

Association of Chlamydia Pneumoniae-Specific Antibody and Angiographically Demonstrated Coronary Artery Atherosclerosis in a Sample of Iranian Patient

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Abstract: Coronary heart disease remains a leading cause of morbidity and mortality and several risk factors have been shown that play role in its pathogenesis. Several studies suggested that there is an between Chlamydia pneumoniae and coronary artery disease as a curable etiology. The aim of this study was to evaluate the relationship between Chlamydia pneumoniae-specific antibodies and angiographically demonstrated coronary artery disease in Iranian patients. This study performed on 180 subjects, admitted for coronary artery angiography in Mazandaran Heart Center, Sari, Iran, in 2006. Patients divided in two case and control groups according to evidence of atherosclerosis in angiography. Both groups were matched from the viewpoints of age, gender, cigarette smoking, diabetes mellitus, dyslipidemia and hypertension. The presence of IgG and IgA antibodies to Chlamydia pneumoniae in patient's serum assessed with enzyme-linked immunosorbent assay. Out of 180 patients, 90 (27 men and 60 women) with mean age of 55.32 ± 10 years in case group and 90 patients (36 men and 64 women) with mean age 54.32 ± 10 years in control group examined. According to angiography in case group 86 cases (95.5%) had stenosis in Right Coronary artery, 63 cases (70%) in Left circumflex artery and 67 (74.4 %) in Left Anterior descending artery. In the case group, Chlamydia antibody was detected in 46 (51.1%) subjects whereas in control group in 34 (37.8%). The difference between 2 groups was significant ($p = 0.04$). Our study showed that Chlamydia pneumoniae-specific antibodies were associated with ischemic heart disease, which indicated Chlamydia pneumoniae may be has a role in pathogenesis of atherosclerosis.

Key words: Atherosclerosis, chlamydia pneumonia coronary artery disease

INTRODUCTION

Ischemic heart disease is known as the most common, serious, chronic and life-threatening disease among developed societies of the world and the most common cause of myocardial ischemia is coronary artery atherosclerosis (Libby *et al.*, 2005). It is estimated that cardiovascular diseases especially coronary atherosclerosis will have been the leading cause of mortality and early morbidity around the world until 2020 (Libby *et al.*, 2005). Up to now, there have been several studies over potential etiologic factors, diagnosis and treatment of atherosclerosis. A variety of causes, such as smoking, hypercholesteremia, low HDL, hypertension, diabetic mellitus, etc are known as independent risk factors in cardiovascular disease (Libby *et al.*, 2005). The risk factors can disturb normal function of vascular endothelial cells by interfering with the local control of vascular tension, disturbance in maintenance of suitable level of coagulant factors and defense against inflammatory

cells. Disturbance in vascular endothelial function associated with abnormal function of monocytes and platelets leading to aggregation of lipid, smooth muscle cells, fibroblasts, inflammatory cells and interstitial matrix and atherosclerosis plaque. After atherosclerosis plaque formation, diameter of vessels decreases to form tissue ischemia. However, the mentioned risk factors have not been efficient enough to predispose coronary artery insufficiency events. As a result, assessment of other risk factors seems necessary (Auer *et al.*, 2002). This fact, that inflammation is a main part of atheroma phenomenon and associated with activation and proliferation of macrophages, endothelial cells, smooth muscles, production of cytokines and growth factors and activation of complements, were considered by scientists. So that, they were interested to the role of inflammatory agents, especially microorganisms (Muhlestein anderson, 2003; Epstein *et al.*, 1999). Chlamydia pneumonia is one of the microorganisms that were highly focused by scientists (Schumacher *et al.*, 2001). Chlamydia is a gram negative,

obligatory intracellular parasite. It is a common cause of respiratory infections among children and adults. At first, theory of relationship between chlamydia pneumoniae and coronary artery diseases was discussed in 1988 (Saikku *et al.*, 1988). Afterwards, several studies were performed in the field of epidemiology (Grayston, 2000; Mendall *et al.*, 1995), pathology (Vink *et al.*, 2001; Maass *et al.*, 2000; Ericson *et al.*, 2000) and animal models (He *et al.*, 1999). The results obtained from various studies were different and elusive. Some researches were pointed to Chlamydia pneumoniae as completely accidental cause of the disease, whereas others reported it as an important high-risk predisposing factor (Schumacher *et al.*, 2001; Danesh *et al.*, 2000; Selzman *et al.*, 2003). Because of different results presented and mismatching between case and controls in most previous series, considering an actual relationship between Chlamydia pneumoniae infection and atherosclerotic plaques is impossible (Sander *et al.*, 2004; Jaremo *et al.*, 2004). Existence of IgA antibody on mucosal surface is an important humoral immune reaction against pathogens such as Chlamydia pneumoniae and represents active or recurrent infection. In the other hand, the presence of IgG show the previous contact of patient with pathogen (Vijayvergiya *et al.*, 2006). Consequently, in this study we evaluate the association and significance of Chlamydia pneumoniae infection in coronary artery atherosclerosis by measuring serum IgA and IgG in symptomatic patients referred to Mazandaran cardiac center of Sari/Iran in 2006. If there is a relationship between serology of Chlamydia pneumoniae and atherosclerosis, the results of this research will make a new field for further interventions such as vaccination and antibiotic therapy, in order to reduce severity of disease or recovery of the patients.

MATERIALS AND METHODS

This double-blind case-control study was performed on 180 symptomatic patients who referred to Mazandaran Cardiac Center of Sari for coronary artery angiography in 2006. A cardiologist did coronary angiography of the patients after 12 h of fasting. The Case group had coronary artery atherosclerosis that defined by presence of at least one stenosis with each degree in one of coronary artery (Aucer *et al.*, 2002). The control group was consisted of patients without coronary artery disease in angiography. Exclusion criteria were consisted of positive past history of malignancy, connective tissue disorders, immune system deficiency and using immunosuppressive drugs. Both of the case and control groups were matched from the viewpoints of age, gender, cigarette smoking, diabetes mellitus (FBS > 126 mg dL⁻¹

twice), dyslipidemia (total cholesterol > 240 mg dL⁻¹) and hypertension (Systolic Blood Pressure (SBP) > 140 mmHg and Diastolic Blood Pressure (DBP) > 90 mmHg). Severity of coronary artery atherosclerosis was determined according to number and degree of each involved vessel. Thus, 0-3 score for 0-3 vessels involvement and 0-3 score for degree of each vessel stenosis (0 without stenosis, 1 stenosis lower than 50% of vessel diameter, 2 stenosis between 50-75% of vessel diameter and 3 stenosis more than 75% of vessel diameter) were considered. Then, 3 mL of venous blood was taken from all the subjects regardless of fasting state. Immediately serum was separated from blood in the laboratory and then frozen under -20°C, after collection of specimens. Sera were defrosted under environmental temperature. Finally, presence of anti Chlamydia IgA and IgG in sera (medac kit, Germany) was assessed by using Enzyme-Linked Immunosorbent Assay (ELISA) Attention to presence of IgA anti Chlamydia pneumoniae can show recent infection and presence of IgG anti Chlamydia pneumoniae indicates of at least once involvement by the agent and remains for all the life (Liu *et al.*, 2005). In this study, presence of IgG and IgA or both of them were considered seropositive. Numeric data were presented by means of values±standard deviation. Spss (11) software was used to evaluate the differences between the case and control groups. P values lower than 0.05 were considered significant statistically.

RESULTS

One hundred eighty of the patients (90 case and 90 control) including 63 (35%) men, 117 (65%) women, with mean age 54.82±10 were assessed, that all of the cases were in age range of 21-77. Mean age of studied subjects were 55.32±10 in case group and 54.32±10 in control group. There was no statistical difference between 2 studied groups. On the bases of gender distribution, 36 (40%) men and 54 (60%) women were in case group and in control group 27 (30%) were men and 63 (70%) women, without significant statistical differences. About 35 (38.8%) patients in case group and 35 (38.8%) in control group had positive history of dyslipidemia. There was no significant statistical difference between two groups. Diabetes mellitus were seen in 29 patients (32.2%) in case group and 30 patients (33.3%) in control group. The difference between two groups was not significant. Six patients (6.6%) in case group and 9 patients (10%) in control group were smokers who did not have any significant statistical difference between them. Hypertension was seen in 40 (44.4%) of patients in case group and 44 (48.8%) patients in control group, which

Table 1: Prevalence of cases according to matching of intervening variables in two case and control studied groups

Variables	Control group (number = 90)	Case group (number = 90)	p value
Age(year)	10±54.32	10±55.32	0.1
sex:			
Female	63(70%)	54(60%)	
Male	27(30%)	36(40%)	
Diabetes	(3/33 %)30	(2/32 %)29	0/5
Dyslipidemia	(8/38 %)35	(8/38 %)35	0/5
Hypertension	(8/48 %)44	(4/44 %)40	0/3
Smokers	(10 %)9	(6/6 %)6	0/2

Table 2: Prevalence of cases according to Chlamydia pneumoniae serology in case and control studied groups

Serology test	Control group (number = 90)	Case group (number = 90)	p value
Seropositive	34 (8/37%)	46 (1/51%)	0/04
Seronegative	56 (2/62%)	44 (9/48%)	
Total	90 (100%)	90 (100%)	

means statistical difference between 2 groups were not significant (Table 1). We found anti Chlamydia pneumoniae immunoglobulin in 46 (51.1%) sera of case group (IgA or IgG or both) and in 44 (48.9%) were negative (IgA and IgG). Also in control group, 34 (37.8%) were seropositive and 56 (62.2%) seronegative. Statistical analysis showed a significant difference between two groups ($p = 0.04$) (Table 2).

Left Anterior Descending (LAD) coronary artery stenosis were seen in 86 (95.5%) of case group, with mean of severity of stenosis of $66.6 \pm 21\%$. Left Circumflex (LCX) coronary artery stenosis were observed in 63(70%) of case group with mean of severity of stenosis 44.9 ± 32 . Also 67 (74.4%) of Right Coronary Artery (RCA) stenosis with mean severity stenosis of 49.8 ± 32 were found. Statistical analysis presented that 18 (20%) of case group had one main vessel involvement, 18 (20%) with 2 vessels and 54 (60%) had all three main vessels obstruction. In case group, the mean of LAD coronary artery stenosis in seropositive patients were 67.39 ± 24.1 and 64.78 ± 18.5 in seronegative patients. As a result, there was not significant statistical difference between 2 groups. Also, the mean severity of stenosis of LCX coronary artery were 48.52 ± 32.6 in seropositive patients and 41.41 ± 32.1 in seronegative patients. This was not statistically significant, either. Furthermore, the RCA severity of stenosis was 55.33 ± 32.2 in seropositive patients and 44.85 ± 32.2 in seronegative patients. Statistical analysis did not show a significant difference between 2 groups (Table 3).

Among seropositive patients in case group, 10 (21.7%) cases had 1 involved vessel, 10 (21.7%) 2 vessels and 26 (56.6%) had three main vessels involvement. From seronegative patients, 8 (18.2%) had 1 involved vessel, 8 (18.2%) 2 involved vessels and 29 (63.6%) had three main vessels involvement. There was not significant statistical difference between two groups (Table 4).

Table 3: Mean and standard deviation of severity of coronary artery stenosis (%) in case group of two-studied group

	Seronegative (number = 44)	Seropositive (number = 46)	p value
Left Anterior Descending(LAD)	5/18±78/64	1/24±39/67	0/5
Left Circumflex (LCX)	1/32±41/41	6/32±52/48	0/3
Right Coronary Artery(RCA)	2/33±85/44	2/32±55	0/1

Table 4: Prevalence cases according to number of involved coronary artery vessels in case group of studied groups

	seronegative (number = 44)	seropositive (number= 46)	p value
One main vessel involvement	8(2/18%)	10 (7/21%)	
Two main vessels involvements	8(2/18%)	10 (7/21%)	0/7
Three main vessels involvements	28(6/63%)	26 (6/56%)	
Total	44 (100%)	46 (100%)	

DISCUSSION

Cardiac disease has known as the most common cause of mortality and morbidity in the world. Coronary artery atherosclerosis is considered as the main cause of cardiac disease. In recent decades, the hypothesis of influence of infectious factors on incidence and progression of atherosclerosis has been considered as the most interesting subject for research (Carratelli *et al.*, 2006). Based on this hypothesis, infectious agents can have a role in inducing inflammatory response, facilitating of cell death and necrosis during atherosclerosis (Blessing *et al.*, 2002). Furthermore, considering high incidence of Chlamydia pneumoniae infection and the induced role of bacterial infection on platelet activity, it seems that this organism can make a role in both early phase (proliferate) or lateness phase (vascular thrombotic stenosis) (Kalvegren *et al.*, 2003). Related studies have presented different results, as in several studies the role of one or more infectious agents of producing atherosclerosis were clearly determined (Saikku *et al.*, 1988; Sessa *et al.*, 1999; Kaski *et al.*, 1999). However, some studies could not prove this relationship (Danesh *et al.*, 2000; Sessa *et al.*, 1999; Hoffmeister *et al.*, 2000; Ridker *et al.*, 1999). Besides, a set of studies represented that antibiotic therapy for Chlamydia pneumoniae can influence on the incidence of coronary artery disease (Gupta *et al.*, 1997; Gurfinkel *et al.*, 1997).

Results of the recent study were shown that mean serum level of anti Chlamydia pneumoniae IgA and IgG immunoglobulin in case group were more than control group. Presence of anti Chlamydia pneumoniae IgA indicated recent infection of the disease and anti Chlamydia pneumoniae IgG indicated at least once acquiring of the infection in life and will remain in the

body until lifetime, Therefore, in this study, Presence of IgA and/or IgG were considered seropositive. Entirely, without considering acute or chronic infection, coronary artery disease patients had more Chlamydia pneumoniae infection and more serum immunoglobulin level.

In a study by Danesh *et al.* (2000) association of coronary artery disease with chronic Chlamydia pneumoniae infection symptoms were assessed. Their results did not show a significant relationship between Chlamydia pneumoniae IgG titer and onset of cardiovascular disease (Danesh *et al.*, 2000). Besides, in the controlled study on a group of coronary artery disease patients, in order to determine the relationship between the disease and Chlamydia pneumoniae infection, reported that there was not significant relationship between serum levels of IgG and IgA and ischemic heart disease (Wald *et al.*, 2000). This study was performed only on men. All patients divided in 2 case and control groups according to evidence of anti Chlamydia pneumoniae antibody. Death as a consequent of atherosclerotic vascular disease was assessed in the patients prospectively. Difference in the results of these studies with ours may be due to difference in studied population, number of assessed cases and method of selection the control group, or gender distribution of the patients.

In a study by Jaremo and Richter (2004) had presented that the more serum level of IgG, the more coronary artery involvement (Jaremo and Richter, 2004). In this study, patients divided in two case and control groups according to high or low level IgG and rate of coronary artery involvement. Difference between the results of this study with ours may be due to different method of study. Carratelli *et al* (2006) were studied the relationship between coronary artery disease and inflammatory factors. They reported that serum level of anti Chlamydia pneumoniae IgG and IgA were higher in patients with cardiac disorders (Carratelli *et al.*, 2006). They divided patients in 2 case (60 patients) and control (20 patients) groups. Sampling method for case and control groups was similar to our study, but the 2 groups were not matched for intervening variables such as gender, diabetes, hypertension and dyslipidemia. Because of intervening variables, the relationship between Seropositive Chlamydia pneumoniae and incidence of atherosclerotic disease is not a direct association (Carratelli *et al.*, 2006). In our study both case and control groups, were matched and controlled completely from the viewpoints of direct associations such as age, gender, past history of diabetes mellitus, dyslipidemia, hypertension and cigarette smoking and there was an

association between anti Chlamydia pneumoniae seropositivity and incidence of coronary atherosclerosis. This topic can make a new field for further interventions such as vaccination and antibiotic therapy, in order to reduce of disease or recovery of the patients. Therefore, further more complete and comprehensive studies with more cases will consider necessary.

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