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Chlamydia Pneumoniae and *Helicobacter Pylori* Serology – Importance in Patients with Coronary Heart Disease

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ORIGINAL PAPER

ABSTRACT

Background: Chronic infections in CHD are due to one or both of the organisms Chlamydia pneumoniae and Helicobacter pylori. **Aim:** To examine the association between serum markers of Chlamydia pneumoniae and Helicobacter pylori infection and markers of myocardial damage. in patients with acute coronary syndrome (ACS), with chronic coronary artery disease (CAD) and in–control group. **Material and methods:** Sera were taken from a total of 153 subjects. Subjects were divided in three groups: 64 patients with ACS; 53 patients with CAD and a group of 35 conditionally healthy individuals. Analysis of patients' sera for IgG antibodies to H. pylori and markers for myocardial damage was done on the Immulite system.. The presence of specific IgG and IgA antibodies to C. pneumoniae was determined with MIF, Sero FIA (Savyon Diagnostics, Israel). Statistical analysis of data was done using the statistical program SPSS (Statistical Package for Social Sciences), version 13. **Results and discussion:** There was a high significant difference in troponin levels between the three groups of subjects (p=0.0000). Levels of creatine kinase isoenzyme (CK-MB) were highest in the ACS group (500.0 ng/mL). There was a statistically significant difference between CG subjects and ACS patients due to more frequent detection of antichlamydial IgA antibodies in patients with acute coronary syndrome. Positive serum immune response for Helicobacter pylori was 17 (53.1%) and 29 (80.6%), respectively. **Conclusion:** Increased IgA antibody titers for C. Pneumoniae, increased CRP values as well as classic markers of myocardial damage are risk factors for coronary events.

Key words: markers of cardiac damage, chronic infections, Chlamydia pneumoniae and Helicobacter pylori.

1. INTRODUCTION

Elevated levels of specific antibodies and markers such as CRP, myoglobin and creatine kinase are associated with pathological changes, and hence their values give useful information for exact diagnosis and therapy. Chronic infections in CHD (coronary heart disease) are speculated to be due to serum antibody concentrations of one or both of the organisms (*Chlamydia pneumoniae* and *Helicobacter pylori*). Data for *Helicobacter pylori* are difficult to interpret due to the confounding factor of childhood poverty and studies conducted in transplant recipients. Data for *Chlamydia pneumoniae* appear stronger with elevated IgG antibody titers (>= 64) as a risk factor. Larger prospective studies are warranted to determine an association with CHD before treatment of these chronic infections.

2. AIM

The aim of this study was to examine the association between serum markers of chronic *Chlamydia pneumoniae* and *Helicobacter pylori* infection and classic markers of myocardial damage: CRP, myoglobin, troponin and creatine kinase isoenzyme MB (CK-MB) in patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CAD) and in healthy individuals – control group.

3. MATERIAL AND METHODS

Sera were taken from a total of 153 subjects (78.9% males, 21.1% females). Subjects were divided in three groups: 64

patients with ACS; 53 patients with CAD and a group of 35 conditionally healthy individuals. Mean age of the control group of individuals was 59.03 years, 61.38 years of the patients with ACS, and 64.11 years of the patients with CAD. Analysis of patients' sera for presence of specific IgG antibodies to *H. pylori* and markers for myocardial damage (myoglobin, troponin, CK-MB) along with determination of CRP level was done on the Immulite system, DPC (Diagnostic Products Corporation), Los Angeles, USA. The presence of specific IgG and IgA antibodies to *C. pneumoniae* was determined with MIF, Sero FIA tm-Chlamydia IgA, IgG (Savyon Diagnostics, Israel).

The study was approved by the local medical Ethics Committee, and all subjects gave informed consent to participate.

4. RESULTS

All subjects were examined for presence of elevated levels of specific IgA and IgG antibodies to *C. pneumoniae* using the world recognized method, the microimmunofluorescence method (MIF). Fluorescence presence in defined sera solutions of the subjects was a sign of a positive result.

CRP values in relation to cardiovascular events are interpreted as follows:

- < 1 mg/l value is considered to be a low risk for cardiovascular disease;
- 3 mg/l value is considered to be a medium risk for cardiovascular disease;
- > 3 mg/l value is considered to be a high risk for cardiovascular disease.

Myglobin level higher than 25 ng/ml was considered to be elevated. Creatine kinase isoenzyme MB level higher than 5.7 ng/ml was considered to be elevated. If IgG antibody titer to cHSP60 was 1:50 it was considered to be negative and if it was higher than 1:50 it was considered to be positive.

Statistical analysis

Statistical analysis of data was done using the statistical program SPSS (Statistical Package for Social Sciences), version 13.

We present the results obtained by statistical analysis of the data. There was a high significant difference in **troponin** levels between the three groups of subjects (p=0.0000). There was a high statistically significant difference in troponin levels between CG and ACS groups, between CG and CCAD groups and between ACS and CCAD groups. Significantly higher levels of troponin were registered in patients with ACS than in healthy subjects and CCAD patients. In addition, higher troponin levels were also found in CCAD patients in comparion with healthy subjects (Table 1).

Levels of one of the myocardial injury markers, **creatine kinase isoenzyme (CK-MB)** were the lowest in the CG (0.5 ng/mL) and the highest in the ACS group (500.0 ng/mL). Statistical differences in the CK-MB levels between the analyzed groups were found to be highly significant (p=0.0000). (Table 2.).

CK-MB levels that exclude from the normal ones were registered in only one subject from CG, in 34 (53.1%) ACS subjects and in 5 (9.6%) CCAD subjects. Differences in the described distributions were confirmed to be highly significant between CG and ACS groups and between ACS and CCAD groups. (Table 3 and 4)

Kruskal-Wallis test: H (2. N= 151) =36.64, **p =0.0000**						
TPI (troponin)	Mean	Median	Min	Max	Std.Dev	Stand. Err.
CG	1.33	0.2	0.2	20.0	4.59	0.76
ACS	44.16	7.26	0.2	180.0	66.03	8.25
CCAD	8.02	0.2	0.2	180.0	32.09	4.49

Table 1. Troponin levels in all examined groups: control group (CG), patients with acute coronary syndrome (ACS), and patients with chronic coronary artery disease (CCAD)

Pearson Chi-square: 14.9140, df=2, p=0.000578					
TPI		CG	ACS	CCAD	Total
Number	Normal levels	34	23	41	98
%		94.44	35.94	80.39	
Number	Increased levels	2	41	10	53
%		5.56	64.06	19.61	
Total	All groups	36	64	51	151

Table 2. Troponin levels in all examined groups – CG/ACS/CCAD. control group (CG) patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), CG/ACS Chi-square= 32.18 df=1, p=0.00000, CG/CCAD Chi-square = 3.5 df=1, p=0.061, ACS/CCAD Chi-square = 22.73 df=1, p=0.000001

Mann-Whitney U Test Marked tests are significant at p <,05000					
CK-MB	Rank Sum	Rank Sum	U	Z	p-level
CG/ACS	1121.0	3929.0	455.0	-5.0	0.000001
CG/CCAD	1354.5	2561.5	688.5	-2.1	0.035688
ACS/ CCAD	4529.5	2256.5	878.5	4.36	0.000013

Table 3. Creatine kinase isoenzyme MB levels compared in all examined groups: control group (CG), patients with acute coronary syndrome (ACS), and patients with chronic coronary artery disease (CCAD)

CK-MB		CG	ACS	CCAD	Total
Number	Normal levels	35	30	47	112
%		97.22%	46.88%	90.38%	
Number	Increased levels	1	34	5	40
%		2.78%	53.13%	9.62%	
Total	All groups	36	64	52	152

Table 4. Creatine kinase isoenzyme MB levels in all examined groups – CG/ACS/CCAD. control group (CG), patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), CG/ACS Chi-square=25,6716, df=1, p=,000000, CG/CCAD Chi-square = 1,56542, df=1, p=,21, ACS/CCAD Chi-square = 24,3364, df=1, p=,000001

Pearson Chi-square: 1.00026, df=2, p=.61					
Myoglobin		CG	ACS	CCAD	Total
Number	Normal levels	13	11	12	36
%		36.11	17.46	23.08	
Number	Increased levels	23	52	40	115
%		63.89	82.54	76.92%	
Total	All groups	36	63	52	151

Table 5. Myoglobin – in all examined groups : CG/ACS/CCAD. control group (CG) patients with acute coronary syndrome (ACS) patients with chronic coronary artery disease (CCAD) CG / AKS Chi-square=4.34 df=1, p=0.037 CG /CCAD Chi-square = 1.78 df=1, p=0.18 ACS/CCAD Chi-square = 0.56 df=1, p=0.4

Anti hsCRP		CG	ACS	CCAD	Total
Number	<1.0 mg/l	19	10	20	49
%		52.78	15.63	38.46	
Number	1.1 – 3.0 mg/L	7	15	14	36
%		19.44	23.44	26.92	
Number	>3 mg/L	10	39	18	67
%		27.78	60.94	34.62	
Total	All groups	36	64	52	152

Table 6. Anti hsCRP – in all examined groups: CG/ACS/CCAD. up (CG), patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), CG/ACS Chi-square= 16.3 df=2 p=0.0029, CG/CCAD Chi-square =1.71 df=2 , control grop=0.41, ACS/CCAD Chi-square =9.97 df=2 p=0.006

IgA		CG	ACS	CCAD	Total
Number	Negative	35	49	47	131
%		97.22	76.56	88.68	
Number	Positive 1:32	1	12	3	16
%		2.78	18.75	5.66	
Number	High positive 1:64	0	3	3	6
%		0.00	4.69	5.66	
Total	All groups	36	64	53	153

Table 7. Detection of IgA antibodies to C. pneumoniae in all examined groups: CG/ACS/CCAD. control group (CG), patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), Chi-square=7.32 df=1 p=0.0068 CG/ACS, Yates correct. d=1.14 df=1 p=0.28 CG/CCAD Chi-square=2.89 df=1 p=0.089 ACS/CCAD

IgG		CG	ACS	CCAD	Total
Number	Negative	22	31	33	86
%		61.11	48.44	62.26	
Number	Positive 1:64	7	20	11	37
%		19.44	31.25	20.75	
Number	High positive 1: 128/256	7	13	9	27
%		19.44	20.31	16.98	
Total	All groups	36	64	53	153

Table 8. Detection of IgG antibodies to C. pneumoniae in all examined groups: CG/ACS/CCAD. Control group (CG), patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), CG/ACS Chi-square=1.49 df=1, p=0.22, CG/CCAD Chi-square=0.01 df=1, p=0.91, ACS/CCAD Chi-square=2.24 df=1, p=0.13

Of all examined parameters, **myoglobin** > 25 ng/mL was found in 54 (82,54%) patients with ACS without significant difference among the groups.

In majority of the analyzed subjects in all groups elevated myoglobin levels were registered. Myoglobin levels higher than the normal ones were found in 23 (63.9%) healthy subjects, in 52 (82.5%) patients with acute coronary disease and in 40 (76.9%) patients with chronic coronary disease. Tested difference in the distribution of subjects with normal and elevated levels between the examined groups was statistically significant between healthy subjects and patients with ACS. Patients with ACS were more frequently found to have increased myoglobin levels than healthy subjects (Table 5).

Table 6 presents the distribution of subjects regarding

Anti *Helicobacter pylori*		CG	ACS	CCAD	Total
Number	Negative	8	15	7	30
%		25.81%	46.88%	19.44%	
Number	Positive	23	17	29	69
%		74.19%	53.13%	80.56%	
Total	All groups	31	32	36	99

Table 9. Detection of IgG antibodies to Helicobacter pylori in all examined groups: CG/ACS/CCAD. control group (CG), patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), CG/ACS Chi-square=3.02 df=1, p=0.082, CG/CCAD Chi-square = 0.39 df=1, p=0.53, ACS/CCAD Chi-square = 5.82 df=1, p=0.016

Risk factors	Univariate analysis		
	OR	95% CI	
TPI	30.304	6.663	137.831
CK-MB	39.667	5.119	307.357
Myoglobin	2.672	1.043	6.847
hsCRP			
hsCRP (1)	4.070	1.252	13.243
hsCRP (2)	7.410	2.635	20.837
C. pneumoniae IgA	10.714	1.352	84.925
C. pneumoniae IgG	1.673	0.729	3.837
Helicobacter pylori			
Helicobacter pylori (1)	0.498	0.162	1.534
Helicobacter pylori (2)	0.200	0.041	0.971

Table 10. Risk factors for ACS - Binary Logistic Regression. * Sig. Dependent variable: healthy/ACS patients

the **hsCRP** antibody levels, analyzed as normal, low positive and high positive.

Predominant subjects in the CG group (19 or 52.8%) were those with anti-hsCRP antibodody titer less than 1.1 mg/L. In the group of patients with acute coronary disease 39 (60.9%) had hsCRP antibody titre higher than 3 mg/L whereas in the group of patients with chronic coronary disease 20 (38.5%) patients had titre <1.0 mg/L.

Tested difference in the distribution of subjects with normal, low positive and high positive hsCRP antibodies was statistically significant between healthy subjects and patients with ACS, as well as between patients with ACS and CCAD.

Distribution of healthy subjects and patients with coronary disease regarding seropositivity for **antichlamydial IgA antibodies** is given in Table 7. Positive antibodies were found in only one subject from CG (titre 32) and in 21 (17.95%) patients with coronary disease, of whom 15 were low positive and 6 high positive. In the ACS group 15 (23.44%) patients were seropositive for these antibodies, 12 being with low seropositivity and the remaining 3 patients with high seropositivity. In the CCAD group 6 (11.32%) patients were seropositive for IgA antibodies, of whom 3 with low and 3 with high seropositivity.

Reagarding seronegative and seropositive patients, there was a statistically significant difference between CG subjects and ACS patients due to more frequent detection of IgA class antibodies in patients with acute coronary syndrome.

Fourteen (38.88%) healthy controls had **IgG** antibodies

Risk factors	Univariate analysis		
	OR	95% CI	
TPI	0.137	0.058	0.323
CK-MB	0.094	0.033	0.267
Myoglobin	0.705	0.282	1.763
hsCRP			
hsCRP (1)	0.467	0.163	1.336
hsCRP (2)	0.231	0.09	0.592
IgA	0.417	0.149	1.166
IgG	0.569	0.271	1.194
Helicobacter pylori			
Helicobacter pylori (1)	3.571	1.134	11.253
Helicobacter pylori (2)	3.857	0.938	15.865

Table 11. Risk factors for CCAD - Binary Logistic Regression, patients with acute coronary syndrome (ACS), patients with chronic coronary artery disease (CCAD), * Sig. Dependent variable: ACS/CCAD

for *Chlamydia pneumonia*, among whom in 7 (19.4%) the titre was >1:64 and in the other 7 patients it was elevated (>1:128/256).

In the ACS group seropositive for IgG antibodies were 33 or more than half of the subjects. Twenty (31.2%) had antibody titer >1:64 and 13 (20.3%) had antibody titer >1:128/256.

In the CCAD group seropositive for IgG antibodies were 20 (31.7%) subjects, of whom the majority (11 or 20.75%) were with low seropositivity and only 9 (16.9%) with high seropositivity.

Tested differences regarding IgG antibodies between healthy subjects and patients with coronary disease, between healthy subjects and patients with acute coronary disease and chronic coronary disease, as well as between patients with acute and chronic coronary disease were statistically non-significant (Table 8).

Positive serum immune response for ***Helicobacter pylori*** was registered in 23 (4.2%) healthy subjects and 46 (67.65%) patients with coronary disease. Among patients with acute coronary disease and chronic coronary disease 17 (53.1%) and 29 (80.6%) were seropositive for *Helicobacter pylori*, respectively.

Tested difference in seropositive and seronegative subjects for Helicobacter pylori was statistically insignificant between CG and ACS, CG and CCAD patients while statistically significant was between ACS and CCAD patients, which was due to the more frequent presence of antibodies for *Helicobacter pylori* in patients with chronic coronary disease compared to patients with acute coronary disease (Table 9).

Univariate analysis has confirmed the following significant risk factors for acute coronary syndrome (ACS): troponin (TPI), creatine kinase – isoenzyme (CK-MB), myoglobin, anti-hsCRP antibodies, IgA antibodies for *Chlamydia pneumoniae* (Table 10).

Multivariate model for Binary regression analysis has confirmed hsCRP antibody levels higher than 3 mg/L to be independent significant factors or predictors for ACS.

Table 11 lists the results obtained in the univariate regression analysis on risk factors that have predictive role or influence on development of **chronic coronary disease** (CCAD) when comaring the groups with ACS and CCAD.

Univariate analysis has confirmed the following risk factors to be significant for chronic coronary disease: troponin (TPI), creatine kinase – isoenzyme (CK-MB), anti-hsCRP antibodies, and antibodies for *Helicobacter pylori*.

Subjects with antibody titers for *Helicobacter pylori* between 1.1 and 7 had a 3.6-fold significantly higher risk for chronic coronary disease in comparison with subjects with titer less than 1.1.

5. DISCUSSION

Risk factors for onset of coronary heart diseases (CHD) may be **variable**: diet, smoking, hypertension, hyperlipidemia, diabetes mellitus, alcohol, poor physical activity, stress, or **non-variable**: age, sex, race, genetic inheritance.

Reagarding the classic markers of cardiologic injury, individuals with elevated TPI levels in this investigation had 30.3-fold significantly greater risk for ACS than individuals with normal values. Elevated **CK-MB** levels significantly increased the risk (for 39.7-times) for acute coronary disease. Patients with elevated myoglobin levels had a 2.6-fold significantly greater risk for ACS than subjects with normal myoglobin levels.

Subjects with **hsCRP** levels higher than 3 mg/L were at 12.05-fold significantly greater risk for coronary disease than subjects with hsCRP levels lower than 1 mg/L.

Our results are in agreement with those reported by Kiechl et al. (2006) (9). The association between chronic infection and risk for onset of atherosclerosis is increased by higher CRP levels (>1mg/L). In the study conducted by Gattone et al. (2001) (5) concomitant seropositivity for *C. pneumoniae* and CMV was associated with increased CRP levels and risk for myocardial infarction. Today atherosclerosis is recognized as an inflammatory disease (24) and several prospective studies have shown that CRP is a strong independent predictor for future coronary events (22, 23). Subjects in our investiation with hsCRP levels ranging from 1.1 mg/L to 3 mg/L had a 4-fold increased risk for coronary disease compared to subjects with hsCRP levels less than 1 mg/L. On the other hand, subjects with hsCRP levels higher than 3 mg/L had a 7.4-fold significantly increased risk for coronary disease compared to subjects with levels less than 1 mg/L.

Chlamydia pneumoniae

Diagnosis of infections caused by *C. pneumoniae* can be made with serologic tests for detection of IgM, IgG and IgA specific antibodies, with a microimmunofluroscence (MIF) or an ELISA test. Identification of *C. pneumoniae*-specific DNA is done with PCR reaction. MIF, the test we used in this investigation, is the most sensitive and most specific test since it enables differenatiation between active and past infection according to the detected antibody class (7). Positive result is considered if there is a 4-fold rise in specific antibodiody titers (IgG>32, IgM>16, IgA>16) (7). Detection of IgA class correlates with chronic infection.

In our investigation positivity for specific chlamydial IgA antibodies found in ACS patients significantly increased the risk (for 10.7 times) for acute coronary disease.

Several studies support the theory that elevated IgA antibody titers are a better marker for *C. pneumoniae* chronic

infection than the IgG antibody titers. IgA titer is more strongly associated with CHD and is an increased risk for further coronary events (2, 9, 14, 15, 25).

Two theories suggest an association between *C. pneumoniae* and CHD. The first theory takes into consideration production of chlamydial lipopolysaccharide immune complexes (11, 12) that are due to chronic Chlamydia infection. Ciruclating immune complexes may be responsible for tissue injury. The second theory explores intracellular capability of *C. pneumoniae* and its detection in coronary atherosclerotic plaques (1, 10, 16, 18, 21, 26). Microorganism incorporation into macrophages may result in chronic infection of atherosclerotic lesion thereby contributing to onset of atherosclerosis.

Helicobacter pylori

Diagnosis of infection can be made by one of several methods. The simplest is a serologic test, such as enzyme-linked immunosorbent assay (ELISA), which measures IgG antibody titers (8, 20). Another method is the rapid urease test, which detects production of ammonia generated by the organism. However, this requires upper endoscopy to obtain biopsy tissue from the stomach. The last method is the urea breath test during which a patient ingests carbon-labeled urea; if *H. pylori* is present the urea disassociates into ammonia and labeled carbon dioxide that can be detected in a breath analyzer.

Symptomatic infection is curable with appropriate antimicrobial therapy (3, 4). There are no recommendations for diagnosis and treatment of asymptomatic *H. pylori* infections.

One study hypothesized that chronic infection with *H. pylori* or *C. pneumoniae* contributes to the risk of CHD by increasing the concentration of acute-phase proteins (fibrinogen, sialic acid) or activating factor VII antigen (11). The results agree with those of the previous trial (17) that even after controlling for confounding factors, the correlation of infection with CHD was not diminished. Compared with men with neither infection, the presence of both infections correlated with the presence of CHD even more than with infection with one organism. Fibrinogen levels, regardless of whether unadjusted or adjusted for risk factors, were significantly higher ($p<0.05$), to a similar degree, in patients seropositive for *H. pylori* or *C. pneumoniae*. Increased total leukocyte count was higher in patients seropositive for *H. pylori* than those positive for *C. pneumoniae*. Factor VII antigen concentrations were increased frequently in patients with *C. pneumoniae* but not in those with *H. pylori*. Infection with *H. pylori* was strongly related to socioeconomic status in childhood, whereas *C. pneumoniae* was more related to smoking. Since fibrinogen alone cannot explain the connection, other factors must be explored to determine the mechanism by which *H. pylori* may increase the risk for CHD.

The first prospective, longitudinal (11-year follow-up) study assessed whether *H. pylori* was associated with an increased risk of CHD and stroke later in life (19). Of 408 men, 272 developed a major cardiovascular event; myocardial infarction in 135 and stroke in 137. For patients with myocardial infarction, when adjustments were made for age, the risk associated with *H. pylori* was increased ($p=0.02$).

In our investigation the multivariate model for logistic regression analysis of independent significant factors, that is predictors of CCAD, when dependent variables are ACS and CCAD groups, has proven only *Helicobacter pylori* antibodies. Subjects wtih *Helicobacter pylori* antibody titres between 1.1 and 7 had a 3.6-fold significantly increased risk for coronary artery disease in comparison with subjects having titers less than 1.1.

6. CONCLUSION

The results obtained in this study have shown that increased IgA antibody titers for *C. Pneumoniae*, increased CRP values as well as classic markers of myocardial damage such as troponin, myoglobin and creatine kinase, are risk factors for coronary events, especially when their levels are persistantly increased and not transitory. Coronary risk is associated with increased antibody titers, along with increasd CRP levels and classic cardiologic markers. In conclusion, chronic infection, autoimmunity and risk factors caused cardiovascular events in the examined subjects.

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