RELATION OF *CHLAMYDIA PNEUMONIAE* INFECTION TO DOCUMENTED CORONARY ARTERY DISEASE

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Abstract- The possibility that infectious agents may trigger a cascade of reactions leading to inflammation, atherogenesis and vascular thrombotic events has recently been raised. *Chlamydia pneumoniae* is an infectious agent that has received the most attention with respect to coronary artery disease (CAD). To determine the relationship between C. *pneumoniae* and CAD, a case control study was conducted on 167 subjects (81 women and 86 men) who underwent coronary angiography at cardiac catheterization laboratories of our hospital. We measured IgG and IgA antibodies to C. *pneumoniae* antigens by ELISA method in baseline serum samples from 109 cases (mean age 57 years) who had at least one coronary artery lesion occupying 50% or more of the luminal diameter on coronary angiography and from 58 matched controls (mean age 50 years) who had documented normal coronary arteries. The prevalence of IgG and IgA antibodies to C. *pneumoniae* showed no case-control differences for IgG (82.6% vs 74.1%) or IgA (23.5% vs 16.7%). These results suggest that C. *pneumoniae* is not associated with documented CAD. More studies are needed to clarify the possible different effects of C. *pneumoniae* on atherosclerosis.

Acta Medica Iranica, 42(5): 319-324; 2004

Key words: *Chlamydiae pneumoniae*, atherosclerotic lesions, coronary artery disease, IgA, IgG

INTRODUCTION

High blood pressure, elevated serum cholesterol and smoking are considered as the major risk factors for the development of atherosclerosis and coronary heart disease (CHD). In addition, age, sex, obesity, diabetes mellitus, high serum triglyceride and low levels of high density lipoprotein (HDL) predispose patients to CHD.

Inflammation is also involved in the pathogenesis of atherosclerosis and myocardial infarction. Slightly elevated serum C-reactive protein (CRP) concentration is considered as a marker of systemic inflammation that can predict coronary events in studied population (1-3). The possibility that infectious agents may trigger a cascade of reactions leading to inflammation, atherogenesis and vascular thrombotic events has recently been raised. *Chlamydia pneumoniae* is one of infectious agents that has received the most attention. The association of C. *pneumoniae* with atherosclerosis has been detected in some seroepidemiologic studies (4-14), but some reports are against the hypothesis of the pathogenic role of C. *pneumoniae* in coronary artery disease (CAD) (15-18).

The technique most widely used for the detection of antibodies to C. pneumoniae in serum has been microimmunofluorescence. Recently introduced enzyme-linked immunosorbent assay kits are now available which are more accurate.

This study was undertaken to determine the seroepidemiology of C. pneumoniae infection in Shiraz, Southern Iran, and to elucidate the association of this microorganism with angiographically documented CAD.

Received: 28 May 2003, Revised: 9 Nov. 2003, Accepted: 3 Mar. 2004

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MATERIALS AND METHODS

A case-control cross sectional study was conducted on 167 subjects (86 men and 81 women). Coronary angiography was used as the key standard diagnostic method in diagnosis of atherosclerotic plaques. Cases (n=109, mean age of 57 years), defined as patients who presented clinically as stable angina pectoris or acute coronary syndromes and had at least one coronary artery lesion occupying 50% of the luminal diameter or more on coronary angiography, were recruited through the Shiraz University of Medical Sciences cardiac catheterization laboratories from January 15, 2002 to December 15, 2002. Those who had no demonstrable lesion on angiography were served as controls (n=58, mean age of 50 years).

Acute coronary syndromes included acute myocardial infarction (AMI) and unstable angina. AMI was diagnosed as continuous ischemic chest pain within 24 hours of presentation, elevation of creatine kinase to twice the upper limit of normal for at least two times and characteristic electrocardiographic changes in the ST segment (ST elevation > 1 mm, ST depression > 1 mm). When serum enzymes were normal, the same findings in association with characteristic anginal chest pain was considered as unstable angina.

Catheterization reports and medical records, description of the coronary artery lesion, family history of unstable angina or AMI, hypertension, diabetes mellitus and any habit of smoking were recorded in a questionnaire.

Hypertension was defined as the presence of elevated systolic (> 160 mm Hg) and/or diastolic (>95 mm Hg) blood pressure and/or the current use of antihypertensive drugs. Diabetes mellitus was defined as history of hypoglycemic treatment and/or fasting plasma glucose of >126 mg/dl, total cholesterol > 220 mg/dl, low density lipoprotein (LDL) >130 mg/dl and high density lipoprotein (HDL)< 35 mg/dl were each considered as hyperlipidemia status in patients.

Immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies against C. *pneumoniae* were tested using the DIA pro C. *pneumoniae* enzyme-linked immunosorbent assay (Milan, Italy) according to manufacturer's instructions.

Serum samples were diluted and then incubated with the highly purified C. *pneumoniae* outer membrane protein antigens coated in the microwells with negative and positive controls. After washing, the bounded IgGs were further complexed with anti-human IgG and IgA antibodies labeled with HRP. A substrate solution was used reacting with HRP to produce color correlating with the presence of anti C. *pneumoniae* IgG or IgA in the sample. The results were determined by calculating an index value from optical density values relative to control materials. An index of ≥ 0.9 was considered reactive, and <0.9 was considered negative. Seropositivity was defined as the presence of either IgG or IgA antibodies.

Fasting serum samples were analyzed for levels of blood sugar. Total cholesterol, triglycerides (TG), HDL cholesterol and LDL cholesterol were also determined before coronary angiography procedures. In AMI cases, the lipid profile was determined during 12 hours of presentation.

The two groups were matched in relation to HDL, LDL, TG, smoking habits and total cholesterol. Independent 2-sample *t* test. ANOVA, Fisher exact test and Chi square test were applied for statistical analysis. *P* value <0.05 was considered significant.

RESULTS

Table 1 shows the baseline characteristics of the cases and controls. The prevalence of IgG and IgA antibodies between cases and the controls was not statistically significant.

Cases were more likely to be male and more likely to be older compared with the control subjects. Hyperlipidemia, hypertension, smoking and diabetes mellitus were more prevalent in cases compared to control group.

Tables 1 and 2 show the association of seropositivity with those CAD risk factors which were not matched between cases and controls. The results do not show any correlation between age, sex, diabetes mellitus or CAD family history and seropositivity of IgA or IgG, so these factors were not regarded as confounding factors. Table 3 shows that there was no correlation between acute coronary

Table 1. Demographic characteristics, coronary risk factors and C. *pneumoniae* antibody status in patients and controls*

Characteristic	Cases (n= 109)	Controls (n= 58)	*P* value
Age (years)	57.4(11.4)†	50.0(10.8)†	<0.001
Men	68 (62.4)	18 (31.0)	< 0.0001
Women	41 (37.6)	40 (69.0)	< 0.0001
Total cholesterol (mg/dL)	190.8(37.2)†	200.7(44.6)†	0.13
HDL cholesterol (mg/dL)	36.8(8.2)†	39.6(7.2)†	0.029
LDL cholesterol (mg/dL)	120.7 (33.8)†	123.3 (32.7)†	0.65
Triglycerides (mg/dL)	181.5(126.9)†	193.4(124.5)†	0.56
Hypertensives	46 (42.2)	20 (34.5)	0.406
Smokers	33 (30.3)	13 (22.4)	0.36
Diabetics	55 (50.5)	12 (20.7)	<0.0001
CAD family history	6 (5.5)	10 (17.2)	0.02
Antibody status			
IgG	90 (82.6)	43 (74.1)	0.22
IgA	24 (23.5)	9 (16.7)	0.41
IgG or IgA	93 (85.3)	43 (74.1)	>0.95

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; CAD, coronary artery disease.
*Data are presented as number (percent) unless specified otherwise.
† mean(SD).

syndromes (AMI and unstable angina) and seropositivity of lgG or IgA to C. *pneumoniae*. The high prevalence of IgG in cases (82.6%) and in controls (74.1%) shows that prevalence of past infection with C. *pneumonia* in our area is high; prevalence of IgA in cases (23.5%) and in controls (16.7%), shows that acute infection is not infrequent in this area as well.

Table 2. Characteristics of IgG seropositive subjects*

Characteristic	Seropositive (n= 133)	Seronegative (n= 34)	*P* value
Age (years)	55.04±11.48†	54.0±12.61†	0.65
Men	70 (81.4)	16 (18.6)	0.57
Women	63 (77.8)	18 (22.2)	0.57
Diabetics	55 (82.1)	12 (17.9)	0.56
CAD family history	10 (62.5)	6 (37.5)	0.09

Abbreviation: CAD, coronary artery disease.
*Data are presented as number (percent) unless specified otherwise.
† mean±SD.

Table 3. Association of seropositivity of C. *pneumoniae* with acute coronary artery syndromes*†

	Seropositive (n= 56)	Seronegative (n= 12)	*P* value
IgG	55 (80.9)	13 (19.1)	0.61
IgA	21 (30.9)	47 (69.1)	0.51
IgG or IgA	56 (82.4)	12 (17.6)	0.40

*Data are presented as number (percent).
†Acute coronary syndromes include acute myocardial infarction and unstable angina.

DISCUSSION

There are few reports of the seroepidemiology of C. *pneumoniae* infection and its association with CAD in Iran. Although a high prevalence of C. *pneumoniae* was observed in this study, there was no association between C. *pneumoniae* and coronary atherosclerotic lesions.

In our study, the prevalences of IgG and IgA antibodies to C. *pneumoniae* in cases were 82.6% and 23.5%, respectively. Prevalence of IgG was significantly higher in our study compared with reported prevalences from most western countries (19-20), but is similar to the results of Tsai *et al.* (75%) from Taiwan and Tavendale *et al.* (80%) from Scotland (17-18). Following acute infection, the IgG antibody titer increases initially and then usually decreases slowly, whereas the IgA antibody disappears more rapidly. So, our results showed a high prevalence of past infection to C. *pneumoniae* in our area in Southern Iran. Similar to our data, the prevalence of IgG antibody to C. *pneumoniae* was 50% in a study from Japan (21).

Prevalence of IgA antibody to C. *pneumoniae* was 23.5% in our study. It is similar to the seroepidemiology of IgA in Japan, which was around 18% to 24% (21). The prevalence of IgA antibody was higher in Taiwanese patients (80%) and in the Scottish Heart Health Study Cohort (57%). In the Helsinki Heart Study, IgA antibodies were found in 41% to 50% of the serum samples (17-19), which was still higher compared to our results.

Our results suggest that C. *pneumoniae* infection is not associated with angiographically documented coronary lesion which is similar to the results of Taiwanese patients and the Scottish Heart Health study Cohort (17-18). Most previous studies have

used the population controls instead of angiographically negative controls. One exception is the study from Taiwan which was methodologically similar to our study. Two other studies that used angiographically negative controls showed only a weak association between C. *pneumoniae* infection and CAD (20,22). Using angiographically negative controls is more valid because the cases would be more comparable to the controls, except for the presence of CAD.

The Scottish Heart Health study Cohort showed that the presence of antibodies to C. *pneumoniae* in serum at the time of initial screening was unable to predict subsequent coronary events (17). The Physicians Health Study found no association between IgG titers and coronary events (23). Wald *et al.* recently reported a large prospective study in which they found no case control difference in C. *pneumoniae* IgG and IgA concentrations (24). In a case-control study of C. *pneumoniae* and coronary heart disease carried out in women, no relationship was found between C. *pneumoniae* IgG titers and subsequent coronary events (23).

If the results of this study, and the other similar studies mentioned above, are considered together, it is clear that baseline C. *pneumoniae* antibody titers are of little use in predicting subsequent coronary events. IgG past infection and IgA current infection may be pertinent in primary respiratory infection but the relationship between serum antibody titers and a smoldering, intracellular, intra-atheromatous C. *pneumoniae* infection is uncertain. There are conflicting reports on the correlation between the organism detected in atheroma and serum antibody titers (7, 25). It is possible that the assay of circulating C. *pneumoniae* DNA may be of more use in estimating the infective load (26).

Most of the studies showing a positive association between C. *pneumoniae* infection and CAD have used the population controls instead of angiographically documented controls. The ARIC study found a univariate relationship between C. *pneumoniae* IgG titer and subsequent CAD (27). Caerphilly Prospective Heart Disease Study found a positive correlation between baseline C. *pneumoniae* IgA titers and fatal CAD (28) but there was no relationship between CAD and baseline C. *pneumoniae* IgG titers. In a case-control study based on the British Regional Heart Study population, the association between C. *pneumoniae* IgG titers and subsequent CAD was significant (29).

Mild lesions are reported to be positive for the presence of C. *pneumoniae* as are severe lesions (30). C. *pneumoniae* is reported to be a candidate for recent-onset activation of a smoldering inflammatory intraplaque process (5).

In several seroepidemiologic studies, C. *pneumoniae* seropositivity has been shown to be associated with several CAD risk factors, such as age, gender, smoking habits and atherogenic lipid profiles (31-32). These factors are regarded as possible confounders but in this study, there was not any association between these probable confounding factors and C. *pneumoniae* seropositivity. The seropositivity for IgG is higher in males as reported in Taiwanese patients (18).

Although several studies supported the presence of C. *pneumoniae* in diseased blood vessels and some successful antibiotic trials demonstrated that C. *pneumoniae* could modify or exacerbate atherosclerotic process, this study did not support this point of view. Maass *et al.* demonstrated that antibodies to C. *pneumoniae* were not related to the clinical course of unstable angina (25), showing that the microorganism could not promote the process of atherosclerosis (14). Similar to recently reported studies, our result did not show any relationship between markers of C. *pneumoniae* infection in baseline serum samples and subsequent CAD. The correlation, if exists, seems to be more complex. We can conclude that the high prevalence of C. *pneumoniae* infection in Southern Iran is not associated with angiographically documented CAD, and is not considered as a positive predictor for the development of acute coronary syndrome. More studies are needed to clarify the possible different effects of C. *pneumoniae* on atherosclerosis.

Acknowledgement

The authors would like to thank the Office of Vice Chancellor for Research for financial support of this project and Dr. Davood Mehrabani for his editorial assistance and Shiraz Taba Institute for statistical consultation.

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