The Prevalence of *Chlamydia pneumoniae* Antibodies in Thai Patients with Coronary Artery Disease

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Abstract

Blood samples were obtained from 243 consecutive coronary artery 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 positive by an 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 considered, there was no significant difference between CAD patients and healthy controls (OR = 1.29, 95% CI = 0.79-2.10, p>0.05). In cases of positive IgA antibodies alone, there was a significant difference between CAD patients and healthy controls (OR = 1.58, 95% CI = 1.01-2.4, p = 0.029). If both 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 is common in Thai people but chronic *C. pneumoniae* infection is more common in CAD patients than in middle-aged blood donors.

Key word : Prevalence, *C. Pneumoniae*, CAD

Atherosclerosis, with its major manifestation of coronary artery disease (CAD), is the major cause of morbidity and mortality in the Thai population. Several risk factors for coronary heart disease have been well documented, including hyperlipidemia, hypertension, smoking, diabetes mellitus, positive family history for CAD and obesity. However, the traditional risk factors do not explain the development of atherosclerosis in a significant number of patients. In particular, the high

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

Chlamydia pneumoniae is one of the new, emerging infectious agents, with a spectrum of clinical manifestations. It has been tentatively linked to the presence of atherosclerosis[4]. 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[4]. Approximately 50 per cent of adults from many areas of the world possess *C. pneumoniae* antibodies[5,6]. The antibodies are infrequent in children younger than 5 years old 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 70 per cent in older adults. The prevalence is higher in men than 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) tests, ELISA and direct immunofluorescence tests 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.

MATERIAL AND METHOD

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 24 acute myocardial infarction (AMI), 78 unstable angina pectoris and 141 stable angina pectoris. Coronary artery lesions were angiographically verified in all cases. One hundred and fifteen blood donors, none more than 60 years old, were used as control subjects. They were excluded if previous evidence of coronary heart disease was obtained by history taking. The control subjects comprised 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 controls. Fifty μL of positive control, 50 μL of 1:105 diluted specimens and 3 of 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 per cent humidity. After washing 3 times with a buffer, 50 μL of 1:300 diluted horseradish peroxidase (HRP) was added and conjugated with anti-human immunoglobulin. The plate was covered and incubated again for one hour at 37°C in 100 per cent 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 then100 μL of 1 M H_2SO_4 was added to stop the reaction. The strips were measured at 450 nm for which the positive control absorbance value was $\geq$ 1.00 and the average absorbance value of negative control was $>$ 0.10 and $\leq$ 0.40.

Coronary angiography

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

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 ±standard deviation (SD). Epidemiological evaluation of our population was performed with Epi Info Dos 6.04 (Center for Disease Control, Atlanta, Ga.) with 2x2 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. A p value of less than 0.05 was regarded as a significant level.

RESULTS

The demographic data, lipid profile, creatine kinase (CK) and CK-MB results are presented in Table 1. The mean age of healthy controls was less than that of CAD patients because in Thailand blood donors are accepted only if they are less than 60 years old. The triglyceride, low-density lipoprotein cholesterol (LDL-C) and high density lipopro-

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

	Healthy controls (n = 115)	CAD patients (n = 243)	p value
Age, y	47 ± 10	61 ± 10	< 0.001
Men,%	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	12.5 ± 24.8	NS
Coronary angiograph (%)	ND	82*	NS

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. * 200/243 (82%) had at least one disease coronary artery.

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	58/75 (77)	69/98 (70)
	61-80	-	105/140 (75)
	total	80/115 (70)	179/243 (74)
IgA	21-40	17/40 (44)	4/5 (80)
	41-60	32/75 (43)	44/98 (45)
	61-80	-	84/140 (60)
	total	49/115 (43)	132/243 (54)

tein cholesterol (HDL-C) was significantly different between healthy controls and CAD patients. On the other hand, HDL-C in healthy controls was significantly higher than in 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 per cent.

The age-related prevalence of *C. pneumonia* antibodies of healthy individuals and CAD patients is shown in Table 2. The antibody detection rate in healthy controls was 70 per cent for IgG and 43 per cent for IgA. The presence of the antibody in CAD patients was 74 per cent for IgG and 54 per cent for IgA. There were three cases (3%) of recent infection with *C. pneumonia* in healthy individuals, as determined by IgM antibody. In CAD patients only one case (0.4%) was positive for IgM antibody. *C. pneumoniae* IgG antibody was present in 72 per cent of males and 63 per cent of females. For IgA antibody it was present in 48 per cent of males and 41 per cent of females in the healthy control group. In CAD patients, the IgG antibody was present in 74 per cent of males and 68 per cent of females. For the IgA it was present in 56 per cent of males and 44 per cent of females. The odd ratio (OR) and 95 per cent confidence interval (CI) of *C. pneumoniae* antibody in 243 CAD patients compared with 115 healthy controls is shown in Table 3. Since the antibodies to *C. pneumoniae* increased in parallel

Table 3. *Chlamydia pneumoniae* antibodies in 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

with the increasing age of the 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 shows *C. pneumoniae* antibody in 63 cases where the age and sex of the CAD patients were matched with healthy controls. The results were expressed as positive percentage, odd ratio and 95 per cent confidence interval.

DISCUSSION

Known risk factors of CAD account for only 50-70 per cent of the incidence of cardiovascular disease[7,8]. The search for additional risk factors in CAD have 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. 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[9]. Our study used an ELISA method carried out with an automated instrument. The strips were coated with intact *C. pneumoniae* elementary bodies as used in the MIF method. In our study, the antibody was detected in 70 per cent of healthy controls compared with 74 per cent of CAD patients for IgG. The prevalence rate was similar to previous reports by other investigators[5,6,10]. The marker for chronic *C. pneumoniae* infection as presented by circulating IgA, a short lived immunoglobulin with a half life of 5.8 days, was also examined in our study. We found that the prevalence of IgA antibody in CAD patients was higher than in 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 results showed a similar positive rate for IgA as reported by Mazzoli et al and del Piano et al in 1998 and 1995 respectively[11,12]. Circulating specific IgA is undoubtedly a marker of the presence of *C. pneumoniae* and it may indicate that the infection 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 a positive IgA alone or a positive IgG and IgA are valuable for detection of chronic infection by *C. pneumoniae* especially in coronary heart disease. Only three per cent of cases were positive for the IgM antibody. Hence, IgM antibodies are rarely produced in reinfections due to *C. pneumoniae*. Reports from Korea, Italy and Finland indicated that IgM antibodies for *C. pneu-*

moniae were present is 6, 12 and 11 per cent of cases respectively. Our result confirms that acute infection by *C. pneumoniae* is extremely rare in adults because approximately 50 per cent of them have been infected by the age of 20.

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 infection, while 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 in healthy controls. The presence of IgG or IgA antibodies does not prove that a person is prone to develop coronary heart disease but indicates a chronic infection.

In conclusion, the prevalence of IgG and IgA antibodies, as determined by an ELISA method, seems to be common in the Thai population. The sustained IgG and IgA antibodies against *C. pneumoniae* in CAD patients suggest that chronic infection may be more frequent in these patients. The site of *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 is 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 this life-threatening disease.

ACKNOWLEDGMENT

The authors wish to thank Rapport Company Limited for reagent support and Dr. Bob Cowan for critical reading and comments.

(Received for publication on February 11, 1999)

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อุบัติการณ์การติดเชื้อ *คลามีเดีย นิวโมนิเอ* ในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่

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คณะผู้วิจัยได้ทำการศึกษาผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่จำนวน 243 ราย (ชาย 177 ราย, หญิง 66 ราย) อายุระหว่าง 35–78 ปี (เฉลี่ย 61 ปี) ที่รับตัวไว้ในโรงพยาบาลเพื่อทำการตรวจ coronary angiography เปรียบเทียบกับผู้บริจาคโลหิตจำนวน 115 ราย (ชาย 91 ราย, หญิง 24 ราย) อายุระหว่าง 25–60 ปี (เฉลี่ย 47 ปี) พบว่า IgG antibodies ต่อเชื้อ*คลามีเดีย นิวโมนิเอ* ที่ตรวจโดยวิธี ELISA มีถึง 74 เปอร์เซ็นต์ (179/243) ในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ ส่วนในผู้บริจาคโลหิตพบ 70 เปอร์เซ็นต์ (80/115) สำหรับ IgA antibodies พบในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ 54 เปอร์เซ็นต์ (132/243) และพบในผู้บริจาคโลหิตเพียง 43 เปอร์เซ็นต์ (49/115) ในกรณีของ IgG antibodies พบว่าไม่มีความแตกต่างกันทางสถิติระหว่าง 2 กลุ่ม แต่กรณี IgA antibodies พบสูงกว่าอย่างมีนัยสำคัญทางสถิติในกลุ่มผู้ป่วยโรคหลอดเลือดแดงโคโรนารี (OR = 1.58, 95% CI = 1.01–2.40, p = 0.029) ยิ่งในกรณีที่ IgG และ IgA antibodies เพิ่มสูงขึ้นทั้ง 2 ชนิด ยิ่งมีความแตกต่างระหว่าง 2 กลุ่มอย่างเห็นได้ชัดมากขึ้น (OR = 1.80, 95% CI = 0.97–3.36, p = 0.044) โดยสรุปพบว่าการติดเชื้อ *Chlamydia pneumoniae* ในคนไทยพบได้บ่อยไม่แตกต่างกับในประเทศอื่น ๆ ทั่วโลก และพบการติดเชื้อนี้ในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่บ่อยกว่าในผู้บริจาคโลหิต

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