ROLE OF *CHLAMYDIA PNEUMONIAE*, *HELICOBACTER PYLORI* AND CYTOMEGALOVIRUS IN CORONARY ARTERY DISEASE

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ABSTRACT

Coronary artery disease (CAD) is the leading cause of death in many countries. The underlying mechanism of the chronic inflammatory process in atherosclerosis is still unknown. As a possible trigger, different viruses and bacteria may be associated with atherosclerotic diseases. The aim of this work was to investigate the association of chronic infection with *C pneumoniae, H pylori* and cytomegalovirus (CMV) infections and CAD. Fifty patients [20 with acute coronary artery disease (ACAD) and 30 with chronic coronary artery disease (CCAD)] in addition to 15 healthy individuals as a control group were involved in this study. The studied individuals were subjected to complete history taking, thorough physical examination, electrocardiography, echocardiography and coronary angiography (for patients). Assessment of blood glucose level, lipid profile and creatine kinase (CK) was performed. Determination of hsCRP was done by nephlemetry, while *C pneumoniae*-, *H pylori*- and CMV-specific IgG antibodies was done by enzyme immunoassay.

Results showed that the levels of cholesterol, triglycerides, LDL-c and hsCRP were significantly higher, while HDL-c was significantly lower among patients compared to that of the controls. A significantly ($P<0.05$) higher perecentage of patients had *C pneumoniae* and *H pylori*-specific IgG antibodies as compared to that of the controls. Higher percentage of patients had CMV-specific IgG antibody, however, there was no significant difference between the 2 groups. The levels of *C pneumoniae* and *H pylori*-specific IgG antibodies were significantly ($P<0.001$) higher among patients with CAD when compared to that of the controls. CMV-specific IgG level in patients was higher compared to that of the controls, however, the difference was not statistically significant. Among acute CAD patients, *C pneumoniae*-specific IgG was positively correlated with hsCRP ($p<0.05$), cholesterol ($p<0.01$) and HDL-c ($p<0.05$), while *H pylori*-specific IgG was positively correlated with triglyceride level ($p<0.05$). Among patients with CCAD, hsCRP was negatively correlated with HDL-c ($p<0.05$). There was no significant correlation between the levels of CMV-specific IgG and lipid profile or hsCRP.

In conclusion, the level of *C pneumoniae* and *H pylori*-specific IgG antibodies are elevated among CAD patients and their presence was associated with development of the disease. They were significantly correlated to cholesterol level. Moreover, *C pneumoniae*-specific IgG was significantly correlated with hsCRP among ACAD patients, suggesting an important role of these organisms in the development of CAD by altering lipid profile and induction of inflammation.

Keywords: Atherosclerosis, coronary artery disease, cytomegalovirus, Epstein-Barr virus, herpes simplex virus, peripheral artery disease.

INTRODUCTION

Coronary artery disease (CAD) accounts for 20% of deaths worldwide, rising to 50% in developed countries (Gurfinkel and Lernoud, 2006). Atherosclerosis is a multifactorial disease whose age of onset and progression are strongly influenced by inborn and acquired risk factors (Eckardstein, 2004). Many studies showed that 80-90% of patients who developed clinically significant CAD had at least one of four classical risk factors; hypercholesterolemia, hypertension, diabetes mellitus or smoking (Greenland *et al*, 2003). Novel cardio-vascular risk factors have been introduced as predictors of cardiovascular morbidity and mortality, such as C-reactive protein (CRP), lipoprotein (a), fibrinogen and homocysteine. A substantial proportion of patients with CAD do not have traditional risk factors. Infectious diseases may play a role in these cases, or they may intensify the effect of other risk factors (West *et al*, 2009).

Atherosclerosis is an inflammatory disease and an association may exist between its development and infection with certain microorganisms (Watt *et al*, 2003). Some studies have suggested that different viruses and bacteria are associated with atherosclerotic vascular diseases and may be causative factors in the pathogenesis of CAD (Ayada *et al*, 2009). Microbes may act as inciting agents of tissue injury and inflammation, both of which underlie the pathogenesis of atherosclerosis. Increasing evidence supports a link between serological evidence of prior exposure to infectious pathogens, pathogen burden, and the risk for future myocardial infarction and death in patients with CAD (Vercellotti, 2001). It has not been established that bacteria are the causative agent in the

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etiopathogenesis of CAD. However, bacterial agents could have secondarily colonized atheromatous lesions and could act as an additional factor accelerating disease progression (Ott *et al*, 2006).

H. pylori is the commonest bacterial pathogen found worldwide that attaches to mucus secreting cells in the gastric mucosa and initiates inflammation leading to gastritis and peptic ulcer. A relationship may exist between *H pylori* infection and atherosclerotic vascular disease (Franceschi *et al*, 2009 and Gen *et al*, 2010). *C pneumoniae* is an intracellular pathogen which causes respiratory tract infections in humans and more recently it has been associated with chronic diseases such as atherosclerosis (Romano Carratelli *et al*, 2006). *In vitro*, these agents promote a proinflammatory and a procoagulant phenotype in vascular cells (Vercellotti, 2001). Individuals infected with multiple pathogens such as *C pneumoniae, H pylori* and CMV have high CRP levels and a high relative risk factor for CAD (Verecllotti, 2001). Epidemiological studies have suggested that a high titer of CMV antibody was associated with CMV reactivation, and that this condition was a determinant of CAD (Eryol *et al*, 2005). The prevalence of high CMV seropositivity was an independent predictor of CAD and patients with CAD had a high rate of CMV infection (Eryol *et al*, 2005). On the other hand, lack of association between chronic infection with CMV, *C pneumoniae* and *H pylori*, or pathogen burden and endothelial function was observed, suggesting that these agents are not implicated as early etiologic triggers in the genesis of CAD (Khairy *et al.*, 2003).

The aim of this study was to assess the relation of chronic infection with *C. pneumoniae, H pylori* and CMV and occurrence of CAD.

PATIENTS AND METHODS

This study was carried out at King Abdulazez University Hospital, Jeddah, KSA. The samples were collected from the Cardiology Department and intensive care unit (ICU).

I-The studied subjects

This study involved 50 patients suffering from CAD. Their ages ranged between 26-75 years. In addition, 15 apparently healthy subjects were studied as a control group. The ages of the control group ranged between 28-75 years. The studied patients included 20 patients with acute coronary artery disease (ACAD) (13 males and 7 females ranging in age from 26-75 years) and 30 patients with chronic coronary artery disease (CCAD) (22 males and 8 females ranging in age from 40-64 years). All the studied individuals were subjected to complete history taking and thorough clinical examination, ECG, echocardiography. Diagnosis of CAD was established by coronary angiography. Assessment of following laboratory parameters was performed for all the studied individuals: fasting blood glucose, serum lipid profile (cholesterol, triglycerides, HDL-c and LDL-c), cardiac enzymes, hsCRP and *C. pneumoniae-*, *H. pylori-* and CMV-specific IgG antibodies.

II- Sampling

About 10 ml of blood were collected from each individual after 12 hours fasting under aseptic conditions by clean venipuncture without venous stasis. The blood was left to clot at 37^{o}C; then centrifuged at 4000 pm for 10 min. The separated serum was divided into two parts; one was used for immediate assessment of fasting blood sugar, lipid profile, creatine kinase (CK) enzyme and the other part was stored in aliquots at -20^{o}C for estimation of hsCRP and *C. pneumoniae-*, *H. pylori-* and CMV-specific IgG antibodies.

III- Laboratory methods:

Biochemical tests for detection of blood glucose, lipid profile and CK enzyme were performed on synchron CX5 autoanalyser using kits supplied by Beckman (Beckman Instruments, Inc, Fullerton, CA, USA). Assessment of hsCRP was performed on ARRAY 360 using kit supplied by Beckman (Beckman Coulter, Inc.Fullerton, CA). *C. pneumoniae-*, *H pylori-* and CMV-specific IgG antibodies were measured by enzyme immunoassay (EIA) according to Manufactuer's instructions.

1) Estimation of high sensitive C-reactive protein (hsCRP)

The "high sensitivity" refers simply to the lower detection limit of the assay being used. Quantitative assessment of CRP by rate nephelometry was done using Beckman ARRAY 360 System autoanalyser. The rate of increase in light scattered from particles suspended in solution due to complexes formed during an antigen-antibody reaction is measured. Antibody to human C-reactive protein (CRP) is brought into contact with human CRP protein in a sample. The increase in light scattered from the antigen-antibody reaction is converted to a peak rate signal which is a function of the sample hsCRP concentration. Following calibration, the peak rate signal for a particular assay is automatically converted to concentration units by the analyzer (Thomas *et al*, 2003).

2) Assessment of specific IgG antibodies by enzyme immunoassay

1- Assessment of Chlamydia pneumoniae- specific IgG antibody

It was performed using a kit supplied by IBL Gesellschaft Fur Immunchemie und Immunbiologie MBH (Hamburg, Germany).

2-Assessment of Helicobacter pylori- specific IgG antibody

It was performed using a kit supplied by Biohit Diagnostics (Laippatie, Helsinki, Finland).

3- Assessment of CMV-specific IgG antibody
CMV-specific IgG was measured using bioelisa CMV IgG kit supplied by Biokit, SA (Barcelona, Spain).

IV-Statistical analysis
Data were vanalyzed by SPSS statistical package version 11.0 (SPSS Inc, Chicago, IL, USA). One way analysis of variance (F-test) was used for comparison of more than two groups of normally distributed quantitative data followed by LSD post-hoc test for multiple pairewise comparisons of two groups. Kruskal-Wallis test was used for comparison of non-parametric distributed quantitative variables. Student's t test was used for comparison of the means of two groups of quantitative variables, while Mann-Whitney test was used for non-parametric variables. Qualitative data were analyzed by Chi-square (X^2) test and Yates continuity correction test when appropriate. Pearson correlation coefficient (r) was used to measure the association between two quantitative variables. Level of significance was set as p-value <0.05 (Saunders and Trapp, 1994).

RESULTS

Table 1 shows the age, gender and the percentage of hypertensive patients among the studied CAD patients. The levels of fasting blood glucose, cholesterol, triglycerides, LDL-c and CK were significantly ($p<0.001$) higher, while HDL-c was significantly ($p<0.001$) lower among CAD patients compared to that of the controls. CAD patients significantly ($p<0.001$) had higher prevalence of both *C pneumoniae* and *H pylori*-specific IgG antibodies (76% and 62% respectively) compared to the control group (0%). On the other hand, no significant difference in the percentage of CMV-specific IgG seropositivity was found (56% and 33.3% for patients and controls respectively). Table 2 demonstrates that the levels of hsCRP and IgG antibodies specific to both *C pneumoniae* and *H pylori* were significantly ($p<0.001$) higher among patients with acute and chronic CAD as compared to that of the controls. On the other hand, there was no significant difference between the levels of CMV-specific IgG antibody among the studied groups. Our data showed that there was a significant ($p<0.05$ and $p<0.01$) positive correlation between *C pneumoniae*-specific IgG and hsCRP ($r=0.41$) and LDL-c ($r=0.48$), and cholesterol ($r=0.62$). In addition, there was a significant ($p<0.05$) positive correlation between *H pylori*-specific IgG and triglycerides ($r=0.49$). On the other hand, patients with chronic CAD showed a significant ($p<0.05$) negative correlation between hsCRP and HDL-c ($r=0.39$)). There was no significant correlation between the other studied parameters.

DISCUSSION

The role of inflammation in the pathogenesis and progression of CAD has been increasingly discussed, but still remains unclear. Infective pathogens and inflammatory changes in the vessel wall may play an important role in the pathogenesis of atherosclerosis which is the primary cause of CAD (Kowalski *et al*, 2006). In this study, it was found that CAD patients had significantly higher levels of cholesterol, triglycerides, LDL-c and hsCRP and significantly lower level of HDL-c as compared to that of the controls. This result suggest that increased levels of lipids (especially LDL-c) and inflammation play a role in pathogenesis of CAD (Jha *et al*, 2009). Low levels of HDL-c were found to have a consistent relationship with the development of atherosclerosis. It was found that Apo B levels increased with *C pneumoniae* infection, supporting the hypothesis that lipid profiles change to atherogenic lipid profile in chronic infections (Adiloglu *et al*, 2005). However, Mostaza *et al* (2004) indicated that CRP levels were increased in subjects with nonselected hypo-alphalipoproteinemia and that chronic infections with herpes simplex virus type 1, cytomegalovirus, *C. pneumoniae* do not appear to mediate this relationship.

In this study, the level of hsCRP was significantly higher among patients with both acute and chronic CAD patients but the increase was more marked in acute cases. However, there it was negatively correlated with HDL-c in patients with chronic CAD. This finding may suggest an important role of inflammation in pathogenesis of CAD especially in acute disease. Inflammatory markers have been reported to be independent predictors of cardiovascular and cerebrovascular events. Beyond traditional cardiovascular risk factors, markers of inflammation and infections seem to significantly influence the occurrence of cerebrovascular and cardiovascular events (Vahdat *et al*, 2007). A relation between the levels of CRP and the risk of CAD has been demonstrated, however another marker of inflammation, fibrinogen, has also been identified as an independent risk factor for CAD (Corrado and Novo, 2005). Therefore, a possible role of markers of infection and inflammation may be indicated in the development and progression of CAD. Inflammation (indicated by CRP level) and chronic *C pneumoniae* infection have been reported to play an important role in lower limb atherosclerosis and to correlate with the severity of the disease (Kaperonis *et al.*, 2006). A possible link between *C. pneumoniae* and prevalent atherosclerosis was reported in American hemodialysis patients and confirmed the importance of hsCRP as a prognostic indicator (Lentine *et al.*, 2006).

This study showed that the level of *C pneumoniae*-specific IgG was significantly higher among both acute and chronic CAD. Moreover, there was a significant positive correlation between its concentration and hsCRP, cholesterol and HDL-c among patients with acute CAD. These results may indicate that this organism has an important role in pathogenesis of these diseases. The

importance of *C pneumoniae* in atherosclerosis has been reported (Jha *et al.*, 2009). Wang *et al*, (2007) found that circulating *C pneumoniae* DNA was associated with advanced CAD. Diagnostically significant elevation of the serum levels of IgG antibodies to *C. pneumoniae* was associated with CAD progression. Moreover, *C. pneumoniae* has been suggested as the most important pathogen related to the development of atherosclerosis (Sawayama *et al*, 2009). *C pneumoniae* infection in early life may accelerate atherosclerosis, leading to cardiovascular complications. It may affect atherosclerosis either directly or indirectly. Direct effects on vascular wall cells might include cell lysis, transformation, lipid accumulation, proinflammatory changes, and augmentation of procoagulant activity. Indirect systemic effects may involve induction of acute-phase proteins, establishment of a prothrombotic state, hemodynamic stress caused by tachycardia, increased cardiac output, or a regional inflammatory activation in response to systemic endotoxemia and cytokinemia (Mousa *et al*, 2009). The effects of microbial infection, usually in combination with other risk factors (as smoking, hyperlipidemia and family history), might promote atherogenesis and eventually trigger acute coronary events (Hedayat *et al*, 2009).

Other infections, simultaneously occurring with *C. pneumoniae*, may result in a synergistic effect to promote atherosclerosis (Maia *et al*, 2009). In this study, *H pylori*-specific IgG was significantly higher among CAD patients compared to that of the controls and there was a significant positive correlation between its level and triglycerides, suggesting that *H pylori* infection (in addition to *C pneumoniae*) may play a role in pathogenesis of CAD. Niccoli *et al* (2010) demonstrated the presence of both *C pneumoniae* and *H. pylori* DNA in a considerable number of patient's specimens, but not in the control group specimens and supported the hypothesis that these agents have an association with atherosclerosis. The extent of atherosclerosis and the prognosis of patients with atherosclerosis were reported to be increased by the number of infections to which an individual has been exposed (Espinola-Klein *et al.*, 2002). Detection of a broad variety of molecular signatures in all CAD specimens suggests that diverse bacterial colonization may be more important than a single pathogen (Ott *et al.*, 2006). The immunoglobulin-G antibody response to multiple pathogens (pathogen burden) is an independent risk factor for endothelial dysfunction and the presence and severity of CAD. Endothelial dysfunction provides the crucial link by which pathogens may contribute to atherogenesis (Goyal *et al.*, 2007).

It was found that atrophic gastritis may cause hyperhomocysteinemia, which was reported to be an independent risk factor for atherosclerosis and cardiovascular diseases (Kutluana *et al*, 2005). Kanbay *et al* (2005) concluded that *H. pylori* infection may affect lipid metabolism in a way that could increase the risk of atherosclerosis and concluded that *H pylori* infection is an independent risk factor for CAD. Chronic *H pylori* infection is known to increase the pH level of the gastric juice and to decrease ascorbic acid levels, both of which lead to a reduced folate absorption. Low folate hampers the methionine synthase reaction leading to an increased concentration of homocysteine in the blood, resulting in damage of endothelial cells (Corrado and Novo, 2005). Chronic infection with *H pylori* may be involved in the development of the atherosclerosis *via* endothelial dysfunction and systemic and vascular inflammation (Oshima *et al.*, 2005 and Gen *et al.*, 2010).

In this study, a higher percentage of CAD patients had CMV-specific IgG antibody compared to the controls and the levels of CMV-specific IgG was higher among patients with CAD although the difference was not statistically significant. Moreover, there was no correlation between CMV-specific IgG and hsCRP or lipid profile. This finding may suggest that *C pneumoniae* and *H. pylori* may be more important than CMV infection in the development of CAD. It was reported that serum antibodies to mycobacterial heat-shock protein 65 (mHSP65) were correlated with seropositivity to *C. pneumoniae* and *H. pylori* but not to CMV and there was a strong evidence for a potential atherogenic role of persistent bacterial infection, especially *C. pneumoniae* (Mayr *et al*, 2000). In another study, *H. pylori* DNA was detected in a very small subset of atherosclerotic plaques, whereas CMV DNA was not detected in any of the plaques studied. On the other hand, *C pneumoniae* DNA was found in a significant number of the studied atherosclerotic plaques (Latsios *et al*, 2004). Moreover, Rothenbacher *et al* (2005) suggested that seropositivity to CMV might not be a strong risk factor for recurrent cardiovascular events in patients with manifest CAD, and was not associated with levels of established inflammatory markers. However, high CMV seropositivity was a significant CAD determinant even after adjustment for traditional CAD risk factors (Eryol *et al.*, 2005).

It was hypothesized that CAD risk is associated with the aggregate number of pathogens (pathogen burden) and increased pathogen burden is associated with elevated levels of CRP (Jha *et al.*, 2009). Zhu *et al.* (2006) demonstrated a high prevalence and persistence of antibodies against CMV, *H. pylori* and *C. pneumoniae* in Alaskan Eskimos with CAD and Kwon *et al* (2004) detected CMV, and *C pneumoniae* in therosclerotic plaques. However, Khairy *et al* (2003) and Jia *et al* (2009) found no association between chronic infection with CMV, *C. pneumoniae* and *H. pylori*, or pathogen burden and endothelial function and suggested that these agents are not implicated as early etiologic triggers in the genesis

Table 1: General characteristics and laboratory results of studied groups

The studied parameter	Acute CAD (n=20)		Chronic CAD (n=30)		Controls (n=15)		P value
	Mean	±SD	Mean	±SD	Mean	±SD	
Age (years)	58.95	12.21	50.33	5.90	46.33	15.38	<0.01*
Sex Male Female	 13 7	 (65.0%) (35.0%)	 22 8	 (73.3%) (26.7%)	 9 6	 (60.0%) (40.0%)	>0.05**
Hypertension Yes No	 9 11	 (45.0%) (55.0%)	 6 24	 (20.0%) (80.0%)			>0.05**
FBS (mg/dl)	180.40	94.68	89.93	11.95	74.53	6.978	<0.001*
Cholesterol (mg/dl)	196.25	67.54	201.40	39.37	136.53	25.29	<0.001*
Triglycerides (mg/dl)	210.20	156.0	132.87	40.99	90.13	38.19	<0.001*
HDL-c (mg/dl)	38.39	6.77	35.73	7.25	48.86	4.77	<0.001*
LDL-c (mg/dl)	122.40	44.88	139.12	40.23	71.52	22.85	<0.001*
CK (IU/L)	358.14	341.8	137.7	191.2	17.0	4.9	<0.001***

*ANOVA. **Chi-square test. *** Kruskal-Wallis test
-The age of the studied patients and controls showed a significant difference, however, no difference was detected regarding gender.
-The levels of FBS, cholesterol, triglycerides, LDL-c and CK were significantly higher among CAD patients than controls.

Table 2: The levels of hsCRP and *Chlamydia pneumoniae*-, CMV- and *H. plyori*-specific IgG antibodies among the studied groups

The studied parameter	Acute CAD (n=20)		Chronic CAD (n=30)		Controls (n=15)		p value
	Mean	±SD	Mean	±SD	Mean	±SD	
hsCRP (mg/dl)	2.96	3.91	1.29	0.60	0.33	0.07	<0.001*
Chlamydia pneumoniae-IgG (U/ml)	14.33	3.56	13.14	3.84	5.96	2.64	<0.001**
CMV-IgG (U/ml)	0.98	0.25	0.99	0.18	0.98	0.11	>0.05**
H plyori-IgG (EIU)	46.45	25.89	47.20	35.19	13.47	7.61	<0.001*

* H=Kruskal-Wallis test. **ANOVA test
The levels of hsCRP, and *C pneumoniae* and *H pylori*-specific-IgG antibodies were significantly higher among both acute and chronic CAD patients compared to that of the controls.

of CAD. However, they suggested active involvement at later stages of the pathophysiological process.

In conclusion, there were significantly higher levels of total cholesterol, triglycerides and LDL-c and hsCRP and *C. pneumoniae*- and *H pylori*-specific IgG serum levels among CAD patients. However, CMV-specific IgG level was not significantly higher among patients compared to the controls. In addition, there was a relation between *C. pneumoniae*-specific IgG and hsCRP and lipid profile in acute CAD patients, suggesting that chronic *C. pneumoniae* and *H pylori* infection seems to be linked to the presence of CAD through the ability of modification of serum lipids and induction of inflammation. However, more studies involving large numbers of patients are required to support our findings.

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