ORIGINAL ARTICLE

Is there any relationship between *Chlamydophila pneumoniae* and coronary atherosclerosis among Iranians?

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ABSTRACT

Background: Atherosclerosis is a coronary heart disease, andis the most common cause of death in the industrialized world. Some studies suggested that atherosclerosis may be triggered by infectious agents, mostly *Chlamydophila pneumoniae*. However, the role of *C. pneumoniae* in the pathogenesis of coronary atherosclerosis is still controversial. **Objectives:** This study was performed to evaluate whether there is a significant association between coronary artery atherosclerosis and *C. pneumoniae* by the polymerase chain reaction (PCR) method. **Materials and Methods:** This case-control study was carried out on formalin-fixed paraffin-embedded tissue biopsies of the coronary arteries obtained from 30 patients with coronary atherosclerosis and 30 subjects without atherosclerosis living in Northeast of Iran. All subjects' weight and height were determined, and the body mass index was calculated. We also reviewed the medical history and previous laboratory reports of patients. Deoxyribonucleic acid (DNA) was extracted, and *C. pneumonia* DNA was amplified and detected using PCR assay. **Results:** The age of the patients in the study group was from 18 to 50 years, and the male to female ratio was 5:1. Only oneout of the 30 coronary tissue samples had positive PCR for *C. pneumoniae* (3.3%), while it was negative for patients in the control group. **Conclusions:** This study showed that *C. pneumoniae* infection is not strongly associated with coronary artery atherosclerosis in Northeast of Iran.

Key words: Atherosclerosis, *Chlamydophila pneumonia*, coronary artery, Iran

INTRODUCTION

Atherosclerosis is a coronary heart disease (CHD), and is the most common cause of death in the industrialized world. High blood pressure (BP), hypercholesterolemia, diabetes mellitus (DM) and smoking are considered as the major risk factors for the development of atherosclerosis. In addition, increasing age, male sex, family history and genetic factors predispose patients to CHD.[1] There is some data that have suggested the possible potential role of some infectious agents in the pathogenesis of atherosclerosis.[2-6] The possibility of infectious agents may trigger the process of atherosclerosis by direct or indirect inflammatory effects, especially at younger ages.[5]

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Although different studies have been carried out to prove this hypothesis some infections may contribute in the pathogenesis of atherosclerosis; nothing has ever been conclusively proven. The possible role of some agents such as *Chlamydophila pneumoniae*,[6] *hepatitis C. virus*,[7] *Human immunodeficiency virus*,[7] *hepatitis B virus*,[8] *cytomegalovirus*,[9] *herpes* virus,[9] *Epstein-Barr virus*,[10] *Mycobacterium tuberculosis*,[11] and *helicobacter pylori*,[3] have been considered in the pathogenesis of atherosclerosis. Among these pathogens, *C. pneumoniae* is important, because it could be effectively treated by antibiotics.[12] *C. pneumoniae* is an obligatory intracellular organism, and is responsible for at least 10% of community-acquired pneumonias. This bacterium has also been associated with pharyngitis, bronchitis, otitis, influenza-like illness, sinusitis and myocarditis.[12-14]

Conflicting results have been reported about the relationship

between *C. pneumonia* and atherosclerosis. West *et al.*[15] Apfalter *et al.*,[16] and Zibaeenezhad *et al.*[17] reported no association between *C. pneumoniae* and atherosclerosis but others found significant association between *C. pneumoniae* and atherosclerosis.[6,18,19] The results of Jha *et al.*,[6] Dabiri *et al.*[18] and Sessa *et al.*[19] support the idea that *C. pneumoniae* may have a role in the development of atherosclerosis. This controversy about the role *C. pneumoniae* in the pathogenesis of atherosclerosis emphasizes the importance of more research works.

The aim of this study was to demonstrate whether there is any difference between two group of patients with coronary atherosclerotic plaque and subjects without coronary atherosclerosis for the presence of *C. pneumoniae* DNA by polymerase chain reaction (PCR) method.

MATERIALS AND METHODS

This case-control study was carried out on 60 formalin-fixed paraffin-embedded (FFPE) coronary artery biopsies in the molecular pathology laboratory, Ghaem Hospital, Mashhad, Iran in 2010.

Coronary artery tissues were selected from another study that was performed previously by our colleagues for determining prevalence of atherosclerotic plaques in autopsy cases with non-cardiac death in northeast Iran. In this study, left coronary artery (LCA), right coronary artery (RCA) and left circumflex artery (LCX) had been evaluated grossly and microscopically.

Our materials included 30 coronary artery tissues with atherosclerotic plaques that were defined as study group, and 30 coronary artery tissues without atherosclerotic plaques that served as the control group. Archived slides of the two groups were reviewed by two pathologists for confirmation of diagnosis, tissue adequacy and selection of the best paraffin blocks for DNA extraction. For all subjects, the weight and height were determined, and the body mass index (BMI) was calculated. We also reviewed the medical history and the previous laboratory reports of patients.

Five to seven, 5-µm-thick sectionswere cut from each FFPE specimen under sterile conditions, and then the DNA was extracted by using proteinase K and non-heating DNA extraction method.[20] DNA concentration was determined by using the Thermo Scientific NanoDrop 2000 spectrophotometer, and specimens with low DNA content (<20 ng/µL) were excluded from the study.

PCR was performed for detecting *C. pneumoniae* DNA by using the PCR Kits (DNA Technology (JSC), PCR Kit, Moscow, Russia). 10 µl of PCR master mix and 0.5 µl unit of Taqpolymerase (Hot Start Taq DNA Polymerase) were added into each paraffin sealed tube and were mixed, then 5 µl (100 ng) of DNA samples were added (except for positive and negative controls) and were spun at 1,000 rpm for 3-5 s. The tubes were placed into applied biosystems (ABI) Veriti Thermal Cycler and PCR was performed with the program of 180 s at 94°C for the first step and then 45 cycles was run as follows: 50 s at 94°C, 50 s at 64°C, 50 s at 72°C.

Amplified PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light by gel documentation instrument. PCR was interpreted as positive when the DNA band corresponds to the band of the positive control (254bp) in addition to the internal control band (IC:370 bp).

Results of PCR in control and atherosclerotic groups were analyzed with SPSS version 11.5 by a statistician. We used Fisher's exact test for comparison of categorical variables and independent sample *t*-test for continuous variable in patients and control groups. A *P* value below 0.05 ($P < 0.05$) was considered significant.

RESULTS

The age range in the control group was 20-46 years with a mean (standard deviation) age of 26.0 (6.41) years. In the study group, it ranges from 18 to 50 years with a mean (SD) age of 32.0 (9.46) years and the *t*-test showed no significant difference between the ages of patients in the study and control groups (P=0.97). Twenty-five patients (83.3%) were males and five patients (16.7%) were females. The male to female ratio was 5:1 and no significant difference was seen between study and control groups for gender ($P = 1.00$) by Fisher's exact test. The mean BMI of the patients in the study and control groups were 24.4 (3.77) and 23.9 (2.62) kg/m^2 respectively [Table 1]. The frequency of atherosclerotic plaques in the different vessels of heart of the patients in the study is as shown in Table 2. As a result, the possibility

Table 1: Comparisons between patients with coronary atherosclerosis and controls for weight, height and body mass index

	Patient group			Control group		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Weight	44.00	90.00	67.40	45.00	95.00	72.56
Height	110.00	180.00	166	157.00	185.00	172.53
BMI	20.03	36.36	24.54	17.30	35.16	24.41

BMI – Body mass index

Table 2: The frequency of atherosclerotic plaques in different vessels of the heart in the study group

Atherosclerotic plaque	LCX (%)	LCA (%)	RCA (%)
None	9 (30)	5 (16.7)	7 (23.3)
Fibro-fatty plaque	11 (36.7)	7 (23.3)	14 (46.7)
Advanced plaque	10 (33.3)	18 (60)	9 (30.0)

LCX – Left circumflex artery; LCA – Left coronary artery; RCA – Right coronary artery

of the appearance of advanced atherosclerotic plaques s in LCA was higher than the two other vessels. Concurrent occurrences of different stages of atherosclerosis were seen in our patients. Only one vessel involvement with fibro-fatty morphology (mild) was seen in five patients (16.7%), one vessel with advanced plaque was observed in three patients (10.0%), seven patients (23.3%) had one vessel with fibro-fatty and one vessel with advanced plaque, Two vessels with advanced plaque were noticed in six patients (20.0%), Three vessels with advanced plaque were observed in five patients (16.7%) and four patients (13.3%) had two vessels with advanced plaque and one fibro-fatty morphology.

Positive PCR result for *C. pneumonia* was seen in one (3.3%) sample among the 30 coronary artery tissues with atherosclerosis (advanced plaque), while all the control samples were negative. Fisher's exact test showed no significant difference for detection of *C. pneumoniae* between samples of coronary artery with and without atherosclerotic plaque ($P = 1$).

DISCUSSION

Ischemic heart disease could be observed in the absence of its major risk factors such as increasing age, male gender, hypertension, hyperlipidemia, smoking and DM. It was postulated that inflammation contributes to the initiation and progression of atherosclerotic lesions. Inflammatory cells are seen during all steps of atherogenesis, and some infectious agents may trigger this inflammation.[1,13]

Although some studies showed that *C. pneumoniae* can increase the risk of atherosclerosis,[6,18,19] but we did not find any significant difference in the frequency of *C. pneumoniae* DNA isolation in the coronary arteries with and without atherosclerosis by PCR method. We believe that at least two important factors can account for this discrepancy in the results from previous studies: Methodology and epidemiology. *C pneumonia* infection can be diagnosed by various methods such as serology with an increase in serum antibodies against the organism, DNA detectionby PCR, Immunocytochemistry, electron microscopyand *C. pneumonia* isolation by culture.[14]

In other studies, the most widely used method for identifying of *C. pneumoniae* infection was based on antibody detection. The frequency of antibody against *C. pneumoniae* starts to rise in children and is observed approximately in 50% of adolescents.[12-14] Somesero-epidemiologic studies have shown a relationship between *C. pneumoniae* and atherosclerosis. Saikku *et al.,*[21] Podsiadły *et al.*[22] and Romano *et al.*[23] detected a higher level of antibody against *C. pneumoniae* in CHD compared to control group but Zibaeenezhad *et al.,*[17] Romeo *et al.,*[24] and Ericson *et al.,*[25] found that the presence of antibodies was unable to predict coronary artery events.

We used PCR for detection of *C. pneumonia*; this assay appears to be more sensitive than cell culture.[14,26] Our finding was in concordance with those of West *et al.,*[15] Jantos *et al.*[27] and Satpathy *et al*.[28] Similarly, Reszka *et al.,*[29] studied presence of *C. pneumonia* DNA in aortic vessels, Voorend *et al.*[30] in cerebral vessels, and Kwon *et al.*[31] in carotid arteries and could not establish any significant difference in PCR results between the study and control groups. These findings suggest that serology could detect past as well as present infection, whereas PCR detects current C*. pnuemoniae* infection. Considering the study of West *et al.*[15] and Satpathy *et al.*[28] who showed a higher *C. pnuemoniae* IgG antibody in patients with CHD compared to control group but could not detect the organism by nested PCR assay in any specimen strengthens our hypothesis. We believe that the lack of standardized methods for the detection of *C. pnuemoniae* infection used in the aforementioned studies is probably one of the important reasons for the discrepancies in their results.

C. pnuemoniae is a common human pathogen; the majority of infected subjects have few or no symptoms. It seems that this infection has endemic and epidemic distribution. The frequency of antibodies to *C. pneumoniae* is approximately 50% in the northern hemisphere.[14,32] Exposure to *C. pneumoniae* is probably common in Iran. Moghaddam *et al.*[33] reported 38% seropositivity for *C. pneumonia* antibodies (IgG and IgM) in healthy subjects by ELISA method in Tehran, Iran. Studies carried out in Iran on the association between *C.* pneumonia infection, and atherosclerosis revealed conflicting results; while some studies showed the association between *C. pneumonia* infection, and atherosclerosis[18,34] others didn't demonstrate any significant associationbetween *C.* pneumonia and atherosclerosis.[17,35]

The anatomical localization of vessels may also be a factor influencing the results from previous studies. Jha *et al.*[6] showed that *C. pneumoniae* may be associated with CHD and coronary artery was more susceptible to *C. pneumoniae* as compared with carotid artery.

Bahrmand *et al.*[25] and Ericson *et al.*[34] examined the relationship of *C. pneumoniae* infection to the severity of coronary atherosclerosis by PCR and serology respectively. They examined coronary artery samples with two different methods, and both revealed a high rate of reactivity to this bacterium in severe atherosclerosis and a much lower rate in mild atherosclerosis; therefore, they concluded that severity (stage) of atherosclerosis can be presumed as an important factor in diagnosis of *C. pneumoniae* infection.

Our study was performed on 30 archived FFPE coronary artery tissues of patients with atherosclerosis. This sample

size may be too small for final decision; therefore, future study with a larger number of case groups and also using fresh specimens are helpful.

In conclusion, we did not find any significant association between *C. pneumoniae* infection and coronary artery atherosclerosis by PCR method. Further studies involving a greater number of patients and also using more specific methods for detecting living *C. Pneumonia* organisms by culture can be helpful.

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