

ORIGINAL ARTICLES

Hyperhomocysteinemia associated with *Chlamydia pneumoniae* infection in ischemic stroke: A hospital based study from South India

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Abstract

Background and objective: While *Chlamydia pneumoniae* infection and hyperhomocysteinemia have been shown to contribute independently to the atherosclerotic risk, recent evidence has linked the association of *C. pneumoniae* positivity and hyperhomocysteinemia in patients with established atherosclerosis. The aim of this study was to investigate whether such a relationship can be replicated in India, where both infections and hyperhomocysteinemia are prevalent. **Methods:** Patients of acute ischemic stroke enrolled consecutively and prospectively in the Nizam's Institute Stroke Registry, Hyderabad, India (NISHI) were subjected to thorough clinical and neuroimaging evaluation. Blood was drawn in fasting state for estimation of homocysteine level and the titers of *C. pneumoniae* antibodies (IgG and IgA) by microimmunofluorescence method. **Results:** Of the 200 stroke patients, 72 (36%) were tested positive for *C. pneumoniae* antibodies, and 128 (64%) tested negative. The percentage of subjects with hyperhomocysteinemia, smoking, hypercholesterolemia and C-reactive protein positivity was higher in *C. pneumoniae* positive group compared with *C. pneumoniae* negative group. Multiple logistic regression analysis showed that hyperhomocysteinemia was an independent variable in the *C. pneumoniae* positive group (Odds ratio 4.71 95% CI 2.2-9.8).

Conclusion: This study has shown that *C. pneumoniae* seropositivity is linked with hyperhomocysteinemia in patients with ischemic stroke in a sample of South Indian population.

INTRODUCTION

Hypertension, diabetes and hyperlipidemia are the conventional risk factors for the development of atherosclerosis, but there is a growing interest in the newly described risk factors.¹ Many studies have shown that *C. pneumoniae* infection^{2,3} and hyperhomocysteinemia⁴ contribute independently to the atherosclerotic diseases in coronary and cerebral vessels. Recent evidence has linked *C. pneumoniae* positivity and hyperhomocysteinemia in patients with established atherosclerosis.⁵⁻⁸ There is no study published so far from India, on hyperhomocysteinemia associated with *C. pneumoniae* infection in ischemic stroke. This study was aimed to investigate the relationship between *C. pneumoniae* seropositivity and hyperhomocysteinemia in patients with acute

ischemic stroke in a sample of South Indian population.

METHODS

Study population

We included 200 consecutive patients of acute ischemic stroke with stroke onset less than 72 hours; between January 2004 to December 2006 who were enrolled in Nizam's Institute Stroke Registry, Hyderabad, India (NISHI). Nizam's Institute of Medical Sciences is the main referral hospital in Hyderabad, one of the major metropolitan cities in South India. Stroke was defined according to 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 that of vascular origin".⁹ Cerebral infarction was diagnosed on the basis of results of the first CT (Computer tomography) or MRI (Magnetic Resonance Imaging) brain scan. In patients with normal CT scan brain, ischemic stroke was diagnosed based on diffusion weighted MRI scan. Standard methods were used to measure blood pressure, height and weight. Fasting blood samples were used to estimate lipids (including total cholesterol, LDL, HDL, VLDL, and triglycerides), homocysteine and glucose for all patients.

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 2 blood pressure measurements¹⁰ or a patient's self-reported history of hypertension or antihypertensive use, supported by documents and /or evidence of end organ damage. Diabetes was diagnosed if fasting plasma glucose was >110 mg/100ml or patient was on anti-diabetic medications.¹¹ In accordance with the guidelines of National Institute of Health (NIH), patients having serum cholesterol levels >200mg/100ml or those on anti-cholesterol medication were considered as having hypercholesterolemia.¹² Hyperhomocysteinemia was defined as serum homocysteine >15mg/100ml, in those below 60 years¹³ and >20mg/100ml serum in those above 60 years.¹⁴ Smokers were defined as those reporting daily smoking. Ex-smokers and occasional smokers were classified as non-smokers.¹⁵ Alcoholics were defined as those in whom the alcohol consumption was >50 g/day (equivalent to 500ml [2 drinks] of wine, 1,000ml of beer, or >5 drinks [units] of spirits).¹⁶ Body Mass Index (BMI) values from 25.0 -30.0 was taken as overweight and BMI values >30 was taken as obese.¹⁷

Blood collection

At the time of enrollment of cases, 5ml blood sample was obtained. The obtained blood samples 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* with use of microimmunofluorescence.¹⁸

Identification of *C. pneumoniae*

The identification of *C. pneumoniae*-specific IgG and IgA antibodies in plasma was determined

by indirect immunofluorescence test using Euroimmun BIOCHIP slide kit. This kit is manufactured in Germany and is approved by FDA. An IgG titer of 1:100 and an IgA titer of 1:100 were judged to be positive and were interpreted as an evidence of a current or earlier *C. pneumoniae* infection.

Statistical analysis

Statistical analysis was performed using SPSS 14.0 window software (statistical package for the Social sciences, SPSS Inc). Continuous variables were presented in titer of mean and \pm SD. Categorical variables were expressed as proportions. The student 't' test was performed to test the differences in continuous variables, and χ^2 test was used to study the association in proportions. To estimate the Odds ratio (OR) and the resulting 95% CI for the matched positive negative pairs, multiple logistic regression was performed, before and after adjustment for potential confounders. All tests were two sided and p value <0.05 were considered statistically significant.

The design of the study was approved by the Ethics Committee of Nizam's Institute of Medical Sciences. All patients gave their written informed consent to take part in the study. The participants who were not able to consent for themselves because of severe illness or unconsciousness gave consent through their next of kin or guardian.

RESULTS

In this prospective study, 200 consecutive patients (men 149, women 51) with acute ischemic stroke of less than 72 hours duration were recruited. Mean age was 47.8 \pm 15.1years (age range 10-82 years). Out of 200 patients, 72 patients were tested positive for IgG and IgA *C. pneumoniae* antibodies (Men 49 and Women 23). No significant differences were found between seropositive and seronegative groups regarding age (p=0.5), hypertension (p=0.3), diabetes (p=0.8) and alcoholism (p=0.9). In *C. pneumoniae* positive group hyperhomocysteinemia (p<0.001), smoking (p<0.001), hypercholesterolemia (p=0.005) and C-reactive protein (CRP) positivity (p<0.001) were significantly higher compared with *C. pneumoniae* negative group (Table 1). Multiple logistic regression analysis showed that hyperhomocysteinemia was independently associated with stroke in the *C. pneumoniae* seropositive group (odds ratio: 4.71; 95% CI: 2.2-9.8). In patients with *C. pneumoniae* seropositivity,

Table 1: Comparison of baseline characteristics between *C. pneumoniae* negative and positive stroke patients

Parameters	<i>C. pneumoniae</i> positive N=72	<i>C. pneumoniae</i> negative N=128	p value
Men, no. (%)	49 (68)	100 (78.1)	0.6
Women, no. (%)	23 (32)	28 (21.9)	0.3
Mean age in years (SD)	48.8 (15.1)	47 (17.4)	0.5
Age range in years	10-76	18-82	
Hypertension, no. (%)	40 (55.5)	61 (47.6)	0.3
Diabetes, no. (%)	15 (20.8)	27 (21)	0.8
Smoking, no. (%)	26 (36.1)	18 (14)	<0.001
Alcoholism, no. (%)	14 (19.4)	24 (18.7)	0.9
Hypercholesterolemia, no. (%)	30 (41.6)	23 (17.9)	0.005
Hyperhomocysteinemia, no. (%)	30 (41.6)	20 (15.6)	<0.001
CRP positive, no. (%)	35 (48.6)	4 (3.1)	<0.001

CRP: C-reactive protein

CRP was found to be an independent factor for stroke (Odd 24.8, 95 % CI: 8.2-74.4). In this study, both IgG (p<0.001) and IgA (p=0.004) positivity was significantly more prevalent in group with hyperhomocysteinemia than with normal homocysteine shown in Table 2.

DISCUSSION

Atherosclerosis is a global problem causing cardiovascular and cerebrovascular diseases. It is being recognized as a major cause of mortality and morbidity in developing countries.¹⁹ The synergistic interaction between established risk factors like hypertension, diabetes and hypercholesterolemia is well known but the relation between newer risk factors like *C. pneumoniae* and hyperhomocysteinemia, even though observed,

has not been well understood.⁵ Our results indicate that *C. pneumoniae* seropositivity is linked with hyperhomocysteinemia in patients with ischemic stroke in a sample of south Indian population. This correlation was statistically significant for both IgG and IgA antibodies. We used a predefined cutoff limit of 1:100 for IgG and IgA antibodies as a significant titer based on criteria mentioned in the Euroimmune kit, which was determined using a panel of samples from healthy blood donors.

It is not clear if the positivity for *C. pneumoniae* is the cause or the effect of hyperhomocysteinemia although the latter seems plausible.²⁰ The theoretical basis of this association rests on the fact that, in vitro, the growth of *C. pneumoniae* is enhanced in serum-free media and particularly by depletion of lysine or methionine.²¹ In human metabolism, homocysteine is produced

Table 2: *C. pneumoniae* antibodies associated with hyperhomocysteinemia

	Hyperhomocysteinemia (N=50)	Normal homocysteine (N=150)	p value
<i>C. pneumoniae</i> IgG positive, no. (%)	26 (42%)	38 (25.3%)	<0.001
<i>C. pneumoniae</i> IgA positive, no. (%)	12 (24%)	8 (5.3%)	0.004

by demethylation of methionine and defects in the recycling pathway (in which homocysteine is remethylated to methionine) can cause hyperhomocysteinemia.²²

C-reactive protein and C. pneumoniae in stroke

C-reactive protein (CRP) is an inflammatory marker of acute and chronic inflammation.²³ Several studies have found that *C. pneumoniae* infection could contribute to elevation of CRP levels and to the instability or progression of atherosclerotic plaques.^{24,25} In this study both *C. pneumoniae* and CRP positivity was found in 48.6% patients compared to 3.1% in *C. pneumoniae* negative group. The significant association of CRP with *C. pneumoniae* positivity suggests that the mechanism by which *C. pneumoniae* leads to atherosclerosis may be through inflammation.

The main strength of our study is that it is a prospective study of consecutively recruited stroke patients. Another strength is that we have used standard methods to estimate homocysteine and antibody titers in all the study population. Serological testing with MIF, used in our study, is the “gold standard” for clinical diagnosis of *C. pneumoniae* infection. Other postulated tests, such as PCR and flow cytometry, remain under investigation. Multiple logistic regression test was used to study whether positivity of *C. pneumoniae* antibodies is an independent risk factor or associated risk factor for stroke.

There are several limitations in our study. First, the sample size was small. Second, the secondary causes of hyperhomocysteinemia like vitamin B6, B12, and folic acid deficiency, and genetic factors for hyperhomocysteinemia were not evaluated. Third, this study was 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.

Although the clinical implications of this association of *C. pneumoniae* and hyperhomocysteinemia are not clear at present, it is reasonable to hypothesize that in future, the propensity to develop chlamydia infection may be minimized by identifying and correcting hyperhomocysteinemia. However further studies are required to conform this association of *C. pneumoniae* seropositivity

and hyperhomocysteinemia in ischemic stroke patients.

In conclusion, we found significantly elevated *C. pneumoniae* IgG and IgA antibodies in acute ischemic stroke with elevated homocysteine. Large scale studies are required to explore these findings.

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