Original Article

Association of *Chlamydia pneumoniae* Serology and Ischemic Stroke

Zaki Noah Hasan, FICMS (Neurology)

Background: Stroke is one of the major causes of morbidity and mortality throughout the world. Reports about the role of *Chlamydia pneumoniae* infection in the development of atherosclerosis have been reported in many studies. The aim of the study was to evaluate the association between *Chlamydia pneumoniae* infection and ischemic stroke.

Methods: We evaluated 50 patients with ischemic stroke (32 males and 18 females) and 40 control subjects (22 males and 18 females). They were age- and sex-matched. All enrolled subjects underwent an enzyme-linked immunosorbent assay (ELISA) serologic test for IgG and IgA antibodies for *Chlamydia pneumoniae*.

Results: Eighteen (36%) patients with ischemic stroke have positive IgA, in comparison with 6 (15%) among the control group (OR 3.18; CI 1.12–9.04; $P = 0.03$). This translates into the fact that there was a more than three-fold risk of developing ischemic stroke in those with *Chlamydia pneumoniae* infection compared to those who without. The IgG seropositivity was increased in patients with ischemic stroke, but it did not reach statistical significance (OR = 2.32; CI = 0.97–5.58; $P = 0.078$).

Conclusions: Chronic *Chlamydia pneumoniae* infection demonstrated by positive IgA–type antibody can be considered a significant risk for ischemic stroke.

Key Words: atherosclerosis, *Chlamydia pneumoniae*, risk factors, stroke

Thrombotic strokes are a result of in situ occlusions on atherosclerotic lesions in the cerebral arteries, typically proximal to major branches. Thrombogenic mechanism may include injury to and loss of endothelial cells, platelet activation, activation of the clotting cascade, inhibition of fibrinolysis, and blood stasis.[1,2]

Chlamydiae are obligate intracellular bacteria; *Chlamydia pneumoniae* is a common cause of atypical pneumonia, bronchitis, and sinusitis. Chlamydiae are susceptible to tetracycline and the macrolide antibiotics.[3] The anti-*C. pneumoniae* IgG antibodies may be considered markers of completed *C. pneumoniae* infection and they are synthesized for 3–5 years after *C. pneumoniae* infection. The anti-*C. pneumoniae* IgA antibodies may be considered markers of a persistent chronic *C. pneumoniae* infection and stay in the circulation for 3–5 days.[4] IgM is generally considered to signify acute primary infection.

Evidence about the association between *C. pneumoniae* and atherosclerosis came from animal models[5] and from serology studies. Polymerse chain reaction showed an association between circulating *C. pneumoniae* antibody titers and coronary artery disease.[6] The association also was proven by isolating the organism from coronary atheroma at autopsy.[7,8]

Subjects and Methods

The study was conducted at the Department of Internal Medicine (general medical ward and neurology units) in Baghdad Teaching Hospital - Medical City between November 2008 and March 2010. Following a predefined protocol, two groups of subjects were recruited.

Key Points

- Correlation of *C. pneumoniae* and atherosclerosis came from animal models and isolation of the organism from coronary atheromas at autopsy. Serology and polymerse chain reaction showed an association between circulating *C. pneumoniae* antibody titers and coronary artery disease.
- Chronic *C. pneumoniae* infection demonstrated by positive IgA–type antibody can be considered a significant risk factor for ischemic stroke.
- IgG seropositivity was increased in patients with ischemic stroke, but did not reach statistical significance.

From the Department of Neurology, Al-Kindey College of Medicine, University of Baghdad, Jadiriya Baghdad, Iraq.

Reprint requests to Zaki Noah Hasan, FICMS (Neurology), Al-Kindy Medical College, Nahdha Square, POB 47188, University of Baghdad, Jadiriya Baghdad, Iraq. Email: zaki_nooh@yahoo.com

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First group (stroke patients): 50 patients with ischemic stroke (proved by brain CT scan and/or MRI) (32 males and 18 females) aged 58.02 (±9.5) years. Exclusion criteria were[9]

1. Patients with any cardiac risk of stroke. By clinical examination, ECG and echocardiography to exclude cardio embolic source.
2. Clinical or radiological evidence of active pulmonary disease.
3. Known connective tissue disease, and other autoimmune disease, as the stroke cause is known.
4. Suspicion of brain tumor.

Second group (control subjects): consists of 40 control subjects (22 males and 18 females) aged 56.15 (±10.2) years. The control subjects were recruited from the patients' relatives who accompanied the patient to the hospital. The same exclusion criteria were applied on the control group.

Other risk factors for atherosclerosis have been studied for both groups which include age, sex, body mass index, smoking, diabetes, hypertension, and hypercholesterolemia.

A blood sample was taken immediately during the first hour of admission to avoid recruitment of patients with hospital-acquired infection. At least 2 mL of serum were obtained from each subject (stroke patients and controls), were stored at −20° before laboratory analysis, and were tested using an enzyme-linked immunosorbent assay (ELISA) serologic test for *C. pneumoniae* IgG, IgA (EUROIMMUN, Medizinische Laboradiagnostika AG). This kit has a sensitivity of 91.3% and a specificity of 96.2%, according to the manufacturer's guidelines.[10] The cutoff point for seropositivity was ≥1.1 for both IgA and IgG.

Statistical Analysis

Results are expressed as numbers, percentage (%) or mean ± 1 standard deviation (±SD). Significance of difference between groups was assessed by unpaired Student *t* test for continuous variables and chi square test (χ^2 test) for proportions. The statistical analysis was performed using GraphPad InStat version 3.06-2003.[11] *P* values of less than 0.05 were considered statistically significant. Relative risk was defined by a ratio of the probability of the event occurring in the exposed group versus a non-exposed group.

Table 1. Frequency of cerebrovascular risk factors among cases and controls

	Cases		Controls		
Risk factors	**n**	**%**	**n**	**%**	***df, P***
Hypertension					
Present	24	48	21	52.5	1, 0.671
Absent	26	52	19	47.5	
Diabetes mellitus					
Present	20	40	18	45	1, 0.633
Absent	30	60	22	55	
Abnormal cholesterol level					
Present	20	40	16	40	1, 1.000
Absent	30	60	24	60	
Smoking					
Present	37	74	30	75.0	1, 0.914

Table 2. *Chlamydia pneumoniae* IgA seropositivity among cases and controls

	Subjects	
***C. pneumoniae* serology**	**Cases**	**Controls**
IgA		
Positive	18 (36%)	6 (15%)
Negative	32 (64%)	34 (85%)
Total	50	40

P = 0.03, relative risk = 1.54, odds ratio = 3.18, 95% confidence interval = 1.12–9.04.

Results

There were no significant differences between cases and controls regarding sex ratio and mean age. Table 1 shows the frequency of cerebrovascular risk factors in cases: hypertension 48%, diabetes mellitus 40%, hypercholesterolemia 40%, and smoking 74%.

Tables 2 and 3 show that serum levels of anti-*C. pneumoniae* IgA were higher in stroke patients than in controls, whereas there was no statistically significant difference in serum levels of anti-*C. pneumoniae* IgG between stroke patients and controls. Where IgA was concerned, 35% of cases were seropositive and 15% of controls (*P* = 0.03). This difference was less striking for IgG, as 72% of cases were seropositive and 52.5% of controls (*P* = 0.078). The IgA seropositivity was associated with stroke risk (OR 3.18; CI 1.12–9.04; *P* = 0.03) as well as IgG seropositivity (OR 2.32; CI 0.97–5.58; *P* = 0.078).

Discussion

The notion that infection may be partly responsible for vascular occlusive disease is not new.[12] Different studies

Table 3. *Chlamydia pneumoniae* IgG seropositivity among cases and controls

	Subjects	
***C. pneumoniae* serology**	**Cases**	**Controls**
IgG		
Positive	36 (72%)	21 (52.5%)
Negative	14 (28%)	19 (47.5%)
Total	50	40

P = 0.078, relative risk = 1.48, odds ratio = 2.32, 95% confidence interval = 0.97–5.58.

yielded conflicting results.[12] We tried in this study to see whether our population had any special features.

The present study showed an association between the titers of anti-*C. pneumoniae* antibodies and the risk of ischemic stroke in Iraqi stroke patients. It further suggests that this association may be more important for IgA than for IgG antibodies. This is in agreement with Elkind et al[13] and Njamnshi et al,[14] who found a positive association between IgA but not IgG seropositivity with the occurrence of ischemic stroke.[13,14]

In this study, the percentage of seropositive ischemic stroke patients and controls is comparable with other studies, in which the percentage of seropositive *C. pneumoniae* ranged from 12.7% to 86.6% and in controls from 1.5% to 79%.[4,13,15–17] Our study's mean age was 58.02 ± 9.5 years, contrasting the Wimmer et al[4] and Haider et al[18] studies, which showed mean age as 34.6–36.5 years.

Several previously published studies focused on the correlation between the *C. pneumoniae* infections represented by the elevated serum levels of anti-*C. pneumoniae* IgA, IgG, and IgM antibodies and stroke occurrence.[9,13,14] A positive association between *C. pneumoniae* infection and stroke was demonstrated in eight studies.[4,9,13,14,16–19] The association between the levels of anti-*C. pneumoniae* antibodies was not found in other reports.[12,20]

Other studies support a hypothesis of an association between the aggregate number of chronic infectious burden of atypical respiratory pathogens such as *C. pneumoniae, Mycoplasma pneumoniae,* and *Legionella pneumophila* and the occurrence of stroke.[6]

Differences between these studies can be attributed to different inclusion criteria, different subtypes of stroke, variable degrees of statistical adjustment for confounding factors and different serological methods with different cutoff titers for seropositivity.

The present study used ELISA to detect the index levels of anti-*C. pneumoniae* IgA and IgG antibodies. Microimmunofluorescence was used in most of the previously published papers.[4,13,14,16,19] This study recruited only patients in the first hour of admission to avoid including patients with hospital-acquired infection; this resulted in a small sample size.

Conclusion

This study suggests that patients with ischemic stroke have an increased seropositive *C. pneumoniae* IgA antibody.

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