

The Prevalence of *Chlamydia pneumoniae* Antibodies in Coronary Artery Diseases

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Abstract

Blood samples were obtained from 243 consecutive coronary heart disease (CAD) patients (177 male, 66 female) aged 35–78 years (mean, 61 years) admitted for coronary angiography because of suspected coronary heart disease and from 115 blood donors (91 male, 24 female) aged 25–60 years (mean, 47 years). Of the 243 CAD patients, 179 (74%) were identified by ELISA method for *Chlamydia pneumoniae* antibodies IgG and 132 (54%) were positive for *C. pneumoniae* IgA. Among the 115 blood donors, 80 (70%) were positive for *C. pneumoniae* IgG and 49 (43%) were positive for *C. pneumoniae* IgA. When IgG antibodies were concerned, there was not significant difference between CAD patients and healthy controls (OR = 1.29, 95% CI = 0.79–2.10, $p > 0.05$). In case of positive IgA antibodies alone, there was significant difference between CAD patients and healthy controls (OR = 1.58, 95% CI = 1.01–2.4, $p = 0.029$). If IgG and IgA antibodies were positive, OR was increased from 1.58 to 1.80 (95% CI = 0.97–3.36, $p = 0.044$). In conclusion, *C. pneumoniae* infection was common in Thai people but chronic *C. pneumoniae* infection was more common in CAD patients than in middle-aged blood donors. (*J Infect Dis Antimicrob Agents* 2000;17: 1-6.)

INTRODUCTION

Atherosclerosis, with its major manifestation of coronary artery disease (CAD), is the major cause of morbidity and mortality in Thai population. Several risk factors for coronary heart disease have been well documented, including hyperlipidemia, hypertension, smoking, diabetes mellitus, positive family history for

CAD, obesity and inactivity. However the traditional risk factors do not explain the development of atherosclerosis in a significant number of patients. Particularly, the high prevalence of CAD in developing countries is associated with a relatively low frequency of smoking and normal or low total cholesterol levels.^{1,2} Several new risk factors, such as homo-

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cysteinemia, elevated plasma levels of lipoprotein (a) [Lp(a)] and chronic infection are being described in the pathogenesis of coronary arteriosclerosis.³⁻⁵

Chlamydia pneumoniae is one of the new, emerging infectious agents, with a spectrum of clinical manifestations. It has recently been tentatively linked to the presence of atherosclerosis.⁶⁻⁹ This obligate intracellular bacterium is characterized by its extraordinarily high seroepidemiologic prevalence which indicates that virtually everyone becomes infected at least once during their lifetime.⁸ Approximately 50 percent of adults from many areas of the world possess *C. pneumoniae* antibodies.¹⁴⁻¹⁹ The antibodies are infrequent in children younger than 5 years but increase rapidly up to 15 years of age, and then increase more slowly throughout the rest of life. The prevalence of IgG antibodies reaches up to 70 percent in older adults. The prevalence is higher in men than that in women. Infection with *C. pneumoniae* is usually mild or asymptomatic, but it can be severe, especially in the elderly.

Since *C. pneumoniae* is difficult to cultivate in a cell culture system, PCR assay, microimmunofluorescence (MIF) test, ELISA and direct immunofluorescence test have been used to describe the infection caused by *C. pneumoniae*. Little is known about the prevalence of *C. pneumoniae* antibodies in healthy individuals and the association between *C. pneumoniae* and coronary artery disease (CAD) in Thailand. This study aimed to evaluate the prevalence of *C. pneumoniae* antibodies in healthy individuals and CAD patients by an ELISA method.

MATERIALS AND METHODS

Study populations

We studied 243 consecutive CAD patients who attended Her Majesty's Cardiac Center at Siriraj Hospital; 177 were men and 66 were women. These patients comprised of 24 acute myocardial infarction (AMI), 78 unstable angina pectoris and 141 stable angina pectoris. Coronary arteries lesions were angiographically verified in all cases. One hundred and fifteen blood donors were used as control subjects. They were excluded if previous evidence of coronary artery disease was obtained by history taking. The control subjects comprised of 91 men and 24 women.

Antibodies to *Chlamydia pneumoniae*

An ELISA method (SeroCP IgG, IgA and IgM tests: Savyon Diagnostics, Ashdod, Israel) was used to measure *C. pneumoniae* antibodies in CAD patients and control group. Fifty μL of positive control, 50 μL of 1:105 diluted specimens and 3 x 50 μL of negative control were added to the microtiter strips which are coated with intact *C. pneumoniae* elementary bodies. The ELISA plate was covered and incubated at 37°C for one hour in 100 percent humidity. After washing 3 times with a buffer, 50 μL of 1:300 diluted horseradish peroxidase (HRP) were added and conjugated with anti-human immunoglobulin. The plate was covered and incubated again for one hour at 37°C in 100 percent humidity. After washing 3 times with the buffer, 100 μL of tetramethylbenzidine (TMB) substrate was added and the plate was incubated at room temperature for 15 minutes. Then the reaction was stopped by adding 100 μL of 1 M H_2SO_4 . The strip was measured at 450 nm for which positive control absorbance value should be ≥ 1.00 and the average absorbance value of negative control should be > 0.10 and ≤ 0.40 .

Coronary angiography

Coronary angiography was performed on 243 CAD patients. Of those 243, 200 had at least 1 diseased coronary artery, defined as a reduction of the vessel diameter of at least 50 percent.

Statistical analysis

Mean values or proportions for baseline risk factors were calculated for CAD patients and control subjects. A difference in mean values was tested with the Student *t*-test. Continuous values were expressed as mean \pm standard deviation (SD). Epidemiological evaluation of our population was performed with Epi Info Dos 6.04 (Center for Disease Control, Atlanta, Ga.) with 2 x 2 table and related χ^2 with the Yates correction. Fisher's exact two-tailed test was used for cases with expected cell values less than five. The *p* value of less than 0.05 was regarded as significant level.

RESULTS

The demographic data, lipid profile, creatine kinase (CK) and CK-MB were presented in Table 1. The mean age of healthy control was less than that of

Table 1. Demographic data, lipid profiles, CK, CK-MB and coronary angiography of CAD patients and healthy controls.

	Healthy controls (n=115)	CAD patients (n=243)	P value
Age, year	47 ± 10	61 ± 10	< 0.001
Mean, percent	76	73	NS
Cholesterol (mg/dL)	203.9 ± 36.9	208.8 ± 48.5	NS
Triglyceride (mg/dL)	184.2 ± 123.5	152.2 ± 76.6	< 0.01
LDL-C (mg/dL)	120.7 ± 36.7	138.3 ± 43.1	< 0.001
HDL-C (mg/dL)	46.3 ± 11.5	39.1 ± 10.7	< 0.001
CK (U/L)	120.3 ± 81.9	119.2 ± 250.9	NS
CK-MB (U/L)	16.9 ± 7.3	32.5 ± 24.8	NS
Coronary angiography (%)	ND	82	ND

Data presented are mean value ± SD. CAD = coronary artery diseases; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; CK = creatine kinase; CK-MB = creatine kinase MB; NS = no significant difference; ND = not determined.

CAD patients because in Thailand, blood donors were accepted only if they were less than 60 years old. The triglyceride, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were significantly different between healthy controls and CAD patients. This finding confirms the basic knowledge that in CAD patients LDL-C was a major risk factor for atherosclerosis, on the other hand HDL-C in healthy controls was significantly higher than the CAD patients. The CK and CK-MB were not significantly different between the two groups because the majority of CAD patients were diagnosed as unstable angina and stable angina. Coronary angiography was performed on 243 patients. Two hundred of the 243 patients (82%) had at least one diseased coronary artery as defined by a reduction of the vessel diameter of at least 50 percent.

Table 2. *Chlamydia pneumoniae* antibodies in healthy controls and CAD patients by age.

Antibodies	Age (years)	Healthy controls (%)	CAD patients (%)
IgG	21 - 40	22/40 (55)	4/5 (80)
	41 - 60	48/64 (75)	69/98 (70)
	61 - 80	10/11 (91)	105/140 (75)
	total	80/115 (70)	179/243 (74)
IgA	21 - 40	17/40 (44)	4/5 (80)
	41 - 60	24/64 (36)	44/98 (45)
	61 - 80	8/11 (67)	84/140 (60)
	total	49/115 (43)	132/243 (54)

The age-related prevalence of *C. pneumoniae* antibodies of healthy individuals and CAD patients were shown in Table 2. The antibody detection rate in healthy controls was 70 percent for IgG and 43 percent for IgA. The presence of the antibody in CAD patients was 74 percent for IgG and 54 percent for IgA. There were three cases (3%) of recent infection with *C. pneumoniae* in healthy individuals, as determined by IgM antibody. In CAD patients there was only one case (0.4%) positive for IgM antibody. *C. pneumoniae* IgG antibody was present in 72 percent of male and 63 percent of female. For IgA antibody it was present in 48 percent of male and 41 percent of female in healthy controls. In CAD patients, the IgG antibody was presented in 74 percent of male and 68 percent of female. For the IgA it was presented in 56 percent of male and 44 percent of female. The odd ratio (OR) and 95 percent confidence interval (CI) of *C. pneumoniae* antibody in 243 CAD patients compared with 115 healthy controls was shown in Table 3. Since the antibodies to *C. pneumoniae* increased in parallel with the increasing age of population, age and sex of CAD patients were matched with healthy controls to reduce the effect of age and sex on *C. pneumoniae* antibody. Table 4 showed *C. pneumoniae* antibody of 63 cases

Table 3. *Chlamydia pneumoniae* antibodies of 243 CAD patients compared to 115 healthy controls.

Specific antibodies	Healthy controls (n=115)	CAD patients (n=243)	OR (95% CI)	P value
IgG (%)	70	74	1.29 (0.79 - 2.10)	NS
IgA (%)	43	54	1.58 (1.01 - 2.47)	0.029
IgG and IgA (%)	62	75	1.80 (0.97 - 3.36)	0.044

Table 4. *Chlamydia pneumoniae* antibodies in 63 CAD patients and 63 healthy controls which were matched for age and sex.

Specific antibodies	Healthy controls (n=63)	CAD patients (n=63)	OR (95% CI)	P value
IgG (%)	70	81	1.84 (0.80 - 4.20)	NS
IgA (%)	38	65	3.03 (1.47 - 6.26)	0.002
IgG and IgA (%)	29	54	2.93 (1.40 - 6.13)	0.003

whose age and sex of CAD patients were matched with healthy controls. The results were expressed as positive percentage, odd ratio and 95 percent confidence interval.

DISCUSSION

Known risk factors of CAD account for only 50-70 percent of the incidence of cardiovascular disease.²⁰⁻²² The search for additional risk factors in CAD had led to the re-evaluation of the role of infection in the development of atherosclerosis. Strong evidence, including the direct demonstration of the organism in atherosclerotic plaques and the results from seroepidemiological studies, now exists to link *C. pneumoniae* infection with CAD.^{23,24} Most of these seroepidemiological studies detected *C. pneumoniae* antibodies by MIF which has to be interpreted by experienced microscopists and poor reproducibility might be encountered.²⁵ Our study used ELISA method that was done by automated instrument and the strips were coated with intact *C. pneumoniae* elementary bodies as used in MIF method. In our study, the antibody was detected in 70 percent of healthy controls compared with 74 percent of CAD patients for IgG. The prevalence rate was similar to previous reports by other investigators.^{16-19,26,27} The IgG antibody to *C. pneumoniae* was not protective but useful for prevalence study. There was not significant difference between healthy controls and CAD patients for the rate of IgG detection. The marker for chronic *C. pneumoniae* infection as presented by circulating IgA, a short lived immunoglobulin with half life of 5.8 days, has also been examined in our study. We found that the prevalence of IgA antibody in CAD patients was higher than healthy controls [24% vs 43 %, OR = 1.58 (1.01-2.47), p = 0.029]. When we combined positive results for IgG and IgA, the OR increased from 1.58 to 1.80 (95 % CI 0.97-3.36,

p = 0.044). Our result showed the similar positive rate of IgA as reported by Mazzoli et al and del Piano et al in the year 1998 and 1995 respectively.^{28,29} Circulating specific IgA is undoubtedly a marker of the presence of *C. pneumoniae* and it may indicate that the infections is chronic, hence its presence implies that the antigen is still present.

When we matched 63 CAD patients and 63 healthy controls by age and sex, the ORs of positive IgA alone or both the IgG and IgA increased from 1.58 to 3.03 and 1.80 to 2.93 respectively (Table 4). This implies that we can use the positive of IgA alone or IgG and IgA for detection of chronic infection by *C. pneumoniae* especially in coronary heart disease. Only three percent of the cases were positive for the IgM antibody. Hence IgM antibodies are rarely produced in reinfections due to *C. pneumoniae*. The reports from Korea, Italy and Finland of IgM antibodies for *C. pneumoniae* were 6, 12 and 11 percent respectively.^{26,28,60} Our result confirms that acute infection by *C. pneumoniae* is extremely rare in adult because approximately 50 percent of them have been infected at the age of 20.³¹

In this hospital-based study, *C. pneumoniae* was found to be highly prevalent in Thai people, as it is in western countries. Teenagers were found to have a peak of primary infections, while the older people had a peak of reinfection. High prevalence of IgG antibodies to *C. pneumoniae* seems to be common both in CAD patients and healthy controls but prevalence of IgA is more common in CAD patients than healthy controls. The presence of IgG or IgA antibodies does not prove that a person is prone to be coronary heart disease but indicates a chronic infection and inflammation as confirmed by Gabriel et al.³²

In conclusion, the prevalence of IgG and IgA antibodies, as determined by ELISA method, seems to be common in Thai population. The sustained IgG

and IgA antibodies against *C. pneumoniae* in CAD patients suggest that chronic infection may be frequent after an initial infection. The site of colonization for chronic *C. pneumoniae* infection may be in the alveolar macrophages of the lung. Further studies at a cellular level and prospective epidemiological studies are warranted. If causal association are confirmed, there would be important implications for the management and eventual prevention of CAD. The use of specific antibodies could be implemented both to treat and prevent a common infectious agent, thereby reducing the incidence of a common life-threatening disease.

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