Seroprevalence of *Helicobacter pylori* and *Chlamydia pneumoniae* among Patients with Coronary Artery Diseases at Mansoura University Hospital, Egypt

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A B S T R A C T

Coronary artery diseases (CAD) are a global health problem. Its etiology is associated with various risk factors like hypertension, diabetes mellitus (DM) and dyslipidemia. Infections may represent an additive risk factor. The aim of the present study was to investigate the seroprevalence of specific immunoglobulins G for Chlamydia pneumoniae (*C. pneumoniae*) and Helicobacter pylori (*H. pylori*) among Patients admitted in intensive Coronary Care Unit of Mansoura university hospital. This study included 80 patients previously diagnosed to have CAD according to medical records and electrocardiographic (ECG) changes. The second group compromised 80 healthy control subjects. Samples from each subject were subjected to full biochemical laboratory tests, including complete lipid profile. Enzyme-linked immunosorbent assay (ELISA) was done for immunoglobulin (IgG) antibodies to *H. pylori* and *C. pneumonia*. Multiple logistic regression analysis were used to find out statistically significant results for *H. pylori* IgG and *C. pneumoniae* IgG and other conventional risk factors including; hypertension, diabetes, obesity and dyslipidaemia. Seroprevalence of specific IgG antibodies for *C. pneumoniae* and *H. pylori* showed significant increase in *C. pneumoniae* IgG and *H. pylori* IgG positivity among patients compared to control (P value <0.001 and 0.015, respectively). Analysis of risk factors associated with CAD revealed a significant association of *C. pneumoniae* IgG (P<0.001) and no association of *H. pylori* IgG (P=0.07) with CAD. Our study showed significant association between seropositivity of *H. pylori* and *C. pneumoniae* and CAD. Risk factors analysis for CAD revealed significant associations of CAD with hypertension, Diabetes mellitus (DM) and *C. pneumoniae* IgG.

Introduction

Coronary artery diseases (CAD) are the main etiology of morbidity and mortality worldwide.

Atherosclerosis remains the most prevalent cause of this disease. Multiple risk factors are involved in atherosclerosis associated coronary artery diseases such as hypertension, Diabetes mellitus (DM), smoking, obesity and hereditary factors (Tewari *et al.*, 2012; Redfors *et al.*, 2015). Moreover, several microbiological agents including, *Chlamydia*
pneumoniae (C.pneumoniae), Cytomegalovirus, Helicobacter pylori (H. pylori), Porphyromonas gingivalis and others have been responsible for the development of atherosclerosis (Kuvinand Kimmelstiel, 1999).

Microorganisms can cause atherosclerosis by several mechanisms; they have direct damaging effects on endothelium and cause systemic inflammatory response due to cytokines release that leads to changes in the lipid profile. Both effects enhance the formation of atherosclerosis plaque or thrombus on the endothelium (Izadi et al., 2013; Gillissen and Paparoupa, 2015).

Infections caused by C. pneumoniae are usually dormant intracellulary leading to chronic inflammatory stimulus (Izadi et al., 2013). Seroepidemiologic findings in patients with CHD have shown an association between the presence C. pneumoniae IgG and atherosclerosis (Thjodleifsson et al., 2008; Monno et al., 2010).

Other pathogen that may be associated with CAD is H. pylori which is a common pathogen affecting up to 90% of adults worldwide (Malfertheiner et al., 2007; Miftahussurur et al., 2016; Bayındır Bilman et al., 2016; Malfertheiner et al., 2017). It is associated with gastroduodenal disorders like gastritis, duodenal ulcer and gastric adenocarcinoma.

This organism also can inhabit in atherosclerotic plaques. H. pylori may be involved in the pathogenesis of atherosclerosis through activation of a systemic and/or local inflammatory reaction leading to the induction of plaque formation resulting in coronary syndromes (Zaki et al., 2016).

Moreover, H. pylori infection may trigger acute coronary syndromes by platelet reactivity enhancement through its effect on von Willebrand factor- and P-selectin activation.

Other mechanisms that can lead to involvement of H.pylori in the pathogenesis of CAD include up-regulation of serum levels of highly sensitive C-reactive protein (hsCRP) (Gesualdo et al., 2016) and alteration of lipid metabolism (Chimienti et al., 2003).

The aim of the present study was to investigate the seroprevalence of specific immunoglobulin G for C. pneumoniae and H. pylori among Egyptian patients with CAD and analysis their role as risk factors for this disease.

Materials and Methods

This study is a case-control retrospective study. Patients admitted in Intensive Coronary Care Unit of Mansoura university hospital, tertiary care teaching hospitals was taken up for the study from January 2015 till May 2016.

The study included 80 patients previously diagnosed to have CAD according to medical records and ECG changes.

The second group compromised 80 healthy control subjects. The study was approved by the ethical committee of Mansoura Faculty of Medicine, Egypt. Signed informed consent was obtained from each participant in the study.

Each subject was subjected to thorough medical history taking full clinical examination. Five millimeters of blood samples were obtained from each subject and sera were separated for full biochemical laboratory tests including lipid profile cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein
Sera were kept frozen at -20ºC for further analysis.

All patients and controls were screened for serum IgG antibodies. H. pylori (H. pylori IgG) and C. pneumoniae (C. pneumoniae IgG) by enzyme linked immunoassay (ELISA). (OriGene Technologies, Inc. 9620 Medical Center Dr., Suite 200, Rockville, MD 20850) (Budzyński et al., 2014).

**Statistical Analysis**

Data was analyzed using Statistical Package for Social Science software computer program version 17 (SPSS, Inc., Chicago, IL, USA). Quantitative data was presented in mean and standard deviation, while qualitative data was presented in number and percentage. Chi-square “χ²” or Fischer’s exact tests, as indicated, were used to compare the qualitative data. P value less than 0.05 was considered statistically significant.

A logistic regression model was developed to assess risk factors for development of CAD including sex, dyslipidemia, hypertension, DM, obesity and seropositivity for C. pneumoniae and H. pylori.

**Results and Discussion**

The study included 160 subjects classified as 80 patients with CAD and 80 healthy subjects.

The demographic, clinical and biochemical data of the studied groups are summarized in table 1. Patients were significantly older in age with mean age SD 54.3±9.9 years compared to the control subjects 46.7±5.7 years (P<0.001).

Hypertension was the predominant risk factor found among patients (82.5%) followed by obesity (68.7%) and DM (33.8%). Lipid profile showed significantly higher levels regarding cholesterol (376.8±136.9), triglycerides (251.8±139.9), LDL (367.2±132.9) compared to control subjects with significantly (P<0.001) lower HDL-cholesterol level (26.3±12.6) in patients compared to control subjects.

Seroprevalence of C. pneumoniae IgG and H. pylori showed significant increase among patients (P < 0.001 and 0.015; respectively) compared to control subjects, table 2.

Seroprevalence of C. pneumoniae had steadily increased in age groups among patients and control till age 60 years, however, the increase was significantly higher in patients compared to control in age range 30-45 and 46-80 (P value 0.02 and 0.01; respectively), table 3.

Seroprevalence of H. pylori had steadily increased in age groups among control until age 60 years, however, the prevalence of IgG was significantly higher in patients compared to control in age range 30-45, 46-60 and 61-80 (P value 0.002, <0.001, and 0.001; respectively), table 4.

Multiple logistic regression analysis showed that C. pneumoniae IgG seropositive, hypertension, obesity, and dyslipidaemia were significant risk factors for CAD, table 5.

Coronary artery diseases represent a global health problem. Several risk factors are associated with such grave conditions such as hypertension, obesity, smoking, hypercholesterolaemia, diabetes and infection. Infections have been studied as potential risk factor for such health problem.

In the present study seroprevalence of two common pathogens (H. pylori and C. pneumoniae) were evaluated in Egyptian patients.
Infection with *H. pylori* is common among adults worldwide. Exposure occurs through contaminated water early in life to reach maximum infection rates in adults, approximately 80% (Das et al., 2016). Seropositivity for IgG antibodies to *H. pylori* was seen in 71.4% in CAD patients compared to the control subjects (20%) with a significant association between the presence of *H. pylori* IgG and CAD, (P < 0.001) Previous data supported the presence of an association between *H. pylori* and pathogenesis of atherosclerosis as the main cause of CAD (Patel et al., 1995; Kowalski et al., 2002). Moreover, it has been suggested that infection with *H. pylori* may represent an independent risk factor for CAD in young age independent of other classic risk factors (Kinjo et al., 2002).

However, on multiple logistic regression analysis independent of other conventional risk factors, the presence of *H. pylori* IgG revealed non-significant association of *H. pylori* IgG with CAD (P=0.07) in the present study. These findings were in agreement with previous reports (Rajasekhar et al., 2002; Sun et al., 2006; Szewczyk-Golecet al., 2016; Goni et al., 2016).

Large population studies with implications of different methods of *H. pylori* diagnosis of active versus non-active infections, *H. pylori* infection can be considered as non-conventional CAD risk factors being of importance in certain patients especially in young age groups

A finding supporting our results that the prevalence of IgG was significantly higher in patients compared to control in the age range 30-45 , 46-60 and 61-80 (P value 0.002, <0.001, and 0.001; respectively).

*H. pylori* may play a role in the pathogenesis of atherosclerosis (Maia et al., 2009). Infection with *H. pylori* may influence atherogenesis through low grade, persistent inflammatory stimulation (Tamer et al., 2009). Other mechanisms for the development of atherosclerosis in relation to *H. pylori* infection have also been studied. Tamura et al. from Japan suggested that *H. pylori*-induced chronic atrophic gastritis decreases plasma vitamin B12 and folic acid levels, thereby increasing homocysteine levels, which is a known risk factor for atherosclerosis (Tamura et al., 2002).

Immunoglobulin G antibody seropositivity for *C. pneumoniae* was present in 53.8% of the studied patients and in 20% in controls. There was significant association between the presence of Chlamydia infection as evidenced by the presence of seropositivity of IgG antibodies and CAD (P<0.001).

In several previous studies, *C. pneumoniae* was reported to be among the infectious agents associated with the risk of ischaemic heart disease (Danesh et al., 1999; Kalayoglu et al., 2002; Pour et al., 2005; Spagnoli et al., 2007; Agarwal, et al., 2007; Azarkar et al., 2011). Serological association was also shown by Agarwatal et al., 2007 with prevalence of 64% in CAD patients and seroprevalence of 38% in the control group. Several studies have supported the role of *C. pneumoniae* in the initiation and continuation of atherosclerosis (Mahony et al., 2001; Sotiropoulos et al., 2006; Pesonen et al., 2009).

*C. pneumoniae* is an intracellular Gram-negative bacterium causing mainly respiratory infections (Mahony et al., 2001).

*C. pneumoniae* infects human mononuclear cells. These infected mononuclear cells into the circulation then infect endothelial cells by cell-to-cell transmission of *C. pneumoniae*. This infection may stimulate a series of immunological cascades leading to
atherosclerosis (Erkkila et al., 2004). Another mechanism for developing of atherosclerosis is the structural homology between C. pneumoniae heat shock protein and the protective protein expressed on endothelium. This homology leads to the endothelial cells destruction and smooth muscles proliferation by the antibodies produced against bacterium protein (Koh et al., 2002).

In the present study, the seroprevalence of C. pneumoniae had steadily increased in the age groups among patients and control until age 60 years, however, the increase was significantly higher in patients compared to control in age range 30-45 and 46-80 (P value 0.02, 0.01 respectively)

Similar finding was reported previously with the rising seropositivity with increasing age from childhood to adulthood, with subsequently stable levels (Kohet al., 2002). The rising of the seropositivity with age suggests that C. pneumoniae infection occurs early in life and the seropositivity is increased in the older age by either reinfections or chronic infections (Tewari et al., 2012).

The interesting finding in the present study was the significant association of C. pneumoniae IgG (P<0.001) with CAD in multinomial logistic factor regression model among other traditional risk factors like hypertension and DM.

The relation between C. pneumoniae seropositivity and other risks factor for development of CAD such as hyperlipidemia, DM and hypertension was reported in previous study (Lin et al., 2009).

**Table.1** Demographic, clinical and biochemical data of the studied groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (n=80)</th>
<th>Control (n=80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean± SD</td>
<td>54.3± 9.9</td>
<td>46.7± 5.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (75%)</td>
<td>40 (50%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Female</td>
<td>20 (25)</td>
<td>40 (50%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>66 (82.5%)</td>
<td>0 (0%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>27 (33.8%)</td>
<td>0(0%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Obesity</td>
<td>55 (68.7%)</td>
<td>30 (37.5%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>376.8± 136.9</td>
<td>123.1± 11.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>251.8± 139.9</td>
<td>114.7± 11.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>26.3± 12.6</td>
<td>39.5± 3.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>367.2± 132.9</td>
<td>183.2± 18.7</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*significance ≤0.05

**Table.2** Seroprevalence of Chlamydia pneumoniae IgG and Helicobacter pylori IgG

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (n=80)</th>
<th>Control (n=80)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia pneumoniae IgG</td>
<td>43 (53.8%)</td>
<td>16 (20%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Helicobacter pylori IgG</td>
<td>57 (71.4%)</td>
<td>42 (52.5%)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*significance ≤0.05
Table.3 Distribution of *Chlamydia pneumoniae* IgG between patients and control according to age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patients (43)</th>
<th>Control (16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-45 years</td>
<td>17 (39.5%)</td>
<td>1 (6.3%)</td>
<td>0.02*</td>
</tr>
<tr>
<td>46-60 years</td>
<td>25 (58.1%)</td>
<td>15 (93.8%)</td>
<td>0.01*</td>
</tr>
<tr>
<td>61-80 years</td>
<td>1 (13.9%)</td>
<td>0 (0%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Significance ≤0.05

Table.4 Distribution of *H. pylori* IgG between patients and control according to age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patients</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-45</td>
<td>14 (24.6%)</td>
<td>1 (2.3%)</td>
<td>0.002*</td>
</tr>
<tr>
<td>46-60</td>
<td>31 (54.4%)</td>
<td>41 (97.7%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>61-80</td>
<td>12 (21.1%)</td>
<td>0 (0%)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Significance ≤0.05

Table.5 Logistic regression analysis of risk factors associated with coronary artery diseases

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Wald $\chi^2$</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>4.5</td>
<td>15.071</td>
<td>&lt;0.0001</td>
<td>783.1</td>
<td>43.2-126.1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4.297</td>
<td>10.902</td>
<td>0.002</td>
<td>82.7</td>
<td>4.94-138.81</td>
</tr>
<tr>
<td>Obesity</td>
<td>5.5</td>
<td>16.51</td>
<td>&lt;0.0001</td>
<td>790.4</td>
<td>49.3-134.2</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.1</td>
<td>0.2</td>
<td>0.9</td>
<td>67.68</td>
<td>0.5-2.3</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em> IgG</td>
<td>1.5</td>
<td>16.1</td>
<td>&lt;0.001</td>
<td>52.95</td>
<td>2.1-7.8</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> IgG</td>
<td>1.01</td>
<td>3.2</td>
<td>0.07</td>
<td>5.77</td>
<td>1.31-3.8</td>
</tr>
<tr>
<td>Dyslipedemia</td>
<td>2.018</td>
<td>6.110</td>
<td>0.013</td>
<td>7.53</td>
<td>1.52-37.29</td>
</tr>
</tbody>
</table>

B= logistic regression coefficient, P= P value calculated using Wald chi-square test, OR= Odds ratio 95% CI = 95% Confidence interval for odds ratio

However, other studies found no association between chronic infection with *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 (Khairy *et al.*, 2003; Jia *et al.*, 2009).

To the best of our knowledge, this is the first report about the seroprevalence of IgG to *C. pneumoniae* and *H. pylori* among Egyptian CAD patients.

The study showed a statistically significant association between seropositivity for IgG antibodies to *H. pylori* and *C. pneumoniae* and CAD. Logistic regression of risk factors for CAD also revealed significant Associations of CAD with hypertension, DM and *C. pneumoniae* IgG. ImmunoglobulinG antibodies often reflect previous exposure rather than ongoing chronic infection. Future studies with larger populations and with other methods for diagnosis of active infections with *C. pneumoniae* and *H. pylori* are required to
estimate the impact of these pathogens on the development of atherosclerosis and if the antibiotics have to be added to reduce the risk of atherosclerosis and CAD and thus offer effective adjuncts to treatment already in clinical use.

References


Tamura, A., Fujioka, T., Nasu, M. Relation of *Helicobacter pylori* infection to plasma vitamin B12, folic acid, and homocysteine levels in patients who underwent diagnostic coronary


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