Chlamydia pneumoniae Antibodies and Angiographically Demonstrated Coronary Artery Disease in Thailand

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Abstract

Recent reports have suggested an association between *Chlamydia pneumoniae* and coronary artery disease. This study investigated the relationship between the presence of immunoglobulin G (IgG), immunoglobulin A (IgA) of *C. pneumoniae* in angiographically diagnosed coronary disease. Patients enrolled were 243 (178 male, 65 female, mean age 61±10 years) with angiographically proven at least one significant coronary artery stenosis. Fifty-eight patients (33 male, 25 female, mean age 57±11 years) with no angiographic evidence of coronary lesions were used as the normal coronary angiogram group. Control subjects (95 male, 92 female, mean age 58±17 years) were used as normal healthy persons who had no history of coronary artery disease. *C. pneumoniae* IgG and IgA antibodies were measured by ELISA method. We found that 179 out of 243 (73.7%) coronary artery disease (CAD) patients were positive for IgG and 132 out of 243 (54.3%) were positive for IgA. In 58 normal coronary angiogram patients, 23 (39.7%) cases were positive for IgG and 6 (10.3%) cases were positive for IgA. Among 187 healthy controls, 111 (59.4%) cases were positive for IgG and 83 (44.4%) were positive for IgA. When *C. pneumoniae* IgG antibodies were considered, there was significant difference between CAD patients and healthy controls (OR = 1.91, 95% CI = 1.27 – 2.88, p = 0.0018). In cases of positive IgA antibodies, significant difference was also found between CAD patients and healthy controls (OR = 1.49, 95% CI = 1.02 – 2.19, p = 0.0257). These findings were also found with higher odds ratio when we compared between CAD patients and normal coronary angiogram patients. The result suggested that *C. pneumoniae* infection is common in Thai people and chronic *C. pneumoniae* infection is more common in CAD patients. Chronic *C. pneumoniae* infection may be associated with the development of atherosclerotic coronary disease and treatment with antibiotics should be considered in ischemic heart disease.

Key word : Chlamydia Pneumoniae Antibodies, CAD, Coronary Angiography

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Since the first report of increased concentration of IgG and IgA antibodies to *Chlamydia pneumoniae* in patients with acute myocardial infarction or chronic coronary heart disease, evidence has accumulated an association between serological markers of this infection and clinically significant atheroma or manifestations of ischemic heart disease[1,2]. Atherosclerosis is considered to be the most frequent pathologic basis for coronary disease[3]. Although numerous independent risk factors for atherosclerosis have been identified, these factors accounted for the majority, but not for the entire observed cases. Furthermore, detection of DNA by polymerase chain reaction (PCR) and detection of antigen by immunocytochemistry (ICC), as well as other techniques such as electron microscopy and bacterial cultivation, have shown evidence of the presence of *C. pneumoniae* in atherosclerotic blood vessels[4-6]. In a recent systematic review, the organism was found in 52 per cent of diseased but only 5 per cent of normal blood vessels[7]. One of these serological and pathological findings was that *C. pneumoniae* has a direct causal role on atherosclerosis.

Investigation of this association has been performed mostly in the U.S. and Europe, while contribution from Asian countries has been scarce. Furthermore, although a considerable amount of seroepidemiological research has been carried out, it has only rarely involved angiographically demonstrated cases. The objective of this study was to evaluate the association between IgG and IgA anti-*C. pneumoniae* antibodies and angiographically demonstrated coronary disease by means of a case-control study.

MATERIAL AND METHOD

Study populations

A total of 488 subjects were determined for presence of *C. pneumoniae* antibodies. The first group of subjects consisted of 243 patients (178 men, 65 women with a mean age of 61±10 years) who displayed a stenotic lesion of larger than 50 per cent in at least one principal coronary artery. The second group consisted of 58 subjects with other diseases (33 men, 25 women with a mean age of 57±11 years) who had no angiographic evidence of coronary lesions. The third group consisted of 187 normal healthy subjects (95 men, 92 women with a mean age of 58±17 years) who had no history of heart disease.

Assay for antibodies to *C. pneumoniae*

C. pneumoniae antibodies were measured by an ELISA method in a microtiter plate (SeroCP IgG, IgA tests: Savyon Diagnostics, Ashdod, Israel). In brief, a 50 μL of positive control, 50 μL of 1:105 diluted specimens and triplicate of 50 μL of negative control were added to the wells that were 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) conjugated anti-human immunoglobulin was added. 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 solution was added, and the plate was incubated at room temperature for 15 minutes before adding 100 μL of 1 M H_2SO_4 to stop the reaction. Color of the test reaction was measured by the spectrophotometer at the wavelength of 450 nm of which the positive control showed an absorbance value of $\geq$ 1.00, and the average absorbance value of the negative controls were between $>$ 0.10 and $\leq$ 0.40.

Statistical analysis

Mean values or proportions of baseline risk factors were calculated for coronary artery disease (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 EpiInfo version 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 significant level.

RESULTS

The demographic data, conventional risk factors and lipid profiles are shown in Table 1. The prevalence of anti *C. pneumoniae* IgG antibodies was 73.7 per cent among CAD patients, 39.7 per cent among the patients with normal coronary angiogram (CAG) and 59.4 per cent among the normal healthy controls. The prevalence of positive *C. pneumoniae* IgA antibodies was 54.3 per cent among CAD patients, 10.3 per cent among the

Table 1. Demographic data, risk factors and lipid profiles of CAD patients, normal coronary angiogram (CAG) patients and controls.

	CAD patients (n = 243)	Normal CAG patients (n = 58)	Healthy controls (n = 187)
Age, years	61 ± 10	57 ± 11	58 ± 17
Men, %	73	55	51
Diabetes mellitus, %	36	26	27
Hypertension, %	49	38	28
Smoking, %	46	24	39
Total cholesterol (mg/dL)	209 ± 23	192 ± 42	213 ± 43
Triglycerides (mg/dL)	153 ± 76	111 ± 55	169 ± 108
HDL-cholesterol (mg/dL)	39 ± 11	47 ± 18	46 ± 11
LDL- cholesterol (mg/dL)	139 ± 42	123 ± 36	133 ± 41

Table 2. The odd ratio (OR) and 95% confidence interval (CI) of anti-*C. pneumoniae* IgG and IgA antibodies among CAD patients, patients with normal CAG and healthy controls.

	Normal CAG patients (n = 58)	CAD patients (n = 243)	Healthy controls (n = 187)
IgG (%)	23/58 (39.7)	179/243 (73.7)	111/187 (59.4)
OR (95%CI)	4.26 (2.34 – 7.74)	1.91 (1.27 – 2.88)	
P-value	<0.0001	0.0018	
IgA (%)	6/58 (10.3)	132/243 (54.3)	83/187 (44.4)
OR (95%CI)	10.31 (4.27 – 24.90)	1.49 (1.02 – 2.19)	
P-value	<0.0001	0.0257	

patients with normal CAG and 44.4 per cent in the normal healthy controls. In case of positive IgG antibodies, the odds ratio of CAD patients compared with healthy controls was 1.91, 95 per cent CI = 1.27 – 2.88, p = 0.0018. When we compared CAD patients and normal CAG patients, the odds ratio was 4.26, 95 per cent CI = 2.34 – 7.74, p < 0.0001. When positive IgA was considered, the odds ratio of CAD patients compared with healthy controls was 1.49, 95 per cent CI = 1.02 – 2.19, p = 0.0257. When we compared CAD patients and normal angiogram patients, the odds ratio was 10.31, 95 per cent CI = 4.27 – 24.90, p < 0.0001. (Table 2) The seroprevalence of anti-*C. pneumoniae* IgG and IgA antibodies among CAD patients, patients with normal CAG and healthy controls is shown in Fig. 1.

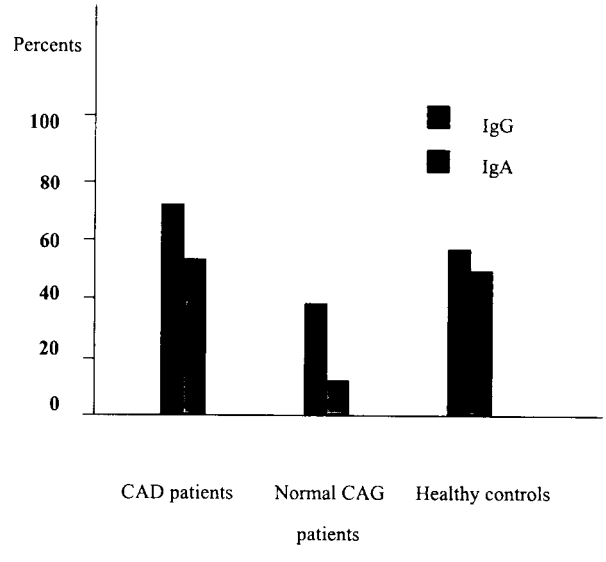

Fig. 1. The seroprevalence of anti-*C. pneumoniae* IgG and IgA antibodies among CAD patients, patients with normal CAG and healthy controls.

DISCUSSION

Given the increasing evidence of an association between *C. pneumoniae* and cardiovascular

diseases, it has become mandatory to define a method for the identification of long-term *C. pneumoniae* carriers. Traditionally, micro-immunofluorescence (MIF) has been considered the diagnostic "gold standard method" for anti-*C. pneumoniae* testing. The limitation of MIF is the cut-off titer for positivity which varies with laboratories, and interpretation of the test needs experienced microscopists to decide which one is a positive or a negative result, so poor reproducibility might be encountered[8]. To solve this problem, we used an ELISA method employing the intact *C. pneumoniae* elementary bodies as the test antigen and the test system was carried out in an automated instrument. Little is known about the prevalence of *C. pneumoniae* antibodies in Thai patients with CAD. Our earlier work showing the associations between *C. pneumoniae* seropositivity and past or present ischemic heart disease is similar to that reported by other investigators from many countries around the world[9-12]. The present study included patients with normal angiogram to confirm that this group had no evidence of coronary artery disease. Coronary angiography was not done in the healthy control group. Based on prevalence of anti-*C. pneumoniae* IgG antibodies our result demonstrated that more CAD patients were infected with *C. pneumoniae* than normal healthy people. When anti-*C. pneumoniae* IgA antibodies were used as the marker for chronic infection we found that chronic *C. pneumoniae* occurred more frequently in CAD patients than in healthy people.

Most previously conducted research, however, has been performed on the basis of cases lacking angiographic confirmation, and has demonstrated an association between seropositivity for *C. pneumoniae* and coronary atherosclerosis[9,11,13]. In fact, research based on angiographically demonstrated cases and controls is quite rare, only one study could detect a significant association (adjust OR = 1.6, 95% CI = 1.0 – 2.7)[14]. Our present study also demonstrated this significant association with OR = 4.26 for positive IgG and 10.31 for positive IgA antibodies.

Our result did support an association between seropositivity for *C. pneumoniae* and coronary artery disease, especially in normal coronary patients who were proved by coronary angiogram to have lower positive IgG and IgA than CAD patients. The limitation of this study is the small number of patients, and with serological studies it is difficult to select controls that are guaranteed to be free of atherosclerosis. There is also a wide range of antibody responses to *C. pneumoniae* due to variation in the infective dose, mode of infection, previous exposure or anamnestic responses to other chlamydiae. If prospective trials designed to clarify the role of antichlamydial therapy in prevention of coronary events have positive results, our findings may have immediate and important implications in the treatment of CAD patients with antibiotics.

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ความสัมพันธ์ระหว่างภูมิต้านทานต่อเชื้อ*คลามีเดีย นิวโมนิเอ* ในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ที่ตรวจยืนยันโดยการสวนหลอดเลือดหัวใจในประเทศไทย

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คณะผู้วิจัยได้ทำการศึกษาผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่จำนวน 243 ราย (ชาย 178 ราย, หญิง 65 ราย) อายุระหว่าง 35-78 ปี (เฉลี่ย 61 ± 10 ปี) ที่รับตัวไว้ในโรงพยาบาลเพื่อทำการตรวจสวนหลอดเลือดหัวใจ เปรียบเทียบกับผู้ป่วยโรคอื่น ๆ ที่ผลการตรวจสวนหลอดเลือดหัวใจพบว่าปกติจำนวน 58 ราย (ชาย 33 ราย, หญิง 25 ราย) อายุระหว่าง 32-83 ปี (เฉลี่ย 57 ± 11 ปี) และอาสาสมัครสุขภาพดี 187 ราย (ชาย 95 ราย, หญิง 92 ราย) อายุระหว่าง 25-80 ปี (เฉลี่ย 58 ± 17 ปี) พบว่า IgG antibodies ต่อเชื้อ*คลามีเดีย นิวโมนิเอ* ที่ตรวจโดยวิธี ELISA มีถึงร้อยละ 73.7 ในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่, ในผู้ป่วยที่หลอดเลือดแดงโคโรนารี่ปกติ พบร้อยละ 39.7 ส่วนในอาสาสมัครสุขภาพดีพบร้อยละ 59.4 สำหรับ IgA antibodies พบในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ร้อยละ 54.3, ในผู้ป่วยที่หลอดเลือดแดงโคโรนารี่ปกติ พบร้อยละ 10.3 ส่วนในอาสาสมัครสุขภาพดีพบร้อยละ 44.4 ในกรณีของ IgG antibodies พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ และอาสาสมัครสุขภาพดี (OR = 1.91, 95% CI = 1.27-2.88, p = 0.0018) กรณี IgA antibodies พบความแตกต่างอย่างมีนัยสำคัญทางสถิติเช่นกัน (OR = 1.49, 95% CI = 1.02-2.19, p = 0.0257) ยิ่งในกรณีเปรียบเทียบกับผู้ป่วยที่หลอดเลือดแดงโคโรนารี่ปกติจะพบความแตกต่างได้ชัดเจนยิ่งขึ้นโดยสรุป พบว่าการติดเชื้อ*คลามีเมีย นิวโมนิเอ* ในคนไทยพบได้บ่อยไม่แตกต่างกับในประเทศอื่น ๆ ทั่วโลก และพบการติดเชื้อนี้ในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่บ่อยกว่าในอาสาสมัครสุขภาพดี และผู้ป่วยที่ได้รับการยืนยันว่าหลอดเลือดแดงโคโรนารี่เป็นปกติโดยการตรวจสวนหลอดเลือดหัวใจ

คำสำคัญ : ภูมิต้านทานต่อเชื้อ*คลามีเดีย นิวโมนิเอ*, ผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่, การสวนหลอดเลือดหัวใจ

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