

ORIGINAL ARTICLES

Association between Chlamydia Pneumonia Seropositivity and Ischemic Stroke: A Case-Control Study

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Abstract:

Background: Apart from traditional risk factors, infectious agent might contribute to ischemic stroke. The aim of this study was to evaluate the association between Chlamydia pneumonia seropositivity and ischemic stroke. **Methods:** 42 ischemic stroke patients diagnosed by history, clinical examination and confirmed by CT scan or MRI of brain selected as case. The same number (42) of age and sex matched subjects having no history or clinical evidence of ischemic stroke were selected as control. Blood samples were collected within 2 to 14 days of ischemic stroke from indoor patients. Controls were collected from both indoor and outdoor patients with neurological disorders other than ischemic stroke. Anti-C. pneumoniae antibodies IgG and IgA were detected by ELISA (enzyme-linked immunosorbent assay) in the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU). **Results:** Among the study population, 66.7% of cases and 45.2 % of control patients were seropositive to C. Pneumoniae IgG (OR: 2.42, 95% CI: 1.00 - 5.85, p = 0.048). Whereas IgA were positive in 81% of case and 57.1% of control (OR: 3.19, 95% CI: 1.19 - 8.52, p = 0.018). Seropositivity to IgA showed more significant results than IgG. **Conclusion:** There was a significant association between Chlamydia pneumonia seropositivity both IgG and IgA with ischemic stroke.

Key words: Chlamydia pneumoniae, Ischemic stroke, Seropositivity etc.

Introduction:

Stroke is one of the major global health problems. Its incidence rises steeply with age and in many developing countries, it is rising because of the adoption of less healthy life style. It is caused by atherosclerotic as well as non-atherosclerotic mechanisms¹. Atherosclerosis is a multifactorial disease. It is a progressive inflammatory disorder of the arterial wall². Infectious agents have been proposed to a contributory factor in the

pathogenesis of atherosclerosis³. Known risk factors for ischemic stroke fail to account for all cases. Besides the conventional risk factors, organisms that cause chronic infections may contribute to the pathogenesis of ischemic stroke through atherosclerosis⁴. The microorganism most strongly implicated in the initiation and progression of atherosclerosis is the obligate intracellular gram negative bacterium *Chlamydia pneumoniae*, which commonly causes respiratory infections⁵. Infectious

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agents that cause chronic infections have been considered as activating factors of chronic inflammation. Additionally, infections may play a direct role in endothelial dysfunction implicated in atherosclerosis and may increase ischemic stroke risk by activation of thrombotic processes⁶. *C. pneumoniae* infection causes an increase in serum triglycerides and a decrease in HDL (high-density lipoproteins), thereby turning the lipid profile toward an atherogenic one³. There is an independent association between *C. pneumoniae* seropositivity and raised fibrinogen levels, thereby facilitate platelet aggregation and thrombus formation⁸. Furthermore, a chronic *C. pneumoniae* infection increases the expression of its own 60kDa heat shock proteins (HsP60), especially when they are persistently elevated. The host immune response to microbial HsP60 may gradually lead or contribute to autoimmunity to human HsP60 and consequently, to the development of atherosclerosis⁹. Serology has been the traditional method of diagnosing infection by *chlamydia pneumoniae*¹⁰. Primary *C. pneumoniae* infection is characterized by a significant immunoglobulin M (IgM) antibody response, a delayed IgG titer and a low IgA level. The presence of elevated IgG antibodies reflects prior infection with *C. pneumoniae* and IgG titres remain elevated for a prolonged period of 3 – 5 years. IgA antibodies last only 3 – 5 days in the circulation and are a marker of recent or persistent infection. The serological pattern of increased IgA and IgG titres has been suggested to indicate chronic persistence of active infection¹¹. Seropositivity usually is first detected at school age and rates generally increase by about 10% per decade. About 50% of individuals have detectable antibody at 30 years of age¹⁰. Evidence for the causal link between *C. pneumoniae* infection and ischemic stroke arise from seroepidemiology, detection of *C. pneumoniae* by PCR in carotid atherosclerotic plug, immunohistochemistry, culturing, and animal models. *C. pneumoniae* is known to cause persistent chronic infection and was found in atherosclerotic plaques in coronary and carotid arteries, and in the aorta by immunohistochemistry and PCR techniques¹². Few previous studies reported on the association

between *C. pneumoniae* infection and stroke risk¹. Case-control studies revealed that specific anti-*C. pneumoniae* antibody levels were significantly higher in patients with cerebrovascular disease than in control patients. So far our knowledge, limited study has been carried out on association between serum *Chlamydia pneumoniae* seropositivity and ischemic stroke patients in Bangladesh. So the aim of the present study was to know seroprevalence of *Chlamydia pneumoniae* infection in Bangladesh and its association with ischemic stroke patients.

Materials and methods:

This was a case-control study conducted in the Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. Following to a predefined protocol, two groups of subjects were recruited. First group (Stroke patients): Forty two ischemic stroke patients (26 males and 16 females) diagnosed by history, clinical examination and confirmed by CT scan or MRI of brain were selected as case. Inclusion criteria were– age > 18 Years and patient or patient's legal guardian willing to participate. Exclusion criteria were– age ≤18 years, patients with hemorrhagic stroke, transient ischemic attack and venous stroke, patients with atrial fibrillation, valvular heart disease, prosthetic heart valve or recent myocardial infarction (<6 weeks) and patients not willing to take part in this study. All ischemic stroke patients were selected from indoor, Department of Neurology and blood samples were collected within 2 to 14 days of ischemic stroke. Second group (control subjects): The same number (26 males and 16 females) of age and sex matched subjects having no history or clinical evidence of ischemic stroke were selected as control. Control subjects were the patients with neurological disorders other than ischemic stroke. Control patients were selected from both indoor and outdoor, Department of Neurology.

Other risk factors for atherosclerosis have been studied for both groups which include hypertension, diabetes, dyslipidemia, smoking and previous vascular events. Hypertension, diabetes mellitus (DM), and dyslipidemia were diagnosed according to established criteria.

With all aseptic precaution 3 to 4 ml venous blood samples were collected by standard venipuncture in a sterile test tube. After collection, samples were sent immediately to the Department of Virology, BSMMU. Sera were separated by centrifugation and stored in -20°C until analysis for anti- *Chlamydia pneumoniae* antibody IgG and IgA. Sera from both patient and control subjects for anti-*Chlamydia pneumoniae* antibody IgG and IgA were detected by ELISA (enzyme-linked immunosorbent assay) method in ETI max-3000 in the Department of Virology, BSMMU. All other relevant investigations were done in the respective Department of Bangabandhu Sheikh Mujib Medical University. Continuous variable was expressed as Mean \pm SD. Categorical variable was presented by frequency and percentage. Qualitative data were analyzed by chi-square test. Quantitative data was analyzed by unpaired t-test. A p-value of < 0.05 was considered statistically significant. Statistical analysis was done using SPSS (Statistical package for social sciences) win version 22 software programme. Approval from the Institutional Review Board (IRB) of BSMMU was obtained prior to the commencement of this study. The aim and objective of the study along with its procedure, risk and benefits were explained to the respondents in easily understandable local language and informed written consent was taken from each. It was assured that all information and record will be kept confidential.

Ischemic Stroke:

An episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction attributable to ischemia, based on

1. Pathological, imaging, or other objective evidence of cerebral, spinal cord or retinal focal ischemic injury in a defined vascular distribution; or
2. Clinical evidence of cerebral, spinal cord, or retinal focal ischemic injury based on symptoms persisting \geq 24 hours or until death, and other etiologies excluded¹³.

Results:

A total of 84 patients were assessed in this study. Forty two ischemic stroke patients were enrolled as case and compared with 42 control subjects. In case group, most 15 (33.3%) were belonged to the age group 51-60 years followed by 14 (31%) patients in the age group 61-70 years. In control group, most frequent age groups were 51 – 60 years and 61 – 70 years, each representing 15(35.7%) patients. The mean (\pm SD) age among cases was 61.74 \pm 10.86 and in control 60.29 \pm 8.40 years (Table-1).

Male and female were equally distributed between case and control groups. Out of 42, male were 26 (61.9%) and female were 16 (38.1%) in each group with a ratio of 1.6:1. In case group out of 42 patients, most 15 (35.7%) were housewife followed by 13 (31%) were businessman. In control group most 16 (38.1%) were businessman (38.1%) followed by 15 (35.7%) were housewife.

Among the study population, diabetes mellitus was present in 59.5% of case and 33.3% of control patients, hypertension was 83.5% and 47.6% in

Table-I
Distribution of the study subjects according to age in case and control (n=84)

Age (years)	Case (n=42)n (%)	Control (n=42)n (%)	p-value
41 - 50	8 (19.0)	8 (19.0)	
51 - 60	14 (33.3)	15 (35.7)	
61 - 70	13 (31.0)	15 (35.7)	
>70	7 (16.7)	4 (9.5)	
Mean \pm SD	61.74 \pm 10.86	60.29 \pm 8.40	0.495

Unpaired t test was done to measure the level of significance and p-value < 0.05 was considered as significant.

case and control respectively. Dyslipidemia was present 81.0% of case and 54.8% of control patients. Hypertension (OR:2.96, 95% CI:1.99-15.13, p=0.001), Diabetes mellitus (OR:2.94, 95% CI: 1.207-15, p=0.016) and dyslipidemia (OR:3.51, 95% CI:1.31-9.36, p=0.010) were statistically significant. Previous vascular event (14.3% of case and 7.1% of control), family history of stroke (16.7% of case and 7.1% of control) and smoking (57.1% of case and 38.1% of control) were more frequent in case than control patients but did not reach the statistically significant level (Table-2).

Chlamydia pneumoniae IgG antibodies were detected in 28 (66.7%) case and 19 (45.2%) control patients. The difference between the two groups was statistically significant (OR: 2.42, 95% CI: 1.00-5.85, p=0.048). The seroprevalence of

Chlamydia pneumoniae IgA were 81% in case compared to 57.1% in control group. This difference was also statistically significant (OR: 3.18, 95% CI: 1.19-8.52, p=0.018). Combined IgG and IgA antibodies were found in 22 (52.38%) among case and only 8 (19.04%) in control, revealed most significant results (OR: 4.67, 95% CI: 1.75-12.45, p=0.0014) (Table-3).

Brain imaging findings among the cases, 36 (85.7%) were MCA territory stroke. PCA territory stroke were 4 (9.5%) and ACA territory stroke and lacunar stroke were only 1 (2.4%) in each (Figure-1). Out of 42 stroke patients, most 36 (85.71%) patients had hemiplegia, followed by speech difficulty in 23(54.71%) patients, facial weakness in 16(38.09%) patients.

Table-II
Risk factor of the study subjects in case and control (n=84)

Risk factors	Case (n=42) n (%)	Control (n=42) n (%)	p-value	OR	95%CI
DM	25 (59.5)	14 (33.3)	0.016	2.94	1.20-7.15
HTN	35 (83.5)	20 (47.6)	0.001	5.50	1.99-15.13
Dyslipidemia	34 (81.0)	23 (54.8)	0.010	3.51	1.31-9.36
Previous vascular event	6 (14.3)	3 (7.1)	0.483	2.16	0.50-9.31
Family history	7 (16.7)	3 (7.1)	0.178	2.60	0.62-10.83
Smoking	24 (57.1)	16 (38.1)	0.081	2.16	0.90-5.18

Chi-square test was done to measure the level of significance and p-value < 0.05 was considered as significant.

Table-III
Chlamydia pneumoniae antibody of the study subjects in case and control (N=84)

Chlamydia pneumoniae antibody	Case (n=42) n (%)	Control (n=42) n (%)	p-value	OR	95%CI
IgG	28 (66.7)	19 (45.2)	0.048	2.42	1.00-5.85
IgA	34 (81.0)	24 (57.1)	0.018	3.18	1.19-8.52
Combined	22 (52.38)	8 (19.04)	0.0014	4.67	1.75-12.45

Chi-square test was done to measure the level of significance and p-value < 0.05 was considered as significant.

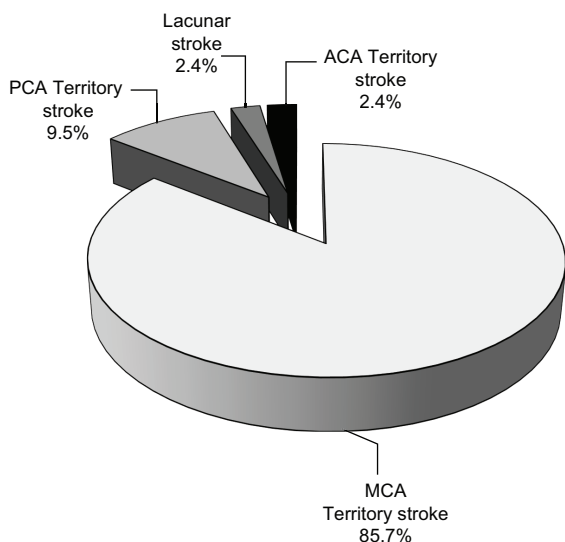


Fig.-1: Pie chart findings CT scan of head/MRI of brain in cases (n=42)

Discussion:

This present study investigated to find out the association between *Chlamydia pneumonia* seropositivity and ischemic stroke. In this case-control study, total 42 cases were enrolled and compared with 42 control subjects. In this study some relevant risk factors of ischemic stroke and some demographic profile like age, sex and occupation were also evaluated. The mean (\pm SD) age among cases was 61.74 ± 10.86 ranging from 40 to 81 years with male female ratio of 1.6:1. The maximum numbers (33.3%) of the patients were in the 51-60 years age group. Kenina et al., (2011)¹⁴ and Alamowitch et al., (2007)⁶ found that mean age of 63.19 ± 11.3 and 66 ± 13 years and a male/female ratio of 1.68:1 and 1.48:1 respectively in European population. Study in Indian population, Rai, et al., (2011)¹⁵ found that mean age was 53.6 ± 14.7 years with male female ratio of 2.18. Stroke prevalence is generally more in male that is found in all of these studies. Regarding mean age of patients, it is variable in different countries because of variation of life expectancy and other co-morbidities. In this study, hypertension was found in 83.5% in cases and 47.6% in control. In the Northern Manhattan stroke study, Elkind et al., (2000)¹⁶ was found hypertension in 75.3% in cases and 49.4% in control group. Another study in

Latvian population, kenina, et al., (2011)¹⁴ was found 84.3% and 37.5% in case and control group respectively. Those studies showed that hypertension was more frequently present in case than control group which was similar to this study. According to Saha, et al (2016)¹⁷ hypertension was the commonest risk factor among the stroke patients which was found all of these studies. Diabetes mellitus was found 59.5% of case and 33.3% of control patient in this study. In the Northern Manhattan stroke study, Elkind et al., (2000)¹⁶ found diabetes mellitus in 36% of case and 17% of control group. Similarly, three case-control studies done by Bandura et al., (2008)¹⁸; Rai et al., (2011)¹⁵; Srivastava et al., (2014)¹⁹ in the Department of Neurology, All India Institute of Medical Sciences found diabetes mellitus in the following frequencies among case and control group: 11.8% and 4.2%; 21% and 11%; 23.4% and 15% respectively which support steady increase in incidence of diabetes mellitus and higher frequency in this study. All of these studies showed diabetes mellitus is more frequent in case group than control group.

In this study, dyslipidemia was found in 81% of cases and 54.8% of control patients. Kenina et al., (2011)¹⁴ found dyslipidemia in the frequency of 47% in cases and 12.5% in control. Srivastava et al., (2014)¹⁸ found hyperchlesterolaemia 29.4% and 18% among case and control group. But no differences was seen (40% in both case and control group) by Hasan, (2011)²⁰. In this study, smoking was found in 57.1% of case and 38.1% of control group. A considerable number (16 out of 42) of patients were female who were non-smoker in each group. So that difference in the frequency of smoking between case and control was not statistically significant in this study. Similar difference in the frequency of smoking was found by Alamowitch et al., (2008)⁶ and Srivastava et al., (2014)¹⁸, 29.3% in case and 21.1% in control; 20.14% in case and 12.2% in control respectively.

In this study, anti-*Chlamydia pneumonia* seropositivity was found significantly higher in cases. In cases, out of 42 patients, 28 (66.6%) were positive for IgG and 19 (45.2%) were positive in control group. This difference was statistically

significant (OR: 2.42, 95% CI: 1.00-5.85, $p = 0.048$). The prevalence of IgA was higher in study population. Seropositivity to IgA was found 81% in case and 57.1% in control group. This difference was statistically more significant (OR: 3.18, 95% CI: 1.19-8.52, $p = 0.018$) than IgG. However, when combined (both IgG and IgA) status was compared between case and control group, a striking differences was found. In case 22 (52.38%) was positive to both IgG and IgA compared to 8 (19.04%) were in control group. That was much more significant (OR: 4.67, 95% CI: 1.75-12.45, $p = 0.0014$). Johnsen et al., (2005)²¹ found similar result, the combined status differences (OR: 1.77, 95% CI: 1.043.00) compared to IgG (OR: 1.28, 95% CI: 0.83-1.95) and IgA (OR: 1.54, 95% CI: 0.96-2.47). Eini et al., (2014)²² found that 30% of cases were IgG positive vs 15% of control ($p = 0.016$) and regarding IgA, 67% were positive in cases compared to 15% in control ($p = 0.0001$). Similarly Piechowski-Jozwiak, et al., (2007)²³ found IgG in 78.7% of case and 52.5% of control ($p = 0.0001$) and IgA were 41.1% in cases and 15.6% in control group ($p = 0.0001$). These studies showed statistically significant results for both IgG and IgA which was found in this study. Furthermore, IgA was more significant than IgG, similar to this study. In Indian study, Rai, et al., (2011)¹⁵ and Srivasta, et al., (2014)¹⁸ showed that seropositivity was significantly higher in stroke patient but difference was less striking for IgG. Only IgA seropositivity yielded statistically significant result (p value were 0.005 and 0.003). Similarly, Alamowitch et al., (2008)⁶; Hasan (2011)¹⁹; Elkind et al. (2006)²⁴; Madre et al., (2002)²⁵; Njamnshi et al., (2006)²⁶; Wimmer et al., (1996)¹¹ found statistically significant differences between case and control group with respect to IgA. In a systematic review and meta-analysis done in China by Chen et al., (2013)²⁷ selected 42 studies performed in case-control and cohort design showed an association between *C. pneumoniae* infection and cerebrovascular disease revealed by serum IgG and IgA as well as PCR technique in peripheral blood cells. (OR: 1.9; 95% CI: 1.17 to 3.07).

Limitation:

Our study was done in short period, with a small sample size. Also, the method of sampling was not random rather purposive. Rather than measurement, only detection of antibodies was performed. So correlation with antibodies level cannot be done. Imaging was not done in all control patients, some control patient could have silent infarcts which may underestimate or overestimate the association between *Chlamydia pneumoniae* seropositivity and ischemic stroke. Patients of neurological diseases other than ischemic stroke were selected as control rather than healthy control.

Recommendation:

Population based study should be done to find out the prevalence of serological marker of *Chlamydia pneumoniae* infection in Bangladeshi population. Further multi-centered large scale studies should be carried out to consider chronic *Chlamydia pneumoniae* infection as a risk factor of ischemic stroke. Study period should be extended. Sample size should be large. Other diagnostic technique like PCR may be included to support this association.

Conclusion:

In conclusion, this study revealed that there was a significant association between *Chlamydia pneumoniae* seropositivity both IgG and IgA with ischemic stroke. This association was stronger for IgA. Moreover, combined seropositivity to IgG and IgA yielded striking significance. So, there may be an increased risk of ischemic stroke in patients seropositive to anti-*Chlamydia pneumoniae* IgG and IgA. Anti-Chlamydial therapy may be a potential preventive measures for stroke risk patients.

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