Chlamydia pneumoniae seropositivity in adults with acute ischemic stroke: A case–control study

N. K. Rai, R. Choudhary[1], R. Bhatia, M. B. Singh, M. Tripathi, K. Prasad, M. V. Padma

Departments of Neurology and [1]Microbiology, All India Institute of Medical Sciences, New Delhi, India

Abstract

Background: Causative role of *Chlamydia pneumoniae* infection in patients with acute ischemic stroke (AIS) remains unresolved till date. **Aim:** To investigate the role of *C. pneumoniae* antibodies in AIS. **Materials and Methods:** Patients with AIS and sex- and environment-matched controls were enrolled. Antibodies to *C. pneumoniae* (IgA, IgG and IgM) were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** A total of 51 patients and 48 controls were enrolled. The IgA seropositivity was significantly associated with AIS (unadjusted odds ratio 3.1; 95% CI 1.38, 6.96; $P = 0.005$), whereas IgG (unadjusted OR 0.44; 95% CI 0.18, 1.09; $P = 0.07$) and IgM (unadjusted OR 1.1; 95% CI 0.36, 3.3; $P = 0.88$) were not. There was no difference in IgA or IgG positivity in different stroke subtypes. On multivariate analysis after adjusting for sex, hypertension, diabetes mellitus, smoking and alcohol, the IgA seropositivity yielded an adjusted OR for stroke (4.72; 95% CI 1.61, 13.83; $P = 0.005$), while IgG seropositivity did not (OR 0.25; 95% CI 0.08, 0.83; $P = 0.23$). **Conclusions:** An increased risk of AIS was demonstrated in patients seropositive for *C. pneumoniae* for IgA antibodies.

Key Words

Acute ischemic stroke, *Chlamydia pneumoniae* serology, carotid artery's intima-media thickness, infections and stroke

For correspondence:

Dr. M. V. Padma, Department of Neurology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, India.
E-mail: vasanthapadma123@gmail.com

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Introduction

Chlamydia pneumoniae, a gram-negative, intracellular bacterium, is one of the most common pathogens in upper respiratory tract infections and is often asymptomatic.[1,2] Frequent epidemics due to *C. pneumoniae* have been reported every 5–7 years, with a 50–70% prevalence of seropositivity (in middle-aged adults).[3] Serological evidence of past infection with *C. pneumoniae* has been found in epidemiological studies to be associated with risk for atherosclerosis and cardiac disease,[4-6] although prospective cohort studies have not always confirmed this association.[7]

Due to the heterogeneity of etiopathogenesis of stroke and stroke subtypes, the association of *C. pneumoniae* infection with ischemic stroke can be more complex. Recent bacterial or viral infection has been shown to be associated with acute ischemic stroke (AIS).[8] The link between *C. pneumoniae* and cerebrovascular disease has been investigated in a number of seroepidemiological and antibiotic intervention studies.[9-19]

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However, the role of *C. pneumoniae* infection to acute stroke as a cause/effect/trigger remains controversial.[20]

Since infectious diseases in general are more common in India compared to Western countries, we aimed to investigate the role (if any) of *C. pneumoniae* antibodies in patients with AIS. Recent reports from South India suggest a positive association of *C. pneumoniae* infection with the occurrence of AIS.[16,18,19] Our aim was to investigate whether this is true also for a geographically and socially different group of patients from North India.

Materials and Methods

Patients with AIS admitted into the neurology services of All India Institute of Medical Sciences (AIIMS), New Delhi, were included in this study, which was approved by the Institutional Ethics Committee.

All the patients enrolled were within 1 week of stroke onset. All cases had undergone computed tomography (CT) or magnetic resonance imaging (MRI). Stroke subtyping was done using the Trial of Org 10172 in acute stroke treatment (TOAST) criteria into large-artery atherosclerosis (L), cardioembolic (CE), small artery occlusion (S), stroke of other determined etiology (O), and stroke of undetermined etiology (U).[21] Patients with acute respiratory tract infection, evident by clinical evaluation or on chest X-ray, were excluded.

As far as possible, age- and sex-matched family members, staying with the patient in the same environment, without history of stroke/TIA or acute respiratory tract infection were taken as controls.

From each case/control, 3 ml of serum was obtained and stored at −70°C until analysis. Samples were tested using *C. pneumoniae* IgG, IgM and IgA enzyme-linked immunosorbent assay (ELISA) method (EUROIMMUN, Lübeck, Germany, *C. pneumoniae* IgG, IgM and IgA ELISAs; Medizinische Labordiagnostika AG) by one blinded investigator. This is a semi-quantitative test, in which results are expressed as a ratio of extinction value of the control or patient sample over extinction value of the calibrator (ratio = extinction of the control or patient sample/extinction of the calibrator). A ratio ≥1.1 was taken as positive. Quantitative values of antibodies were obtained from the standard curve obtained by point-to-point plotting of extinction values against the corresponding units.

Statistical analysis was performed using SPSS 16.0 window software (statistical package for the social sciences, SPSS Inc., New Delhi). Continuous variables were presented in titer of mean and ±SD. Categorical variables were expressed as proportions. The Student's "*t*" test was used to test the differences in continuous variables and χ^2 test was used for categorical values. Multiple logistic regression analysis was performed before and after adjustment for potential confounders. All tests were two sided and *P* value <0.05 was considered statistically significant.

Results

Characteristics of stroke patients and control subjects are summarized in Table 1. Hypertension, smoking and diabetes were more frequent in patients. Only 48 controls could be selected as we could not find sex- and age-matched family members in 3 patients. Percentage of individuals seropositive to *C. pneumoniae* was significantly higher in stroke patients (60.8% of cases were seropositive for IgA antibodies vs. 33.3% of controls) (*P* = 0.005, unadjusted odds ratio 3.1; 95% CI 1.38, 6.96). This difference was less striking for IgG (62.7% of cases were seropositive vs. 79.2% of controls) (*P* = 0.07, unadjusted OR 0.44; 95% CI 0.18, 1.09) and for IgM (15.7% cases vs. 13.7% controls) (*P* = 0.78; unadjusted odds ratio 1.7; 95% CI 0.39, 3.5). After adjusting for sex, hypertension, diabetes, smoking and alcohol, IgA yielded a significant adjusted OR for stroke (4.72; 95% CI 1.61, 13.83; *P* = 0.005), while IgG seropositivity did not (0.25; 95% CI 0.08, 0.83; *P* = 0.23).

Stroke subgroup analyses of different classes of antibodies are given in Table 2. There was no difference in IgA or IgG positivity between different stroke subtypes. Numbers of patients positive for IgM were too small to analyze. Six patients were diabetic and all were positive for IgA, and five of them were positive for IgG. Mean total leukocyte count (TLC) was increased in IgA seropositive patients than those who were seronegative (11,594/mm^3 vs. 9545/mm^3). Erythrocyte sedimentation rate (ESR) was found to be increased in 22 of 31 IgA seropositive patients (70.7%), while it was found to be elevated in 12 of 20 IgA seronegative patients (60%).

Discussion

This case–control study provides evidence for an association between acute infection with *C. pneumoniae* (IgA antibodies) and the risk of AIS. It further suggests that this association was not demonstrable with IgG or IgM antibodies. IgM antibodies have been associated with primary or acute infection.

Many studies demonstrated a positive association between *C. pneumoniae* infection and stroke, yielding an OR of 1.71–8.58, and several did not. Table 3 summarizes the results seen in various studies.[9-11,13,14,20,22-24] *C. pneumoniae* IgG antibodies may be considered as markers of "chronic" infection and they could be in circulation for 3–5 years following infection. The IgA antibodies may be considered as markers of a primary, acute or chronic infection and stay in circulation on an average for 3–5 days or longer.[25] *C. pneumonia*e infection has been implicated to have a role in enhancing systemic inflammation and immune-mediated vascular damage, altering lipid metabolism, inducing the production of cytokines and lipoproteins.[20,26-29] These changes may adversely modify the conventional risk factors and aggravate atherosclerosis. *C. pneumoniae* is phagocytosed by alveolar macrophages and transported by blood stream to the vascular subendothelial region through the injured arterial endothelium.[30,31] Several studies demonstrated the presence of *C. pneumoniae* in atherosclerotic lesions in coronary

Table 2: Seropositivity in different stroke subtypes

	IgA (%)	IgG (%)	IgM (%)
Anterior circulation (45)	28/46 (60.1)	28/46 (60.1)	7/46 (15.2)
Post circulation (5)	3/5 (60)	4/5 (80)	1/5 (20)
Large artery (29)	17/29 (58.6)	19/29(65.5)	5/29 (17.2)
Small artery (17)	9/17 (52.9)	9/17 (52.9)	2/17 (11.8)
Cardioembolic (5)	5/5 (100)	4/5 (80)	1/5 (20)
Unknown etiology (1)	1/1	-	-

Table 1: Baseline characteristics of patients and controls

	Case (*n* = 51)	Control (*n* = 48)	*P* value	OR (95% CI)
Age (years)	53.6 ± 14.7	38.6 ± 13.8	<0.001	(9.3, 20.7)
Males (%)	35 (68.6)	34 (70.8)	0.8	(0.9, 2.1)
Hypertension (%)	23 (45)	3 (6.2)	<0.001	0.08 (0.02, 0.3)
Smoker (%)	23 (45)	7 (14.6)	0.001	0.21 (0.08, 0.55)
Diabetes mellitus (%)	6 (11.8)	2 (4.2)	0.17	0.33 (0.06, 1.70)
Alcohol	9 (17.6)	7 (14.6)	0.68	0.79 (0.27, 2.34)
IgA (%)	31 (60.8)	17 (33.3)	0.005	3.1 (1.38, 6.96)
IgG (%)	32 (62.7)	38 (79.2)	0.073	0.44 (0.18, 1.09)
IgM (%)	8 (15.7)	7 (13.7)	0.78	1.7 (0.38, 6.96)

Table 3: Odds ratios for stroke in relation to the levels of anti-*Chlamydia pneumoniae* IgA and IgG in different case–control studies (95% CI)

Study	Age (years)	Cases/controls (No.)	OR for IgA	OR for IgG
Wimmer *et al.*[13]	18–50	58/52	0.71 (1.08–2.70)[1,2]	1.91 (1.06–3.47)[3]
Cook *et al.*[9]	16–88	176[4]/1518	4.4 (3.0–6.5)[2]	4.2 (2.5–7.1)[2]
Elkind *et al.*[10]	>39	89/89	4.51 (1.44–14.06)[1,2]	2.59 (0.87–7.75)[2]
Heuschmann *et al.*[24]	No limit	145/260	NA	0.86 (0.44–1.67)[2]
Anzini *et al.*[23]	18–46	141/192	8.8 (3.9–19.1)[1,2]	2.2 (1.5–3.9)[1,2]
Ngeh *et al.*[38]	65–98	95/82	0.63 (0.26–1.52)[2]	1.32 (0.66–2.64)[2]
Johnsen *et al.*[42]	50–64	254/254	1.54 (0.96–2.47)[2]	1.28 (0.83–1.95)[2]
Njamnishi *et al.*[14]	26–80	64/64	4.29 (1.84–11.56)[1]	1.46 (0.68–3.22)
Elkind *et al.*[37]	>55	246/474	1.5 (1.0–2.2)[1,2]	1.2 (0.8–1.8)
Piechowski-Jóźwiak *et al.*[15]	<55	94/103	8.95 (4.44–18.07)[1,2]	0.85 (0.53–1.63)[2]
Alamowitch *et al.*[43]	18–85	483/483	1.54 (0.84–2.81)[2]	1.1 (0.80–1.51)[2]
Bandaru *et al.*[16]	3–82	200/200	18.3 (2.3–144.0)[2]	1.9 (1.4–3.2)[2]
Present study	>18	51/48	4.72 (1.61–13.83)[2]	0.25 (0.08–0.83)[2]

OR = Odds ratio; NA = Not applicable; [1]Statistically significant difference; [2]Adjusted OR; [3]Unadjusted odds ratio; [4]Stroke or TIA cases

arteries, aorta, and carotid arteries obtained from autopsy and endarterectomy specimens.[30,32,33] One Indian study which has shown association of high C-reactive protein (CRP) in stroke patients lends further credence to the hypothesis that infection with *C. pneumoniae*, acute or chronic, may be associated with atherosclerosis through the mechanism of low-grade inflammation.[16] This mechanism has been further supported by the finding of increased carotid artery's intima-media thickness (CIMT) in asymptomatic patients who are seropositive for *C. pneumoniae*.[18] Temporary regression of this thickness after antibiotic treatment further supports the role of *C. pneumoniae* in atherosclerosis and stroke.[17] However, two recent, large, randomized, controlled trials did not observe a beneficial effect of antichlamydial antibiotic therapy on the secondary prevention of cardiovascular heart disease. Neither trial used the serological marker of chronic *C. pneumoniae* infection during patient selection. *C. pneumoniae* IgA antibody was assessed in only 32.4% of the participants in one trial and in none of the participants in the other trial. In the absence of acute/chronic *C. pneumoniae* infection among the trial participants, there is little reason to expect a clinical benefit with antichlamydial antibiotic treatment.[34,35] Stroke is far more heterogeneous in nature than acute coronary syndrome and both cannot be equated in totality.

In our study, IgA seropositivity was more prevalent. In most of the patients, blood sample was drawn within 72 hours of stroke onset, minimizing any possibility of post-stroke acute rise in antibodies. In our study, the possibility of raised anti-*C. pneumoniae* antibodies due to acute respiratory tract infection was minimized by excluding patients with clinical or radiological evidence of lung infection. There are some differences between previous studies [Table 3] and our study, which require further explanation. Our controls were younger than the patients. This difference was due to our study design. Controls were selected from the same family, suggesting exposure to common environment, to equalize the exposure risk in both the groups. But it was difficult to get sex- and age-matched controls from the same family. We compromised age-matched controls to get from same sex, community and environment since it has been shown in a previous study that there was difference in seropositivity risk with sex but not with age.[29] One Indian study performed in patients with bronchial asthma has shown no difference of seropositivity rate in different age groups.[36]

Another important issue is the difference in the serum levels of *C. pneumoniae* antibodies in stroke subtypes. In a few studies, pathogenic role of *C. pneumoniae* was implicated mainly in lacunar infarction[37] and in some other studies, it was found to be associated with large artery atherosclerosis.[14,34] Most recently, some authors found increased risk for all subtypes of stroke in patients with elevated *C. pneumoniae* IgA but not IgG.[15,16,23] We did not find any difference amongst different stroke subtypes [Table 2]. Our patients with positive IgA serology had raised mean leukocyte count and raised ESR, pointing toward acute inflammation.

We used ELISA method for detecting the index levels of anti-*C. pneumoniae* IgA and IgG antibodies. Micro Immuno Florescence (MIF) method was used in most of the previously published papers and is considered as a reference standard in the *C. pneumoniae* serology.[38-40] However, an ELISA method is considered to be more objective (i.e. less subjective or operator dependent), and it has reasonably good reproducibility and good correlation with MIF.[41] It has been used in other studies with consistent results.[15,42,43] Our study has the main limitation of a small sample size.

In conclusion, the findings of this study show an association of IgA antibodies to *C. pneumoniae* in patients with AIS from North India. These findings support the theory that in some stroke patients, inflammation and immune reaction secondary to acute infection with *C. pneumoniae* may trigger an acute stroke. Raised IgA antibodies indicating probably recurrent acute infections may have set the stage for the acute ischemic event in connivance with other atherosclerotic risk factors in these patients. This study paves way for future larger studies, preferably a prospective population-based study, and by using other markers of systemic acute and chronic infection of *C. pneumoniae* along with markers of acute inflammation/thrombosis and acute phase reactants. Future role of chronic antibiotic therapy as a stroke prevention measure in high-risk individuals is an attractive concept, especially in a developing

country where infection and cerebrovascular disease contribute significantly to health mortality/morbidity in the society.

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