18%	3%
Arterial hypertension	72%	13%
Hypercholesterolaemia	62%	12%
Nicotinism	18%	33%

Table II
Comparison of pharmacological treatment in patients with unstable angina and controls

Compound	Unstable angina pectoris	Controls
Aspirin	100%	6%
Statins	60%	0
Beta-blockers	90%	0
ACE* – inhibitors	74%	0

*ACE – angiotensin converting enzyme

values measured in 450 nm wave length. The details of this assay are described elsewhere (Rechciński *et al.*, 1997). The sera diluted 1:500 were also tested for optical density (OD450) of IgG reacting with the recombinant cytotoxin-associated protein A CagA using ELISA (A. Covacci, IRS Siena, Italy).

Another method used in this study to assess the presence of IgG antibodies against four highly specific antigens and three specific antigens of *H. pylori* was Milenia blot *H. pylori* IgG test (DPC Biermann GmbH, Bad Nauheim, Germany). The antigens investigated in this kit were: cytotoxin-associated protein A (CagA) of molecular mass 120 kDa, vacuolising cytotoxin A (VacA) – 87 kDa, urease subunit A (UreA) – 30 kDa, urease subunit B (UreB) – 64 kDa, as well as antigens of molecular weight of 35, 26, and 20 kDa. The combination of positive results for separate antigens made it possible to diagnose *H. pylori* infection, as recommended by the manufacturer.

Statistical methods. Chi^2 test was used to assess the significance of differences in results observed between the studied groups.

Results

The IgG antibodies reacting with GE of *H. pylori* antigens were detected by ELISA in all patients with unstable angina and in 60% of symptomless individuals ($p<0.05$). The level of anti-GE IgG expressed as total optical density for the wave length of 450 nm (OD total) ranged in the group of patients with unstable

angina from 0.585 to 13.922 (mean 6.1 ± 3.0) and in the controls – from 0.422 to 8.308 (mean 3.4 ± 3.0) – $p<0.05$ (Table III). Using Milenia blot, 75% of cardiac patients, and 48% of controls were *H. pylori* positive – $p<0.05$. The prevalence of anti-CagA IgG assessed by ELISA was observed in 66% of cardiac patients and in 70% of controls – with the difference being nonsignifficant – $p>0.05$. The total optical density in ELISA test for anti-CagA ranged in the group of patients with unstable angina from 0.09 to 2.489 (mean 0.8 ± 0.70), in controls – from 0.067 to 2.485 (mean 0.6 ± 0.7) and did not vary significantly between two groups (Table IV).

Despite the significant differences between both studied groups in the prevalence of *H. pylori* infection detected by ELISA or Milenia blot, no statistical difference was found in the occurrence of IgG antibodies against selected highly specific antigens when they were analyzed separately (Table V). Antibodies interacting with CagA were detected by Milenia blot in 59% patients with unstable angina and in 48% controls ($p>0.05$), the prevalence of IgG interacting with VacA was 57% and 38%, respectively ($p>0.05$). Also IgGs interacting with two subunits of urease were detected with a similar rate in both groups – 71% vs. 48% for UreA ($p>0.05$) and 50% vs. 38% for UreB ($p>0.05$), respectively. Also, the rate of detection of IgG antibodies against specific 35 kDa protein did not vary significantly between the compared groups: 21% vs. 14%, respectively ($p>0.05$). Antibodies of IgG class against protein of molecular weight of 26 kDa were

Table III
The prevalence of anti-*H. pylori* GE IgG and anti-CagA IgG assessed by ELISA or Milenia blot

Group	Percent of positive results			
	ELISA		Milenia blot	
	GE 14-120kDa IgG	CagA IgG	20-120kDa IgG	CagA IgG
Angina pectoris	100*	66	75^{+}	59
Controls	60*	70	48^{+}	48

* and $^{+}$ – significance $p<0.05$

Table IV
The levels of anti-*H. pylori* GE IgG and anti-CagA IgG expressed as optical density

Group	ELISA			
	total OD 450 1:500–1:128000		OD 450 1:500	
	IgG anti-GE range	IgG anti-GE mean	IgG anti-CagA range	IgG anti-CagA mean
Angina pectoris	0.585 – 13.922	6.1 ± 3.0*	0.09 – 2.489	0.8 ± 0.7
Controls	0.422 – 8.308	3.4 ± 3.0*	0.06 –2.485	0.6 ± 0.7

* – significance $p<0.05$

Table V
The prevalence of serum IgG interactions with selected *H. pylori* antigens detected by Milenia blot *H. pylori* IgG system. Analysis performed for all subjects in compared groups and restricted only to *H. pylori*-seropositive individuals in both groups

Antigens	Percent of positive serum interactions with selected *H. pylori* antigens detected by Milenia blot	
	Angina pectoris all subjects – (seropositive ones)	Controls all subjects – (seropositive ones)
CagA 120kDa	59 – (80)	48 – (100)
VacA 87kDa	57 – (76)	38 – (79)
ure A 30kDa	71 – (95)	48 – (100)
ure B 64kDa	50 – (67)	38 – (79)
35kDa	21 – (29)	14 – (29)
26kDa	71* – (95)	45* – (93)
20kDa	73* – (98)	41* – (96)

* – significance $p<0.05$

detected in 71% of patients with unstable angina and in 45% of controls ($p<0.05$), and the antibodies against 20 kDa protein were found in 73% vs. 41% ($p<0.05$), respectively. However, when the analysis of the occurrence of IgG antibodies specific for 26 and 20 kDa proteins was only limited to *H. pylori*-positive individuals in both groups (Milenia blot), the prevalence of such antibodies ranged from 86 to 98%, and there were no statistical differences between these subgroups.

Discussion

The differentiation of various antigens interacting with the sera of the investigated humans made the serodiagnosis of *H. pylori* infection more reliable (Faulde *et al.*, 1993; Nilsson *et al*., 1997), but it was not used in clinical practice due to the fact that this method seemed to be expensive and laborious. The results of our studies concerning the similar prevalence of anti-CagA IgG in patients with coronary artery disease in comparison with controls, remain in accordance with previous reports (Koenig *et al.*, 1999; Whincup *et al.*, 2000). Such high prevalence of this type of antibodies even in the group of healthy individuals, may be justified by the asymptomatic *H. pylori* infections. In our previous study, the frequency of asymptomatic infections in healthy donors ranged from 50 to 60% as detected by 13C-urea breath test (Wiśniewska *et al.*, 2002). Another possible explanation of this phenomenon is antigenic mimicry with host proteins detectable in both normal and atherosclerotic arteries tissues (Franceschi *et al.*, 2002). In previous studies it was already suggested that generalized elevated levels of IgG, IgA, IgE (but not IgM), help to predict first myocardial infarction or sudden cardiac death in population of healthy men with abnormalities in the lipid profile (Kovanen *et al.*, 1998), but the specificity of these immunoglobulins was not investigated. In our studies it was confirmed that patients with the documented coronary atherosclerosis in comparison with symptomless individuals had higher levels of IgG reacting with *H. pylori* antigens. Previously, we showed that the highest titers of anti-GE IgG were even more prevalent in the patients with atherosclerosis than in *H. pylori*-infected patients with gastroduodenal symptoms (Rechciński *et al.*, 2002; Chmiela *et al.*, 2003). The tendency of the patients with coronary artery disease to intensively produce IgG to various antigens was considered. However, we could not see any significant difference in the production of IgG to mycobacterial Hsp65 among such patients, patients with active tuberculosis, dyspeptic patients and healthy controls (Chmiela *et al.*, 2003). In the present study we did not find any significant difference in the prevalence of serum IgG interaction with *H. pylori* highly specific and specific antigens between the group of coronary atherosclerosis and controls. Obviously, we are not free to assume the absence of atheromatic plaques in coronary vessels of symptomless individuals in the control group, since recently it has been proved that coronary atherosclerosis is a process which begins already in asymptomatic teenagers (Tuzcu *et al.*, 2001). Nevertheless, according to the clinical interview, the plaques in coronary arteries of these individuals, if present, were stable. The role of immunoglobulins in the process of initiation or destabilization of atheromatous lesions may be elucidated by recent studies of Sims *et al.*, 2001. These authors found in their immunohistological studies, that gamma globulin leakage from the lumen into arterial wall is observed when arteries exhibit a breakdown of sub-endothelial lamina, which results in subsequent lipids and inflammatory cells entry. Class G immunoglobulins seem to have the best properties to participate in this process, as they have the smallest sizes and the longest clearance in comparison with IgA and IgM. One cannot rule out the fact that also the size of the reacting antigen may be important for this phenomenon. Interestingly enough, it was previously found, that the smallest antigens of investigated bacteria are recognized by serum antibodies predominantly in the organisms of infected children, in contrast with the infected adults, whose organisms used to recognize mainly the larger bacterial proteins (Chmiela *et al.*, 1998). Although we did not observe such a phenomenon in the patients with atherosclerosis or in symptomless subjects, it is possible that gastrotoxic activity of aspirin used for treating the patients with coronary artery disease may lead to the failure of mucosal barrier and an easier penetration of some bacterial antigens to the circulatory system and the induction of stronger humoral response.

We conclude, that class G immunoglobulins interacting with *H. pylori* surface antigens, and circulating in blood vessels of the patients with unstable angina pectoris, can be characterized by higher concentration than in symptomless individuals, and by a similar specificity. The strong humoral response against *H. pylori* antigens may play a role in the maintenance of coronary heart disease.

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