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Brief Communication (Original)

Association between *Chlamydia pneumoniae* infection and atherosclerosis of cervical or intracranial cerebral vessels in Thai patients

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Background: *Chlamydia pneumoniae* infection is associated with coronary and carotid atherosclerosis. The prevalence of chlamydia infection varies from one country to another. Prevalence is related to socioeconomic status and sites of atherosclerosis differ between different ethnicities.
Objectives: To study the association between chlamydia infections and cervicocerebral atherosclerosis in Thai patients.
Methods: In this observational case–control study, patients with significant extracranial carotid artery or intracranial artery stenosis by ultrasound and magnetic resonance angiography (MRA) were studied and compared with an age- and sex-matched group of control participants without evidence of carotid stenosis by ultrasound. Antibodies to *C. pneumoniae* were studied by microimmunofluorescence. IgG titer ≥1:64 or IgA titer ≥1:16 were considered positive.
Results: We included 75 patient participants in the case group with evidence of significant carotid or intracranial stenosis and 75 control participants. IgA seropositivity for *C. pneumoniae* was found in 12 patients in the case group (16%) and 1 control participant (1.3%) (OR 14.1; $P = 0.001$). In the case group, 43 patients participants (57%) and 40 (53%) control participants were seropositive for *C. pneumoniae* IgG (OR 1.18; $P = 0.62$). Multivariate analysis revealed that IgA seropositivity was significantly associated with both cervical and intracranial cerebral atherosclerosis independent of other vascular risk factors.
Conclusions: *C. pneumoniae* IgA seropositivity is associated with cervicocerebral atherosclerosis in Thai patients. This association is independent of other vascular risk factors and is present in almost all subgroups including those with extracranial carotid, intracranial artery, and combined stenoses.

Keywords: Atherrosclerosis, cerrical, chlamydia pneumonia, intracranial vessels

Chronic infection may play an important role in inflammation-related atherosclerosis. Serological evidence of previous infection with *Chlamydia pneumoniae*, which is a common respiratory pathogen, has been found in epidemiological and case–control studies to be associated with atherosclerosis, ischemic heart disease, and first ischemic stroke [1-6]. *C. pneumoniae* have also been found in atherosclerotic plaques and isolated from coronary and carotid atheromas.

Stroke is a heterogenous disease with various etiologies. Atherosclerosis of cervicocerebral arteries accounts for 20%–30% of ischemic stroke. In a subgroup analysis of previous studies in ischemic stroke patients evidence of chlamydia infection is more likely to be associated with large vessel atherosclerosis and lacunar stroke [6-8]. To our knowledge, there has been no case–control study focusing on atherosclerosis of the cervicocerebral arteries and chlamydia infection in Thai patients. The prevalence of chlamydia infection varies from one country to another. Prevalence is related to socioeconomic status and atherosclerosis sites differ among different ethnicities. For example, intracranial atherosclerosis is much more common in Asians than white people of European descent. The present study aimed to study the association between chlamydia antibody titers and atherosclerosis of cervicocerebral arteries in Thai patients. We also aimed to study the association of the infection with the sites of atherosclerosis.

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Methods

The present observational case–control study was approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University (IRB No. 081/52). All participants provided their written informed consent to be included in the study. Patients who were referred to the King Chulalongkorn Memorial Hospital neurovascular ultrasound laboratory for evaluation of cervicocerebral arteries and met inclusion criteria were screened by carotid duplex and transcranial Doppler ultrasound. Carotid ultrasound was performed using a Corevision sonographic imager (Toshiba Corp., Tokyo, Japan) and a Multi-Dop ultrasound analyzer (DWL, Sipplingen, Germany) was used for transcranial Doppler evaluation. Inclusion criteria were patients with Thai ethnicity ≥20 years old. Eligible patients had evidence of significant (>50%) carotid or intracranial stenosis by standard criteria. Consensus criteria proposed by the Society of Radiologists was used to diagnose significant carotid stenosis, and peak systolic velocities were used for diagnosis of intracranial artery stenosis [9]. Magnetic resonance angiography was performed to confirm the presence and severity of stenosis in all patients. Patients who had clinical evidence of pneumonia or severe infection at the time of the study were excluded. Patients with arterial stenosis from other causes such as arterial dissection, moyamoya disease, and vasculitis were also excluded.

A control group consisted of 75 age- and sex-matched participants without a history of stroke and with no evidence of either cervical or intracranial cerebral atherosclerosis by carotid or transcranial Doppler ultrasound. Sample size was calculated based on existing data from previous studies using a prespecified power calculation of 80%. For all cases and controls, blood samples were collected, centrifuged and frozen at −70°C until *C. pneumoniae* serology. Stored sera were analyzed for IgG and IgA antibody titers to *C. pneumoniae* using microimmunofluorescence (MIF). MIF was performed using commercially available kits (Focus Diagnostics, Cypress, CA, USA). IgG titer ≥1:64 and IgA titer ≥1:16 were considered positive. Laboratory staff were blinded to case and control status.

Statistical analyses

Data were analyzed using SPSS for Windows, version 16 (SPSS Inc, Chicago, IL, USA). Means were calculated for continuous variables, and proportions for dichotomous variables. A McNemar test was used for group comparisons of proportions. Conditional logistic regression was used to estimate the odds ratio (OR) for matched case–control pairs before and after adjustment for potential confounders. Significance was determined at the 0.05 level using 2-sided tests.

Results

We categorized 150 participants and divided them equally into those with significant carotid or intracranial stenosis (case) and age- and sex-matched (control) groups. Carotid duplex and transcranial Doppler ultrasound were performed on all patients. Every patient with significant stenosis underwent magnetic resonance angiography (MRA) of the brain and neck where stenoses were confirmed. Of this group, 55 patients (73.3%) were male. Mean ages in both case and control groups were 68.08 and 66.87 years, respectively.

The three most common risk factors were dyslipidemia, hypertension, and old age (≥60) among case and control groups. Case group patients were significantly more likely to have dyslipidemia, hypertension, and diabetes mellitus than control participants. The proportion of case and control groups were not different in subjects with a history of cigarette smoking, ischemic heart disease, or in those or aged ≥60. Baseline characteristics of the two groups are shown in **Table 1**.

The prevalence of an elevated *C. pneumoniae* IgG titer in the case and control populations was high. *C. pneumoniae* IgG seropositivity with cutoff titer of 1:64 in the case group was found in 43 patients (57%) and 40 (53%) controls (OR 1.18; $P = 0.62$). By contrast, there was far less prevalence of elevated IgA in either group. IgA seropositivity for *C. pneumoniae* with a cutoff titer of 1:16 was found 12 patients in the case group (16%) and in only 1 control (1.3%) (OR 14.10; $P = 0.001$). IgG and IgA seroprevalence in the cases and controls are shown in **Table 2**.

Multivariate analysis revealed that elevated IgA titers were associated with atherosclerosis of both the cervical and intracranial cerebral arteries with significant stenosis after adjusting for diabetes mellitus, hypertension, dyslipidemia, sex, and history of cigarette smoking (OR 20.72; 95% CI 2.10–204.77). Diabetes and hypertension were independently associated with atherosclerosis of both the cervical and intracranial cerebral arteries. Results of the multivariate analysis are shown in **Table 3**.

Table 1. Characteristics of cases and controls matched for age and sex

	Case n (%) or mean (SD)	Control n (%) or mean (SD)	*P*
No.	75	75	
Male	55 (73)	55 (73)	1.0
Weight, kg	63.8 (9.85)	64.5 (11.68)	0.68
Height, cm	162.6 (8.69)	164.3 (7.30)	0.19
SBP, mmHg	143.6 (23.28)	130.4 (19.02)	<0.001*
DBP, mmHg	81.4 (12.25)	76.2 (12.40)	0.01*
Total cholesterol	177.6 (40.99)	197.1 (39.54)	0.003*
HDL, mg/dL	47.9 (13.83)	54.6 (15.11)	0.005*
LDL, mg/dL	107.0 (34.97)	115.5 (32.64)	0.12
FBS	110.5 (29.29)	99.5 (13.26)	0.003*
Dyslipidemia	68 (91%)	55 (73%)	0.006*
Hypertension	62 (83%)	39 (52%)	<0.001*
Age ≥60 y	58 (77%)	53 (71%)	0.35
Diabetes mellitus	31 (41%)	5 (7%)	<0.001*
Ischemic heart disease	13 (17%)	7 (9%)	0.15
Smoking	10 (13%)	7 (9%)	0.44
Alcohol consumption	7 (9%)	13 (17%)	0.15

*$P < 0.05$

Table 2. Prevalence of elevated *Chlamydia pneumonia* antibody titers

Factors	n	IgG ≥1:64, n (%)		IgA ≥1:16, n (%)	
	case/control	case	control	case	control
Overall	75/75	43 (57)	40 (53)	12 (16)	1 (1)
Male	55/55	31 (56)	31 (56)	9 (16)	1 (2)
Dyslipidemia	68/55	39 (57)	29 (53)	11 (16)	1 (2)
Hypertension	62/39	37 (60)	24 (62)	9 (15)	1 (3)
Age e”60 y	58/53	33 (57)	35 (66)	10 (17)	1 (2)
Diabetes mellitus	31/5	16 (52)	4 (80)	5 (16)	0 (0)
Ischemic heart disease	13/7	8 (62)	5 (71)	2 (15)	0 (0)
Smoking	10/7	4 (40)	3 (43)	2 (20)	0 (0)
Alcohol	7/13	5 (71)	7 (54)	2 (29)	0 (0)

Table 3. Association of stroke risk factors with extracranial carotid or intracranial artery stenosis

	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sex	1.15 (0.55–2.41)	2.13 (0.85, 5.39)
Dyslipidemia	3.53 (1.39–8.96)	2.43 (0.86, 6.90)
Hypertension	4.40 (2.08–9.32)	3.40 (1.42, 8.14)
Age ≥60 y	1.42 (0.68–2.95)	0.76 (0.31, 1.87)
Diabetes mellitus	9.86 (3.57–27.27)	7.52 (2.50, 22.64)
Ischemic heart disease	2.04 (0.76–5.43)	1.59 (0.47, 5.40)
Smoking	1.50 (0.54–4.16)	1.77 (0.50, 6.32)
Chlamydia pneumonia IgA	14.10 (1.78–111.42)	20.72 (2.10, 204.77)

The association between IgA titers and atherosclerosis of cervicocerebral arteries was also stratified according to the site of stenosis. In the case group, isolated extracranial carotid stenosis without intracranial stenosis was found in 23 patients (31%) whereas 26 (35%) had isolated intracranial stenosis. We found 26 cases had combined intracranial and extracranial stenoses.

When the sites of atherosclerosis are evaluated, IgA chlamydia seropositivity was found to be significantly associated with all subgroups except for isolated extracranial carotid stenosis. The odds ratio was highest in patients with combined intracranial and extracranial carotid stenosis. There was no correlation between chlamydial IgG serology and either the site of cervical or intracranial cerebral atherosclerosis. Data are shown in **Table 4**.

Discussion

We report a case–control study in a cross-sectional Thai population that demonstrated a significant association between atherosclerosis of cervicocerebral arteries and evidence of *Chlamydia pneumoniae* infection. These findings are consistent with earlier studies in patients with ischemic stroke in which atherosclerotic subtype is more closely related to chlamydia infection [6, 8, 10-12].

The present study differs from previous studies. First, this is a case–control study of a cross-section of Thais focusing on chlamydia infection and atherosclerosis of both the cervical and intracranial cerebral arteries. Second, our cases had definite evidence of atherosclerotic stenosis by ultrasound and MRA. Third, we performed a subgroup analysis of different sites of atherosclerosis. Our findings are consistent with previous studies in ischemic stroke patients in which IgA was found a better predictor for the disease [6, 10]. *Chlamydia pneumoniae* IgA titer, but not IgG titer, was associated with atherosclerosis of both the cervical and intracranial cerebral arteries. Although it remains controversial, it is postulated that IgA is a marker of persistent, chronic infection, whereas IgG may reflect a past or remote infection [6, 13-15].

The proposed mechanism of chlamydia infection related to atherosclerosis includes direct and indirect effects. Several studies identified *C. pneumoniae* in the endothelium, smooth muscle cells, and macrophages within the vascular wall [8, 16]. Direct invasion of the vessels wall by *C. pneumoniae*, and secretion of lipopolysaccharides and heat shock protein 60 may lead to endothelial injury [17]. This results in increased numbers of macrophages at the infected sites and smooth muscle cell proliferation. The second mechanism indirectly effects increased platelet aggregation, increased procoagulant, and decreased anticoagulant activities. *C. pneumoniae* have been demonstrated in the middle cerebral and other large cerebral arteries [8, 18, 19].

There are two commonly used methods to detect antibodies to *C. pneumoniae*: MIF and enzyme-linked immunosorbent assay. Here, we used a MIF method, which is recommended as the criterion standard index method. However, the prevalence of chlamydia infection cannot be directly compared with previous studies, because the method of antibody detection and cutoff points were different [6, 8, 10-12]. A study from Cameroon that used the same method and cutoff point, found an association between *C. pneumoniae* antibody IgA and stroke. However, the prevalence of IgG and IgA seropositivities between our study and that from Cameroon were different. These differences may result from various factors, such as the prevalence of the infection in the community and socioeconomic status of those investigated [10].

Table 4. Subgroup analyses of elevated *Chlamydia pneumoniae* IgA Titers (≥1:16)

Subgroup	n (cases)	Odds ratio (95%CI)	*P*
Total population	75	14.10 (1.78, 111.42)	0.001
All patients with extracranial carotid stenosis	49	14.44 (1.74, 119.52)	0.002
All patients with extracranial carotid stenosis without intracranial stenosis	23	3.36 (0.20, 56.00)	0.37
All patients with intracranial stenosis	52	19.85 (2.47, 159.30)	<0.001
All patients with intracranial stenosis without extracranial carotid stenosis	26	13.46 (1.43, 126.69)	0.004
Combined intracranial and extracranial	26	27.26 (3.16, 235.24)	<0.001

The present study focused on atherosclerosis of the carotid and intracranial arteries. Although the present study was not aimed to investigate the prevalence of intracranial and extracranial carotid stenoses, we found a larger number of patients with intracranial artery disease. This is consistent with previous studies that found intracranial disease is more common in Asia. In the subgroup analysis of the site of atherosclerosis, chlamydia IgA antibody was found to be associated with almost all subgroups including the extracranial carotid, intracranial, combined stenosis, and isolated intracranial stenosis.

A limitation of the present study was serological testing, which was not repeatedly measured over time. Therefore, chronic and past infection could not be reliably determined. Intracranial atherosclerosis was evaluated by transcranial Doppler ultrasound and MRA, but the transcranial ultrasound is not the criterion standard for determining intracranial atherosclerosis and 10% do not have temporal window. Therefore, some cases could have been missed. Neither is MRA the criterion standard for determining intracranial atherosclerosis, it has a tendency to overestimate the degree of arterial stenosis, which could result in an overestimation of the number of cases of intracranial stenosis. To reduce these errors, we used a combination of transcranial Doppler ultrasound and MRA. Because this was an observational study of cross-section of a population, causality cannot therefore be determined. The association emphasizes consistency with previous studies performed in other parts of the world. We consider that the present study provides additional data about chlamydia infection and atherosclerosis of both the cervical and intracranial cerebral vessels, especially for Asian populations.

Conclusion

Chlamydia pneumoniae IgA seropositivity is associated with atherosclerosis of both the cervical and intracranial cerebral vessels in Thai patients. This association is independent of other vascular risk factors and is present in almost all subgroups including extracranial carotid, intracranial artery, and combined stenoses.

Conflict of interest statement

The authors declare that there is no conflict of interest in this research.

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