The association between *Chlamydia pneumoniae* DNA in atherosclerotic plaque and major risk factors in patients undergoing coronary artery bypass grafting

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Abstract

**Background and aim:** This study was conducted to investigate the prevalence of *Chlamydia pneumoniae* pathogen inside the atherosclerotic plaque of patients undergoing CABG by using PCR assay and to determine whether there is any association between the presence of bacteria in atherosclerotic lesions and classical coronary risk factors.

**Methods:** In a cross-sectional study, 102 patients (20 to 79 years old; 73.5% male) undergoing CABG were evaluated in terms of major coronary risk factors and the presence of *Chlamydia pneumoniae*.

**Results:** *Chlamydia pneumoniae* was found in 23.4% of coronary plaque specimens. Of these, two patients had no risk factor and the rest of the patients had 1 to 3 risk factors. Patients with positive PCR were more likely to have hypercholesterolaemia (p = 0.009) and low HDL levels (p = 0.000) in comparison with the PCR-negative group. There were no statistical differences for other risk factors.

**Conclusion:** Our results imply the synergic contribution of *Chlamydia pneumoniae* DNA and known dyslipidaemia to the development of atherosclerotic lesions in patients undergoing CABG.

**Key words:** *Chlamydia pneumoniae*, atherosclerosis, risk factors, PCR, CABG

Introduction

Since 1988, the association between the presence of *Chlamydia pneumoniae* in atherosclerotic lesions and coronary artery disease (CAD) has been described in several investigations [1-8]. It was initially demonstrated in seroepidemiological reports followed by studies in which the organism was identified in vascular tissue of cardiovascular patients by electron microscopy, polymerase chain reaction (PCR), immunocytochemical staining (ICC) or culture [9]. Despite the inconsistency, the interaction of *Chlamydia pneumoniae* with conventional coronary risk factors [10, 11] and its inflammatory role in the development of atheromatous plaques [12-14] have been suggested as some plausible mechanisms in this regard.

Kinjo et al. [10] reported that patients incorporating both IgA titres and a classical risk factor such as obesity, hypercholesterolaemia, or smoking predicted risk for acute myocardial infarction more effectively than single-parameter patients. Other studies suggested the association of *Chlamydia pneumoniae* antibodies with an atherogenic lipid profile [11, 15, 16]. In contrast, in Iran, Bahrmand et al. [17], evaluating the arterial specimens of cases which mostly had an autopsy diagnosis of cardiac death, found that the levels of low density lipoprotein (LDL) and triglycerides were significantly lower in the PCR-positive cases than in the PCR-negative cases. Besides, they noticed that 78.5% of PCR-positive cases had only one risk factor for atherosclerotic cardiovascular disease,
whereas all PCR-negative cases had multiple risk factors. They suggested that *Chlamydia pneumoniae* may be involved in the development of atherosclerosis in humans, especially in cases where classical risk factors are not identified to explain the incidence of atherosclerotic vascular disease. It seems, however, that further studies are needed to clarify the pathogenetic mechanisms underlying the role of *Chlamydia pneumoniae* and its interactions with classic risk factors of CAD [11].

Considering the contradictory results of Bahrmand’s study in Iran, which is in contrast to many previous studies, this study was conducted in Shariati Hospital in Tehran, Iran, in order to assess the prevalence of *Chlamydia pneumoniae* pathogen inside the atherosclerotic plaque of patients undergoing coronary artery bypass grafting (CABG) by using PCR assay and to determine whether there is any association between the presence of bacteria in atherosclerotic lesions and known coronary risk factors in patients undergoing CABG.

**Methods**

**Patients**

This cross-sectional study, approved by the Ethics Committee of Tehran University of Medical Sciences, was conducted in Shariati Hospital in Tehran, Iran. This study conforms to the principles outlined in the Declaration of Helsinki. Coronary atherosclerotic samples were obtained from 102 patients undergoing CABG during an eight-month period.

Variables including age, sex, history of smoking (use of ≥ 3 cigarettes per day within one month of surgery [18, 19]), hypertension (reported history of hypertension or use of an antihypertensive agent or SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg [20]), diabetes mellitus (reported history of diabetes or treatment with an anti-diabetic agent and FBS ≥ 126 mg/dl [21]), hyperlipidaemia (levels of cholesterol ≥ 240 mg/dl, triglyceride > 200 mg/dl, LDL > 130 mg/dl and HDL < 40 mg/dl [22]) and family history of CAD (one of the direct blood relatives including parents, siblings or children has had any of the following at age < 55: angina, myocardial infarction, sudden cardiac death presumed to be from ischaemic heart disease because of no other obvious cause or coronary intervention [23]) were recorded.

**Table I.** Distribution of risk factors among patients

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Frequency n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>75 (73.5)</td>
</tr>
<tr>
<td>female</td>
<td>27 (26.5)</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td></td>
</tr>
<tr>
<td>high total cholesterol</td>
<td>55 (53.9)</td>
</tr>
<tr>
<td>low HDL</td>
<td>32 (31.7)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25 (25.5)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18 (18.3)</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>28 (27.4)</td>
</tr>
</tbody>
</table>

Abbreviations: HDL – high density lipoprotein, CAD – coronary artery disease

**Preparation of samples and PCR amplification**

During the operation, a total of 102 samples were subjected to single-step PCR [24, 25] for direct detection of *Chlamydia pneumoniae*. After longitudinal incision, atherosclerotic cylinders were obtained by blunt dissection of the coronary plaque from the adventitial layer. Under sterile conditions, a specimen of 0.3 cm² was harvested from the involved coronary artery and the sample was immediately collected in a defined *Chlamydia pneumoniae* transport medium.

The specimens in the tube containing Tris EDTA buffer were cut with a sterile blade, as multiple sections, and frozen at –20°C. The specimen sections were treated with a solution containing 10 mM Tris–HCl (pH 8.3), 1 mM EDTA, and 100 mg of proteinase K per ml and incubated at 60°C for 1 h and heated at 96°C for 10 min. DNA was extracted with phenol-chloroform-isoamyl alcohol and precipitated with absolute alcohol. The precipitate was washed with 70% ethanol. The pellet was dried and dissolved in 25 µl of sterile, double distilled water, and 5 µl of the DNA suspension were used for amplification. Each PCR reaction mixture (50 µl) contained 5 µl of genomic DNA, 20 pmol of HL-1 primer (5’-GTT CAT GAA GGC CTA CT-3’), 20 pmol of HR-1 primer (5’-TGC ATA ACC TAC GGT GTT TT–3’), 2.5 units of Taq DNA polymerase (Promega Corporation, USA), 150 µM deoxynucleoside triphosphate mix, 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl2. The reaction mixture was amplified for 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s. Amplification products were electrophoresed by 2% agarose gel containing ethidium bromide, and were visualised under UV illumination.

**Statistical analysis**

All data were analysed using Medcalc software (Belgium, version 9.3.7.0). Qualitative variables and PCR results were compared by Fisher’s exact test. A p value < 0.05 was considered significant.

**Results**

The mean age of patients was 54 ± 6.3 years and 73.5% of patients were men. Table I shows the distribution of risk factors among patients. Twenty-three (23.4%) of 102 coronary specimens were positive for the presence of *Chlamydia pneumoniae* DNA. Of these, two patients had no risk factor but other patients had 1 to 3 risk factors. The characteristics of the patients according to presence of *Chlamydia pneumoniae* DNA and risk factors are shown in Table II. There were no significant differences in hypertension, diabetes,
family history and smoking between PCR positive and negative groups. Patients with positive PCR were more likely to have hypercholesterolaemia (p = 0.009) and low HDL level (p < 0.001) in comparison with the PCR-negative group.

**Discussion**

In this study, *Chlamydia pneumoniae* was detected in 22 (23.4%) of 102 coronary plaque specimens. This is consistent with other studies that have frequently detected *Chlamydia pneumoniae* pathogen in 17 to 100% of lesions [2, 17, 26-31] but rarely reported the organism in non-atheromatous arteries (about 1-9% of subjects) [26, 32, 33]. This variance in the prevalence of presence of *Chlamydia pneumoniae* may be firstly due to absence of a single diagnostic serology, PCR, or ICC assay, which have different sensitivities [9, 34]. Moreover, the assays used for detection of *Chlamydia pneumoniae* are not standardised [9, 35]. However, the presence of chlamydial DNA in atherosclerotic plaques could suggest a direct aetiologic or indirect facilitating role for this organism in the pathogenesis of atherosclerosis [36]. While many studies have imputed a causative role for *Chlamydia pneumoniae* in the onset and progression of vascular disease through different mechanisms [2, 14, 37-40], some others have evaluated the indirect association of *Chlamydia pneumoniae* with known classic coronary risk factors.

In the present study, hypercholesterolaemia (53.9%) and smoking (49%) were the most prevalent risk factors. Similarly, Leinonen et al. [11] reported that chronic *Chlamydia pneumoniae* infections were more common among smokers, and even in acute pneumonia caused by *Chlamydia pneumoniae*, HDL level was lower and triglyceride level higher than in pneumonia caused by viruses and other bacteria. In another study, Maass et al. [41], evaluating the presence of viable *Chlamydia pneumoniae* in coronary samples of patients undergoing myocardial revascularisation, detected chlamydial DNA in 30% of atheromata. Established cardiovascular risk factors in their study were high cholesterol (57%), high systolic blood pressure (70%), diabetes mellitus (36%), and tobacco smoking (24%)

In this study, patients with positive PCR had significantly higher total cholesterol and lower HDL levels in comparison with the PCR-negative group. This is in line with Laurila et al. [15] study in which the serum TG and total cholesterol concentrations were higher in the subjects with a chronic *Chlamydia pneumoniae* infection than in the subjects with no antibodies. In addition, our results support the study of Murray et al. [16], who reported higher total cholesterol and lower HDL cholesterol in seropositive men. They concluded that *Chlamydia pneumoniae* infection is associated with an atherogenic lipid profile in men and altered lipid levels may underlie the association between *Chlamydia pneumoniae* and CAD. The pathogenic mechanism by which the organism may initiate and promote the disease is poorly understood. However, Kalayoglu et al. [42] in their study demonstrated that *Chlamydia pneumoniae* induces macrophages to accumulate excess cholesterol and develop into foam cells, the hallmark of early atherosclerotic lesions. Furthermore, *Chlamydia pneumoniae* interaction with platelets leading to aggregation, production of reactive oxygen species (ROS) and oxidative damage to LDL may play a crucial role in the development of atherosclerotic cardiovascular disease [40].

**Table II. Distribution of patients according to presence of *Chlamydia pneumoniae* DNA in coronary specimens and coronary risk factors**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th><em>Chlamydia pneumoniae</em> DNA</th>
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<tr>
<td></td>
<td>PCR positive (n = 23)</td>
</tr>
</tbody>
</table>
| Hypertension        | 6 (26.0)            | 19 (24.0)           | 0.791
| Diabetes mellitus   | 7 (30.4)            | 11 (13.9)           | 0.115
| Hypercholesterolaemia | 18 (78.2)     | 37 (46.8)           | 0.009
| HDL [mg/dl]         | 41.2 ± 8.4          | 43.4 ± 12.4         | 0.013
| LDL [mg/dl]         | 135.0 ± 45.6        | 116.2 ± 42.3        | 0.010
| Low HDL             | 16 (69.5)           | 16 (20.2)           | < 0.001
| Family history of CAD | 8 (34.7)       | 20 (25.3)           | 0.428

Abbreviations: PCR – polymerase chain reaction, CAD – coronary artery disease, HDL – high density lipoprotein, LDL – low density lipoprotein

Limitations of the study

Use of DNA amplification in our study avoided the potentially lower specificity of immunological detection techniques in other studies. In addition, we used PCR to detect the presence of bacteria in coronary specimens due

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to the difficulty and length of time required to isolate *Chlamydia pneumoniae* by cell cultures. We did not examine non-atherosclerotic samples for the presence of *Chlamydia pneumoniae*. Lack of serological profiles (IgG, IgA and IgM) and lack of follow-up data were other limitations of this study. Also, our patients referred for CABG may not be totally representative of the overall population of patients with coronary atherosclerosis.

In conclusion, our study demonstrates the presence of *Chlamydia pneumoniae* in coronary atheromatous lesions of patients undergoing CABG. Our results also imply the synergic contribution of *Chlamydia pneumoniae* DNA and known coronary risk factors (especially dyslipidaemia) to the development of atherosclerotic lesions and coronary events in patients undergoing CABG.

**Acknowledgments**

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**References**

Związek pomiędzy Chlamydia pneumoniae w blaszkach miażdżycowych a czynnikami ryzyka rozwoju choroby wieńcowej u chorych poddawanych rewaskularyzacji chirurgicznej

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Streszczenie

Wstęp: Od dawna sugerowano istnienie związku pomiędzy rozwojem choroby wieńcowej a infekcją Chlamydia pneumoniae.

Cel: Ocena częstości występowania patogenu Chlamydia pneumoniae w blaszkach miażdżycowych uzyskanych z naczyń wieńcowych chorych poddawanych operacji pomostowania aortalno-wieńcowego (CABG).

Metody: U 102 chorych (20–79 lat, 73,5% mężczyzn), u których wykonano CABG, oceniono czynniki ryzyka choroby wieńcowej oraz zbadaano blaszki miażdżycowe z pobranych fragmentów tętnic wieńcowych na obecność Chlamydia pneumoniae (metoda PCR).

Wyniki: Patogen Chlamydia pneumoniae został wykryty w 23,4% badanych blaszkach i miażdżyce chorych na choroby sercowo-naczyniowe, a u pozostałych występowało 1–3 takich czynników. Chorzy, u których stwierdzono choroby wieńcowe Chlamydia pneumoniae, mieli znacznie częściej hipercholesterolemie (p = 0,009) i niskie stężenie cholesterolu HDL (p = 0,000) niż chorzy bez tego patogenu.

Wnioski: Wyniki naszego badania sugerują synergistyczny wpływ Chlamydia pneumoniae i dyslipidemii na rozwój blaszki miażdżywej u chorych poddawanych CABG.

Słowa kluczowe: choroba wieńcowa, Chlamydia pneumoniae, CABG, miażdżyca

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