ORIGINAL ARTICLE

# Seroprevalence of *Chlamydia Pneumoniae* Antibodies in Stroke in Young

*V.C.S. Srinivasarao Bandaru, D. Babu Boddu, V. Laxmi, M. Neeraja, S. Kaul*

**ABSTRACT:** ***Background:*** Younger patients, aged below 45 years, usually lack the conventional risk factors of stroke whereas infections, especially in developing countries, may play a role. There have been many reports in the last decade about the association of *Chlamydia pneumoniae* (*C.pneumoniae*) and atherosclerosis involving cerebral vessels. ***Objective:*** To investigate the seroprevalence of *C.pneumoniae* IgG and IgA antibodies in patients aged below 45 years with acute ischemic stroke. ***Methods:*** This study was done at a tertiary care hospital in South India between January 2004 and December 2006 where we recruited consecutive patients aged less than 45years with acute ischemic stroke. Age and sex matched controls were recruited from the outpatient department with non stroke diagnosis. All stroke patients underwent CT (Computerized Tomography), MRI (Magnetic Resonance Image), MRA (Magnetic Resonance Angiography), Transthoracic Echocardiography and Carotid Doppler for stroke sub group diagnosis. We measured *C.pneumoniae* antibodies IgG and IgA by microimmunofluorescence technique in all patients and controls. ***Results:*** A total of 120 patients and 120 controls were studied over a period of two years. We found *C.pneumoniae* antibodies in 29.1% (35/120) stroke patients and in 12.5% (15/120) control subjects (p=0.002). *C.pneumoniae* IgG antibodies were found in 27.5 % (33/120) of stroke patients and 12.5% (15/120) of controls (p=0.006). IgA antibodies were observed in 5 % (6/120) of strokes and none in control group (p=0.03). After adjustment of all risk factors *C.pneumoniae* IgG seropositivity showed odds ratio of 2.6; 95% Confidence Interval 1.2-5.6. ***Conclusions:*** *C.pneumoniae* IgG antibodies were found to be associated with ischemic stroke in young.

**RÉSUMÉ: Séroprévalence des anticorps dirigés contre Chlamydia pneumoniae chez les jeunes patients atteints d'un accident vasculaire cérébral.** ***Contexte :*** Les patients de moins de 45 ans ne sont habituellement pas porteurs des facteurs de risque conventionnels de l'accident vasculaire cérébral (AVC) et les infections, spécialement dans les pays en voie de développement pourraient jouer un rôle dans cette pathologie. Plusieurs articles ont été publiés au cours des dix dernières années au sujet de l'association entre le Chlamydia pneumoniae (C.pneumoniae) et l'athérosclérose des vaisseaux cérébraux. ***Objectif :*** Le but de l'étude était d'examiner la séroprévalence des anticorps de classes IgG et IgA antiC.pneumoniae chez les patients de moins de 45 ans qui présentent un (AVC) ischémique aigu. ***Méthodes :*** Cette étude a été réalisée dans un hôpital de soins tertiaires dans le Sud de l'Inde entre janvier 2004 et décembre 2006. Nous avons recruté de façon consécutive les patients âgés de moins de 45 ans qui présentaient un AVC ischémique aigu. Des témoins appariés pour l'âge et le sexe ont été recrutés parmi les patients de la clinique externe qui ne présentaient pas d'AVC. Tous les patients atteints d'un AVC ont subi une tomodensitométrie, une IRM (imagerie par résonance magnétique), une ARM (angiographie par résonance magnétique), un échocardiogramme transthoracique et un Doppler carotidien pour allocation à des sous-groupes selon le diagnostic. Nous avons mesuré les taux d'anticorps de classes IgG et IgA antiC.pneumoniae par micro-immunofluorescence chez les patients et les témoins. ***Résultats :*** Nous avons étudié 120 patients et 120 témoins au cours d'une période de deux ans. Nous avons détecté des anticorps antiC.pneumoniae chez 29,1% (35/120) des patients atteints d'AVC et chez 12,5% (15/120) des sujets témoins (p = 0,002). Des anticorps de classe IgG étaient présents chez 27,5% (33/120) des patients atteints d'AVC et chez 12,5% (15/120) des témoins (p = 0,006). Des anticorps de classe IgA étaient présents chez 5% (6/120) des patients atteints d'AVC et chez aucun des témoins (p = 0,03). Après avoir ajusté les données pour tous les facteurs de risque, le rapport de cotes pour la séropositivité de classe IgG à C.pneumoniae était de 2,6 et l'intervalle de confiance à 95% était de 1,2 à 5,6. ***Conclusion :*** Des anticorps de classe IgG contre C.pneumoniae étaient associés à l'AVC ischémique chez les jeunes patients.

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Etiology of ischemic stroke is multi factorial and infections have emerged as one among them. *C.pneumoniae* is an obligate intracellular gram negative bacterium. It is a common respiratory pathogen[1] causing acute or chronic respiratory diseases and is known to exacerbate cystic fibrosis and asthma.[2] In the last decade several reports have shown the association of chronic *C.pneumoniae* infection with atherosclerosis and thrombosis.[3-6] Many studies have incriminated *C.pneumoniae* in the causation of coronary heart disease, stroke[7-9] and asymptomatic carotid atherosclerosis.[10] In fact, patients having an infection within a week before the onset of stroke might develop cortical middle cerebral artery infarcts, cardioembolic infarcts and arterial dissections suggesting a differential effect of infection.[11] In older patients, various conventional risk factors (diabetes, hypertension, hypercholesterolemia, etc) play an important role in stroke etiopathogenesis. However, younger patients (aged <45

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years) usually lack these risk factors, and infections, especially in developing countries, may assume significance. It is therefore relevant to look for the role of infection in contributing to various subgroups of ischemic stroke in young patients. We aimed to investigate the prevalence of *C.pneumoniae* antibodies (IgG & IgA) in young patients with acute ischemic stroke and its various subgroups.

## Methods

This was a prospective case control study performed at Nizam's Institute of Medical Sciences, Hyderabad in South India between January 2004 and December 2006. The design of the study was approved by the University Ethics Committee. Consecutive patients, aged <45 years, with first ischemic stroke who admitted to our stroke unit within first 72 hours after stroke onset were included. Age and sex matched controls without any evidence of cerebral or coronary artery disease (CAD) were recruited from out patient department and patients admitted to the department of Neurology with non-vascular diseases. Presence of sepsis and acute respiratory infections either in patients or in controls were excluded. All subjects gave their written informed consent to take part in the study. We obtained the consent from their next of kin or guardian if the patients were unable to do so. Data regarding demographic characteristics and vascular risk factors (hypertension, diabetes, hyperlipidemia, ischemic heart disease and smoking) were recorded. Stroke was defined, according to the World Health Organization, as "rapidly developing clinical signs of focal/global disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin".[12] Cerebral infarction was diagnosed on the basis of results of the first CT (Computer Tomography) or MRI (Magnetic Resonance Imaging) scan of the brain. All subgroups of ischemic stroke were included.

Data were collected through face-to-face interviews of cases and control subjects by the supervisor of this project. Physical and neurological examination was performed by the stroke specialist. We have used standard methods to measure blood pressure, height, weight, fasting blood specimen lipids (including Total cholesterol, Low density lipoprotein (LDL), High density lipoprotein (HDL), Very low density lipoprotein (VLDL), and Triglycerides), homocysteine and glucose in all cases and control subjects.

According to the Joint National Committee VI-VII, hypertension was defined as a systolic blood pressure >140mmHg and /or a diastolic blood pressure >90mmHg based on the average of the two blood pressure measurements, or a patient's self-reported history of hypertension or antihypertensive use, supported by documents.[13] Diabetes was diagnosed if fasting plasma glucose was >110 mg/100ml or patient was on anti-diabetic medications.[14] Individuals who had never smoked were classified as non-smokers; those who currently smoked or had quit within the last 12 months were classified as current smokers. Individuals who had quit smoking >12 months before inclusion were classified as ex smokers.[15] Alcoholics were defined as those in whom the alcohol consumption was >50 g/day (equivalent to 500ml [two drinks] of wine, 1000ml of beer , or >5 drinks [units] of spirits).[16] Hyperhomocysteinemia was defined as >15mg/100ml of serum.[17] According to the National Institute of Health U.S.A. guidelines revised in 2001, serum cholesterol <200mg/100ml is desirable, 200-239mg/100ml is considered borderline high, and >240mg/100ml is considered high. We considered serum cholesterol levels >200mg/100ml or those on anticholesterol medication as having hypercholesterolemia.[18] Strokes were classified as extracranial large artery athero-sclerosis, intracranial large artery atherosclerosis, cardio-embolic, small vessel disease (lacunar), stroke of other determinate etiology and stroke of un-determined etiology.[19]

### *Estimation of C.pneumoniae antibodies*

The obtained blood samples of cases and control subjects were centrifuged, and aliquoted into 1 ml specimens. These were frozen at -70°C until the time of analysis for IgG and IgA antibody titers to *C.pneumoniae* using microimmuno-fluorescence.[8] The presence of *C.pneumoniae* IgG and IgA antibodies in serum was determined by indirect immuno-fluorescence test using Euroimmun BIOCHIP slide kit (commercial kit, Germany). IgG and IgA serum titers of 1:100 were judged to be positive as per the manufacturer's instructions and were interpreted as a current or earlier *C.pneumoniae* infection. Serum C-Reactive protein (CRP) titers 1:16 (96mg/l) were diagnosed to be positive as per HUMATEX CRP (Germany) kit specifications.

### *Statistical analysis*

Statistical analysis was done using SPSS 14.0 window software (statistical package for the Social sciences, SPSS Inc). Continuous variables were presented in titer of mean ±SD. Categorical variables were expressed as proportions. The student 't' test was performed to test the differences in continuous variables, and $x^2$ test was used to study the association in proportions. Multiple logistic regression was performed before and after adjustment for potential confounders. All tests were two sided and p value <0.05 was considered statistically significant.

## Results

We studied 120 young patients with acute ischemic stroke and 120 age and sex matched controls over a period of two years. There were no differences between the two groups regarding age (p=1), sex (p=1), diabetes (p=0.2), smoking (p=0.3), alcohol intake (p=0.5), hypercholesterolemia (p=1) and hyperhomo-cysteinemia (p=0.6). Other stroke risk factors like hypertension (p<0.0001) and *C.pneumoniae* seropositivity (p= 0.002) were significantly more in stroke patients compared to control group (Table 1). We observed protein C and protein S deficiency in two patients. We did not encounter arterial dissection and seropositivity for HIV and Syphilis. 2 out of 30 patients with cardioembolic stroke had patent foramen oval (PFO) on transesophageal echo (TEE).

IgG *C.pneumoniae* antibodies were present in 33 patients and 15 controls (p=0.006); IgA *C.pneumoniae* antibodies were present in six patients and none in control group (p= 0.03) (Table 2). In the patient group, IgG antibodies were found in 33, IgA in four and both were positive in two patients (Figure). Multiple logistic regression showed *C.pneumoniae* IgG antibody



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**Table 1: Comparison of baseline data between controls and stroke patients <45 years**

| Parameters | Stroke patients (n=120) | Control patients (n=120) | p value |
|---|---|---|---|
| Men | 90(75%) | 90(75%) | 1 |
| Mean age(SD) | 35.3(9.5) | 35.3(9.5) | 1 |
| Age range | 3-45 | 3-45 | 1 |
| Hypertension | 42(35%) | 13(10.8%) | <0.0001 |
| Diabetes | 14(11.6%) | 8(6.6%) | 0.2 |
| Smoking | 26(21.6%) | 19(15.8%) | 0.3 |
| Alcoholism | 26(21.6%) | 21(17.5%) | 0.5 |
| Hypercholesterolemia | 25(20.8%) | 26(21.6%) | 1 |
| Hyperhomocysteinemia | 30(25%) | 26(21.6%) | 0.6 |
| *C.pneumoniae* | 35(29.1%) | 15(12.5%) | 0.002 |

positivity was an independent risk factor for ischemic stroke in young Indian patients (odds ratio 2.6; 95% Confidence Interval 1.2-5.6).

In sub types of ischemic stroke in young patients, the prevalence of *C.pneumoniae* antibodies was found to be 45% in intracranial large artery atherosclerosis, 30% in cardioembolic stroke, 28.5% in small artery stroke and 25% in extracranial large artery and 29.5% in stroke of other determined etiology, (Table 3). Both *C.pneumoniae* and CRP positivity was found in 29% of patients with stroke compared to 12.5% of controls (p=0.005) (Table 4).

## Discussion

In this study we found an association between the presence of *C.pneumoniae* antibodies and ischemic stroke among young Indian patients. This is in agreement with previous studies where *C.pneumoniae* antibody positivity has been noted in young stroke patients.[20-22] In Cameroon, Njamnshi AK et al demonstrated *C.pneumoniae* infection significantly associated with ischemic stroke in patients in the < 50 years age group compared to matched controls.[23] Piechowski-Jozwiak B et al found *C.pneumoniae* seropositivity had increased the risk of stroke in young patients aged below 55 years (odds ratio 9.35 95% Confidence Interval 4.78-18.2, p<0.001).[24]

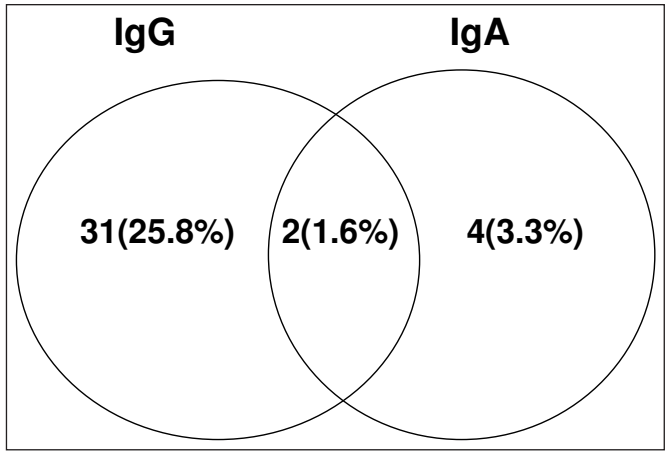


***Figure:*** *Distribution of C.pneumoniae antibodies in young stroke patients.*

In this prospective case-control study, we found that positive serum IgG and IgA antibody titer by microimmunofluorescence (MIF) test, against *C.pneumoniae* was significantly more in ischemic stroke patients compared to age and sex matched control subjects. Even though there are many tests available, the MIF test is the only currently acceptable serologic test for detection of *C.pneumoniae* antibodies, according to the Center for Disease Control and Prevention (CDCP) and the Laboratory Center for Diseases (Canada).[25] The MIF test is widely accepted as the "gold standard" in *C.pneumoniae* serodiagnonosis.[26,27]

### *C.pneumoniae antibodies in Stroke*

Our results have shown that *C.pneumoniae* IgG antibody positivity is more prevalent and found to be an independent risk factor for ischemic stroke in young Indian patients (odds ratio 2.6; 95% Confidence Interval 1.2-5.6), which is comparable to the study done by Hertzen LV et al who have reported raised *C.pneumoniae* IgG titers were a significant independent risk factor for mortality from cerebrovascular disease in Finnish patients (odds ratio 4.93: 95% Confidence Interval 1.4-17.3).[28]

**Table 2: Prevalence of *C.pneumoniae* antibodies**

| Parameters | Stroke patients (n=120) | Control patients (n=120) | p value |
|---|---|---|---|
| IgG antibody | 33(27.5%) | 15(12.5%) | 0.006 |
| IgA antibody | 6(5%) | 0 | 0.03 |



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**Table 3: Percentage of *C.pneumoniae* antibody positivity in various strokes subgroups**

| Subtypes of Ischemic stroke | Number (%) (n=35/120) |
|---|---|
| Extra cranial large artery atherosclerosis | 5/20(25%) |
| Intracranial large artery atherosclerosis | 14/31(45%) |
| Cardio embolism | 9/30(30%) |
| Small artery disease | 2/7(28.5%) |
| Stroke of other determined etiology | 5/17(29.5%) |
| Stroke of undetermined etiology | 0/15 |

Agarwal et al showed in their study that *C.pneumoniae* IgG antibodies were significantly elevated in Indian patients with CAD.[29]

In a previous study, we found IgA anti Chlamydial antibodies were significantly associated with stroke in patients age ranging between 3 to 82 years. We did not analyze separately the *Chlamydia* seropositivity in patients aged below 45 years in that study. In the present study we exclusively studied young patients (age below 45 years) with acute ischemic stroke in whom the conventional risk factors of stroke were less common. Interestingly we found IgG as a significant risk factor for stroke in young which was in contrast to our previous study.[30] The plausible reason could be that many of the patients in the present study had past history of respiratory infections and they were from low socioeconomic status which might predispose them for chronic *C.pneumoniae* infection as evidenced by IgG seropositivity.

Although there are no satisfactory serologic tests available to detect chronic *C.pneumoniae* infection,[25] several researchers have suggested that *C.pneumoniae* IgG antibody indicates past infection and IgA antibody is a marker of chronic persistent infection. It has recently been demonstrated that IgG antibodies titers but not IgA antibodies correlated with the ability to detect *Chlamydia* organism in human coronary arteries at autopsy.[31]

The presence of *Chlamydia* IgG antibody is indicative of *Chlamydia* infection at an undetermined time. IgG antibodies persist for long periods and decline very slowly. High levels of IgG antibodies are of diagnostic value in chronic *Chlamydia* infection. With in 72 hours of stroke onset we collected blood sample for *C.pneumoniae* assay, as IgA half life is six days and IgG half life is 23 days. In our study, overall seroprevalence of *Chlamydia* specific IgG antibodies was 27.5% and IgA antibodies was 5%. Wang et al have recommended in a follow up study of persons with long lasting IgG antibodies, that a large portion of subjects never produce IgA antibodies for unidentified reasons. Therefore, the role of IgA antibody as a marker for chronic *Chlamydia* infection is questionable.[32]

### *C.pneumoniae and stroke subgroups*

In this study *C.pneumoniae* seropositivity was observed in all ischemic stroke subgroups except stroke of indeterminate etiology. Other studies have also described the association of *C.pneumoniae* in various stroke subgroups in all age groups.[8] Another retrospective study showed *C.pneumoniae* infection more prevalent in large artery atherosclerosis.[20] *C.pneumoniae* infection causes atherosclerosis of large arteries by infecting the vascular endothelial cells, activation of the NFκβ,[33] up regulation of procoagulant activity (tissue factor, plasminogen activator inhibitor), increased platelet count, adhesion molecules and finally thrombosis formation.[34] The positivity of *C.pneumoniae* antibodies in nonatherosclerotic strokes like small artery disease and stroke of other determined etiology caused by pathophylogical changes due to lipohyalinosis or hypercoagulability may be an epiphenomenon.

### *C-reactive protein and C.pneumoniae in stroke*

CRP is a marker of inflammation.[35] Several studies have found that *C.pneumoniae* infection could contribute to elevation of CRP levels and to the instability or progression of atherosclerotic plaques.[36,37] To explore the inflammatory mechanism of *Chlamydia* we have estimated CRP. In this study both *C.pneumoniae* and CRP positivity was found in 29% of patients and it was significantly associated with stroke compared to controls subjects (p=0.005).

The strength of our study was that both cases and controls were drawn from the same hospital. Serological testing with MIF, used in our study, is the "gold standard" for clinical diagnosis of *C.pneumoniae* infection. Other postulated tests, such as polymerase chain reaction (PCR) and flow cytometry, remain under investigation. Multiple logistic regression test was used to study whether positivity of *C.pneumoniae* antibodies is

**Table 4: C-reactive protein present in stroke and control subject**

| Parameters | Stroke (n=120) | Controls (n=120) | p value |
|---|---|---|---|
| CRP positive & *C.pneumoniae* positive | 35(29%) | 15(12.5%) | 0.005 |
| CRP positive & *C.pneumoniae* negative | 32(26%) | 28(23%) | 0.8 |



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an independent risk factor or associated risk factor for stroke.

There are limitations in our study. Due to the small sample size of each subgroup of stroke, multiple logistic regression analysis could not be done in subgroups. This study is based on serological tests. Serologic tests detect antibodies to a specific micro-organism, which indicates that infection with the micro-organism, took place at some point in time. However, absolute proof of the micro-organism's actual involvement in the process of atherosclerosis could only come from demonstrating its presence in the vascular wall.

### *Therapeutic Implications*

If persistent *C.pneumoniae* infection contributes to vascular events such as stroke, it is interesting from a therapeutic perspective. Although *C.pneumoniae* may contribute to the risk of stroke directly, in most cases, it acts in concordance with the conventional risk factors. In younger individuals with ischemic stroke in whom *C.pneumoniae* found to be a risk factor, eradication of infection by antibiotic treatment may decrease the risk of stroke.

In conclusion, we found significantly elevated *C.pneumoniae* IgG antibody in various subgroups of ischemic stroke in patients aged <45 years compared to control subjects. Large scale studies are required to explore these findings.

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