Possible Role of Chronic Infection With Chlamydia Pneumoniae in Japanese Patients With Acute Myocardial Infarction

Chiya Kosaka, MD; Katsuko Hara; Yutaka Komiyama, PhD; Hakuo Takahashi, MD

Chlamydia pneumoniae, a common human respiratory pathogen, has been implicated in the pathogenesis of coronary heart diseases (CHD) in several seroepidemiological studies. The present case–control study investigated the relation between serologic evidence of C. pneumoniae infection and CHD in a Japanese population. Two groups of cases were enrolled: 26 patients with acute myocardial infarction (AMI) and 46 patients with effort angina pectoris (e-AP). Their data were compared with 58 age-matched healthy controls and also compared with 53 patients with vasospastic angina (VSA) as pathological control subjects. Anti-C. pneumoniae specific IgA and IgG antibody titers were measured by enzyme-linked immunosorbent assay (ELISA). The mean indices of IgG-type antibody in AMI and e-AP were not significantly different from those in either the normal controls or VSA group. On the other hand, the mean indices of IgA-type antibody in AMI were significantly higher than in the normal controls (1.39±0.83 in AMI vs 0.84±0.58 in controls, p<0.001) and VSA (1.39±0.83 in AMI vs 1.05±0.61 in VSA, p<0.05) group. However, the differences in the IgA titers in the e-AP group compared with the normal controls did not reach a significant level. The odds ratio associated with the seropositivity of IgA for AMI against the normal controls was 3.89 (95% confidence interval: 1.16–13.10) and that against VSA was 6.90 (95% CI: 1.73–27.52) after adjustment for risk factors for CHD and/or age, sex and smoking status. In 6 patients the elevated IgA titers were sustained even at 3 months after the episode of AMI. These results suggest that seropositivity for IgA-type antibody against C. pneumoniae may be a significant risk factor for the development of AMI. The possible mechanisms include chronic inflammation in the coronary artery due to persistent C. pneumoniae infection. (Jpn Circ J 2000; 64: 819–824)

Key Words: Acute myocardial infarction; Chlamydia pneumoniae; Coronary heart disease; Effort angina pectoris; ELISA; IgA; IgG; Infarction; Infection; Vasospastic angina

There is accumulating evidence that infection with Chlamydia (C.) pneumoniae, a common causative microorganism for respiratory infection1 is associated with coronary heart diseases (CHD) (see Reviews2–5). Several seroepidemiological studies, including retrospec-
tive6–8 and prospective ones,9–11 have demonstrated a strong association between CHD and this organism. The association was supported by the demonstration of its specific presence in atherosclerotic lesions by immunohistochemistry, electronmicroscopy and polymerase chain reaction techniques for the DNA of C. pneumoniae.12-14 In addition, an association between C. pneumoniae and cerebrovascular diseases has been reported.15-17 Thus, it has been hypothesized that infection with C. pneumoniae has a causative role in atherosclerosis, and recent animal studies18-20 and clinical interventional studies7,18 strongly support this hypothesis.

The prevalence of antibodies to C. pneumoniae is infrequent in children younger than 5 years, but increases quickly during adolescence, indicating active C. pneumoniae infec-
tion during childhood. It then increases slowly and reaches about 50% prevalence by middle age.19,20 The primary infection induces a time-limited antibody response, and most people are reinfected more than once during their lifetime. Previous studies demonstrate that the prevalence of antibodies for C. pneumoniae in the Japanese population is similar to that in the North American and European populations, except that it starts to increase prominently at an earlier age and reaches a relatively higher plateau level at a younger age.21,22 This appears to coincide with reports that the prevalence of C. pneumoniae is higher in Asia than in Western countries, which is generally considered to be a consequence of the increased population density.23,24 Despite the inference that infection with C. pneumoniae would therefore be common in Japan, reports on the association between CHD and infection with this organism in the Japanese population are extremely limited.25

Methods

Participants

All study samples were taken from volunteers and patients who gave informed consent.

We studied 26 patients with acute myocardial infarction (AMI; 22 men, 4 women; mean age, 61.5±10.7 years), 46 patients with effort angina pectoris (e-AP; 32 men, 14 women; mean age, 63.5±10.8 years) and 53 patients with

(June 5, 2000; revised manuscript received July 24, 2000; accepted August 1, 2000.)

Department of Clinical Sciences and Laboratory Medicine, Kansai Medical University, Osaka, Japan

Mailing address: Professor Hakuo Takahashi, MD, Department of Clinical Sciences and Laboratory Medicine, Kansai Medical University, 10-15 Funzochocho, Moriguchi, Osaka 570-8507, Japan. E-mail: takahashi@tkk.kmu.ac.jp

Japanese Circulation Journal Vol.64, November 2000
Table 1  Clinical Characteristics of Patients and Normal Controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AMI (n=26)</th>
<th>e-AP (n=46)</th>
<th>VSA (n=53)</th>
<th>Normal (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.5±10.7*</td>
<td>63.2±10.8*</td>
<td>60.4±7.9*</td>
<td>55.4±9.5</td>
</tr>
<tr>
<td>Male</td>
<td>22 (84.6%)*</td>
<td>32 (69.6%)</td>
<td>36 (67.9%)</td>
<td>31 (51.7%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>19 (73.1%)*</td>
<td>32 (69.6%)*</td>
<td>26 (49.1%)*</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (50.0%)*</td>
<td>18 (39.1%)*</td>
<td>14 (26.4%)*</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>15 (57.7%)*</td>
<td>20 (43.5%)*</td>
<td>20 (37.7%)*</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>12 (46.2%)</td>
<td>26 (56.5%)</td>
<td>30 (56.6%)</td>
<td>27 (45.0%)</td>
</tr>
</tbody>
</table>

Data are shown as mean±SD for age and as n (%) for other factors. The significance in continuous variables and proportion was examined by ANOVA and chi-square statistics, respectively. *p<0.05 vs normal controls subjects. **p<0.05 vs patients with VSA. AMI, acute myocardial infarction; e-AP, effort angina pectoris; VSA, vasospastic angina.

Results

Characteristics of the Participants (Table 1)

Patients with e-AP were significantly older than the other groups, but there were no significant differences in age between the AMI group, normal controls or VSA group. As most patients with AMI were male, the ratio of males in the AMI group was significantly higher than the other groups. Because carriers of the commonly recognized risk factors for CHD (ie, hypertension, hypercholesterolemia, and diabetes) were not included in the normal controls, there were significant differences in these factors between that group and the others. As expected, patients with AMI had a significantly higher prevalence of hypertension and diabetes than patients with VSA, who were the control subjects for different cardiovascular diseases. Differences in the ratio of hypertension between patients with e-AP and those with VSA were also significant. There were no significant differences in smoking habits among the groups.

Mean Indices of Anti-C. Pneumoniae IgG- and IgA-Type Antibodies

As shown in Fig 1 (right panel), a significant difference in IgG titer was not observed among the groups. In contrast, the mean index of IgA in AMI was significantly higher than in either the normal controls (1.39±0.83 in AMI vs 0.84±0.58 in controls, p=0.0008) or VSA group (1.39±0.83 in AMI vs 1.02±0.60 in VSA, p=0.0274) (Fig 1, left panel). Such a significant elevation in IgA antibody was not observed in the e-AP group who represented another atherosclerotic coronary disease. The mean index of IgA in the e-AP group was either similar to the titer in the normal controls (0.84±0.51 in e-AP vs 0.84±0.58 in the normal controls) or less than in the VSA group (0.84±0.51 in e-AP vs 1.02±0.60 in VSA).

Japanese Circulation Journal Vol. 64, November 2000
**Figure 1.** Mean index of IgA and IgG antibodies to Chlamydia pneumoniae in patients and normal controls. The column represents the mean index ± SD of IgA (left panel) and IgG antibodies (right panel) to C. pneumoniae (CP). *p<0.05 vs normal controls or VSA, **p<0.01 vs normal controls or VSA.

**Figure 2.** Correlation of IgA and IgG antibody titers to Chlamydia pneumoniae with age in patients (A–C) and normal controls (D). Scattergram shows correlation between the indices of IgA and IgG antibodies to C. pneumoniae and age. Open and closed circles represent IgG and IgA antibodies, respectively. The dotted and solid lines represent the coefficient of correlation for IgG and IgA antibodies with age, respectively. (A) AMI (IgG: r=0.290; IgA: r=0.510), (B) e-AP (IgG: r=0.079; IgA: r=0.184), (C) VSA (IgG: r=0.116; IgA: r=0.017), (D) normal controls (IgG: r=0.082; IgA: r=0.030).

**Distribution of IgA and IgG Titers in the Patient Groups**

The age-related distribution of anti-C. pneumoniae IgG and IgA titers for each individual in each group was plotted (Fig 2). All groups showed a similar positive, but weak correlation of IgG with age, supporting previous reports that the prevalence of antibodies to C. pneumoniae increases with age. A positive correlation with age was observed for IgA, except for the normal controls in which a very weak negative correlation was observed (Fig 2D). However, patients with AMI showed a remarkable positive correlation (r=0.51) (Fig 2A). The AMI group had a population with a high IgA titer (>2.0 of index) in the over 60 years age bracket, but there were very few such subjects in the other groups. Therefore, the presence of these elderly subjects with higher IgA titers is likely to contribute to the positive correlation between IgA titer and age, and the elevated mean index of IgA in the AMI group.

**Seropositivity and OR**

We next calculated the OR for the seropositivity of anti-C. pneumoniae antibodies using logistic regression analysis to evaluate the relative risk of developing atherosclerotic coronary diseases in subjects with the antibody compared with those without the antibody. The overall IgG and IgA seropositivities observed in our study were 38.9% (72/185) and 17.8% (33/185), respectively, in accordance with the high exposure rate in the Japanese population as reported previously. Table 2 shows the OR of the AMI and e-AP groups for seropositivity of IgA or IgG antibodies against the normal controls. Significant associations were not observed between these disease states and IgG positivity, or between e-AP and IgA positivity. On the other hand, a significant association was observed between AMI and IgA positivity. After adjusting for age, sex, and smoking habits, the OR was 3.89 (95% CI: 1.16–13.10, p=0.028). More significant associations between AMI and IgA positivity...
Table 2  Odds Ratios for Developing Coronary Heart Disease by Seropositivity of Anti-Chlamydia Pneumoniae Antibodies Against the Normal Controls and the VSA Patients

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th></th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>Positive</td>
<td>Unadjusted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Against normal controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI (n=26)</td>
<td>9 (34.6%)</td>
<td>0.98 (0.37-2.59)</td>
<td>1.22 (0.40-3.73)</td>
</tr>
<tr>
<td>e-AP (n=46)</td>
<td>20 (43.5%)</td>
<td>1.43 (0.65-3.14)</td>
<td>1.42 (0.58-3.51)</td>
</tr>
<tr>
<td>Normal (n=50)</td>
<td>21 (35.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Against VSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI (n=26)</td>
<td>9 (34.6%)</td>
<td>0.75 (0.28-1.98)</td>
<td>0.74 (0.24-2.27)</td>
</tr>
<tr>
<td>e-AP (n=46)</td>
<td>20 (43.5%)</td>
<td>1.08 (0.49-2.41)</td>
<td>1.16 (0.49-2.76)</td>
</tr>
<tr>
<td>VSA (n=23)</td>
<td>22 (47.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios against the normal controls were adjusted for the effects of age, sex, and smoking, and those against the VSA group were adjusted for the effects of age, sex, smoking, and components recognized coronary risk factors including hypertension, hypercholesterolemia, and diabetes. *p<0.05, **p<0.01. AMI, acute myocardial infarction; e-AP, effort angina pectoris; VSA, vasospastic angina.

Table 3  Odds Ratios for Developing Coronary Heart Disease Assessed by Seropositivity for Anti-Chlamydia Pneumoniae Antibodies Subdivided by the Level of the IgA Antibody Titer

<table>
<thead>
<tr>
<th>IgA titer</th>
<th>AMI (n=26)</th>
<th>Normal (n=60)</th>
<th>VSA (n=53)</th>
<th>Odds ratio (95% CI) against normal controls</th>
<th>Odds ratio (95% CI) against VSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.5</td>
<td>23 (68.5%)</td>
<td>41 (68.4%)</td>
<td>45 (84.9%)</td>
<td>3.55 (0.95-13.31)</td>
<td>3.77 (0.91-15.54)</td>
</tr>
<tr>
<td>≥1.0</td>
<td>15 (37.5%)</td>
<td>19 (31.3%)</td>
<td>23 (47.2%)</td>
<td>2.94* (1.14-7.00)</td>
<td>2.21 (0.77-6.30)</td>
</tr>
<tr>
<td>≥2.0</td>
<td>11 (42.3%)</td>
<td>9 (15.0%)</td>
<td>8 (15.1%)</td>
<td>4.16** (1.45-11.90)</td>
<td>3.89* (1.16-13.10)</td>
</tr>
<tr>
<td>≥4.0</td>
<td>9 (36.6%)</td>
<td>1 (1.7%)</td>
<td>41 (7.5%)</td>
<td>31.14** (3.60-264.28)</td>
<td>31.14** (2.90-336.98)</td>
</tr>
</tbody>
</table>

Odds ratio against the normal controls was adjusted for the effects of age, sex, and smoking, and those against VSA were adjusted for the effects of coronary risk factors in addition to age, sex, and smoking. *p<0.05, **p<0.01. AMI, acute myocardial infarction; e-AP, effort angina pectoris; VSA, vasospastic angina.

were observed when patients with VSA were used as controls (Table 2). After adjusting for age, sex, smoking habits, and the commonly recognized risk factors for CHD, the OR against VSA was 6.90 (95% CI: 1.73-27.52, p=0.0062). When subdividing the group by the levels of IgA antibody titer, the association between AMI and IgA increased with increasing titer of IgA against both the normal controls and the VSA group (Table 3). Therefore, the relative risk for the onset of AMI is higher in subjects with higher IgA titers.

Antibody Titers at 3 Months After the Episode of AMI

Blood sampling at 3 months following the AMI was possible only in 6 patients, who all sustained the elevated IgA titers even at 3 months after the episode (from 1.72±0.41 to 1.95±0.48). The IgG titers did not change significantly at 3 months (from 1.10±0.81 to 1.48±0.80).

Discussion

Infection with C. pneumoniae is commonly diagnosed by serology, although generally the clinical significance of the elevated titer has been complicated. With a normal immune system, an acute infection induces elevated levels of IgA- and IgG-type antibodies to C. pneumoniae. The IgG-type antibody level rises gradually and usually decreases slowly, whereas the IgA-type antibody tends to disappear rapidly because of its short half-life (5-6 days). In cases of reinfection, the IgA response is predominant and the elevated level of IgA antibodies occasionally persists for a relatively long time. In addition to reinfection, chronic infection also causes persistent elevation of the IgA titer because Chlamydiae are intracellular parasitic bacteria. Thus, elevated IgA titers are generally considered to indicate chronic persistence of active infection, including C. pneumoniae, whereas IgG titers in the absence of IgA titers may be a sign of past infection.

In the present study, we observed an association between CHD and anti-C. pneumoniae IgA-type antibody, but not with the IgG type. This study is cross-sectional, and therefore, we could not definitely determine if the positive IgA titer represents chronic infection or a transient increase caused by acute infection, including recent reinfection. Further studies with paired samples over years and prospective studies are obviously required to disclose the temporal relationships between infection with C. pneumoniae and CHD. However, acute infection is unlikely, because elevated levels of IgG are more prominent in acute infection and reaches ≥512 by the MIF test, which corresponds to over 9.0 on the IgG index by the ELISA system! In the present study, all patients with AMI had an IgG index less than 3.0. In addition, the presence of an older-age population with high IgA titers, but not high IgG titers, only in the AMI group, also leads us to assume that the high IgA titer must be the result of persistent infection not recent reinfection. In support of this, 6 patients with AMI retained the elevated IgA and IgG antibody titers for 3 months after the acute episode; if it was related to an acute infection, the IgA titer should have been decreased in a few months. Similarly, it has been demonstrated that a significant portion of subjects with high levels of IgA antibodies sustain their elevated seropositivity. Therefore, at least some of the AMI patients with positive IgA titers in the present study could be chronically infected with C. pneumoniae.

As the persistent inflammatory response in the vascular wall induced by chronic infection or occasional reinfection is supposed to be especially important as the mechanism whereby chlamydial infection could contribute to athero-

Japanese Circulation Journal Vol.64, November 2000
genesis., the elevation in IgA-type antibody may serve as a significant marker for the development of CHD including the acute coronary syndrome. The reason for the absence of a significant association with IgG titers is unknown, although several previous reports have demonstrated significant elevations in IgG or both IgG and IgA titers in atherosclerotic diseases, including AMI., In contrast, reports on the association of IgA-type antibody, but not IgG, with C. pneumoniae infection have been previously demonstrated in CHD adult atherosclerotic and cerebrovascular diseases. The high prevalence rate of IgG in the general adult population may obscure its predictive value. These results, including ours, suggest that chronic infection demonstrated by raised levels of IgA antibody is a significant risk factor for AMI.

In the present study, we assumed patients with VSA were pathologist control subjects with a different mechanism for the angina, in addition to the normal controls. Although in this particular study, smokers were included because patient groups also include smokers, the normal controls were limited to healthy subjects who had neither risk factors for CHD nor had been hospitalized. Use of such subjects would afford a more strict comparison for the role of chlamydial antibodies on atherogenesis. On this point, the significant elevations in the IgA titers and in the OR for the AMI group against the VSA group clearly indicated that infection with C. pneumoniae was, at least in part, associated with the development of AMI. Unexpectedly, no significant association of IgA titers for the e-AP group was observed against either the normal controls or patients with VSA, although previous reports have demonstrated significant elevations of IgG antibodies against C. pneumoniae in chronic CHD and coronary stenosis. The reason for the discrepancy is unknown, but may reflect differences in race, study design, or the sensitivity of the assay system. In support of this, seropositivity in European patients is obviously higher than that in Japanese, as shown in the present study and by Miyashita et al who used the MIF test, not ELISA, for measurement of antibody titers.

The e-AP patients included in the present study had the coronary stenosis confirmed by angiographic findings, not by clinical signs only, and therefore they may not be at the pre-stage of myocardial infarction. Besides, chlamydial infection may effect mainly the onset but not the development of atherosclerosis. In fact, in vitro experiments have suggested that the infection triggers the acute phase of atherosclerosis-related events through the expression of tissue factors, which would increase the risk of thrombosis of the plaque, or the induction of matrix-degrading metalloproteinases which would trigger rupture of plaques.

In conclusion, IgA titers, but not IgG, against C. pneumoniae were higher and more often positive in patients with AMI than in the normal controls and patients with VSA. Therefore, the elevation in IgA antibody may serve as a significant marker for the development of an acute coronary syndrome. The current study adds another piece to the evidence of an association of the organism with CHD using a different method and a different study population in Japan.

Acknowledgments

The authors wish to thank the staff in the Cardiovascular Center, Kansai Medical University Hospital for providing us with sera samples, Dr Toshitake Morikawa, Pharmaceutical Research Laboratory, Hisachi Chemical Co. Ltd for generously providing the Chlamydia pneumoniae-specific ELISA kit, and Dr Terako Ueda for the useful discussion on the statistical analysis. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (Hokue Takahashi. #18774720, and #10577253).

References


Japanese Circulation Journal Vol. 64, November 2000


