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Chlamydia pneumoniae antibodies in various subtypes of ischemic stroke in Indian patients

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Abstract

Background: As infections occur more frequently in developing countries, we carried out this prospective case-control study, to establish the association, if any, between *C. pneumoniae* antibodies and ischemic stroke particularly in relation to its subtypes.
Design: Antibodies (IgG and IgA) to *C. pneumoniae* in serum were measured by microimmunofluorescence test in 200 consecutive ischemic stroke patients and 200 age and sex matched controls.
Results: Seventy two out of 200 ischemic stroke patients (36%) had positive *C. pneumoniae* antibodies (IgG or IgA), compared to 35 out of 200 controls (17.5%) ($p<0.0001$). IgG antibody was positive in 64/200 (32%) ischemic stroke patients, compared to 34/200(17%) controls ($p<0.0001$) and IgA was positive in 20/200(10%) ischemic stroke patients compared to 1/200(0.5%) controls ($p<0.0001$). Logistic regression analysis showed statistically significant association between *C. pneumoniae* antibody positivity and ischemic stroke, thereby establishing it as an independent risk factor. Prevalence of *C. pneumoniae* antibodies was significantly higher in all stroke subtypes (except the stroke of undetermined etiology) compared to controls.
Conclusion: Significant and independent association was found between *C. pneumoniae* antibodies and ischemic stroke in this sample of south Indian population. The association was found in all ischemic stroke subtypes, except stroke of undetermined etiology.

Keywords: *C. pneumoniae*; Ischemic stroke; Stroke subtypes; IgG antibody; IgA antibody; Microimmunofluorescence

1. Introduction

Chlamydia pneumoniae (*C. pneumoniae*) is a common respiratory pathogen [1] and often causes asymptomatic infections [2]. Many reports in the last decade have associated chronic infection with *C. pneumoniae* to atherosclerosis and thrombosis [3] and thereby incriminated *C. pneumoniae* in the causation of coronary heart disease and stroke [4]. Due to the heterogeneity of underlying mechanisms, the association with ischemic stroke is more complex. Patients who have had an infection within a week prior to stroke onset have been shown more likely to have cortical middle cerebral artery (MCA) infarcts, cardioembolic infarcts, and arterial dissections, suggesting a differential effect of infection dependent on stroke subtype [5]. Since infectious diseases in general are more common in India compared to western countries, we aimed to investigate the frequency of *C. pneumoniae* antibodies in patients with ischemic stroke and its various subtypes. There is no study, so far, on the association of chronic infection with *C. pneumoniae* with increased risk of stroke in Indian patients.

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2. Patients and methods

2.1. Study population

Two hundred patients of acute ischemic stroke enrolled consecutively (one hundred patients above and one hundred patients below 40 years of age) in Nizam's Institute Stroke Registry, Hyderabad, India (NISHI) , the premier university hospital and a major referral centre in south Indian state of Andhra Pradesh were included in the study. Two hundred age and sex matched control subjects attending the department of Neurology for non-vascular diseases were also investigated. This study was approved by the Institutional Ethical Committee. The study period was from January 2004 to December 2006.

2.2. Selection of cases

Subjects were enrolled in the study if they met the following criteria: first ischemic stroke, stroke treatment in the hospital, and admission to the hospital within the first 72 h after stroke onset. Stroke was defined according to World Health Organization as "rapidly developing clinical signs of focal/global disturbance of cerebral function, with symptoms lasting 24 h or longer or leading to death, with no apparent cause other than of vascular origin" [6]. Cerebral infarction was diagnosed on the basis of history, examination and results of the first CT scan (Computer tomography). All subtypes of ischemic stroke were included.

Data were collected through face-to-face interviews of case and control subjects by the trained research fellow and supervisor of this project, medical record review, physical and neurological examination by the study physicians and in-person measurements of fasting blood specimens for lipid, and glucose [7]. When the subjects were unable to provide answers, proxy or his/her close blood relation knowledgeable about the subject's history was interviewed. Standardized questions were adapted from the behavioral risk factor surveillance system, [8] by the Centers for Disease Control and Prevention regarding the following conditions: hypertension, diabetes, hypercholesterolemia, peripheral vascular disease, transient ischemic attack, cigarette smoking, and cardiac conditions such as myocardial infarction and coronary artery disease. Standard techniques were used to measure blood pressure, height, weight, fasting blood specimen lipids (including total cholesterol, LDL, HDL, VLDL, and triglycerides) and glucose [9]. If the etiology was not clear then additional tests like, fibrinogen, antithrombin III, protein C, protein S, and anticardiolipin antibodies were also done. Serum homocysteine estimation was done in all patients. For control subjects also, fasting glucose, lipids panels (including total cholesterol, LDL, HDL, VLDL, and triglycerides) and homocysteine was evaluated. Clinical, imaging and all laboratory investigations were entered into study forms. Controls were interviewed in person and evaluated in the same manner as cases.

According to Joint National Committee VI–VII, hypertension was defined as a systolic blood pressure >140 mm Hg and/or a diastolic blood pressure >90 mm Hg based on the average of the 2 blood pressure measurements, or a patient's self-reported history of hypertension or antihypertensive use, supported by documents [10]. Diabetes was diagnosed if fasting plasma glucose was >110 mg/100 ml or patient was on anti-diabetic medications [11]. In accordance with the guidelines of National Institute of Health (NIH), patients having serum cholesterol levels >200 mg/100 ml or those on anti-cholesterol medication were considered as having hypercholesterolemia [12]. Hyperhomocysteinemia was defined as serum homocysteine >15 mg/100 ml, in those below 60 years [13–15] and >20 mg/100 ml serum in those above 60 years [16]. Smokers were defined as those reporting daily smoking. Ex-smokers and occasional smokers were classified as non-smokers [17]. Alcoholics were defined as those in whom the alcohol consumption was >50 g/day (equivalent to 500 ml [2 drinks] of wine, 1000 ml of beer, or >5 drinks [units] of spirits) [18]. BMI values from 25.0–30.0 was taken as overweight and BMI values >30 was taken as obese [19].

2.3. Assessment of stroke subtypes

All stroke patients underwent brain imaging by CT scanning and when clinically appropriate, Magnetic Resonance Imaging (MRI). Transthoracic echocardiography (TTE) or Transesophageal echocardiography (TEE), non-invasive vascular imaging (Extracranial duplex Doppler, Transcranial Doppler or Magnetic Resonance Angiography) was done in all patients. Additional tests were performed when required. The project supervisor and co-supervisors reviewed the data. Ischemic stroke subtypes were classified as extracranial large artery atherosclerosis, intracranial large artery atherosclerosis, cardioembolic, small vessel disease (lacunar), stroke of other determinated etiology and stroke of undetermined etiology [20].

Blood collection was done at the time of enrollment of cases and control subjects; 5 ml blood sample was obtained. The obtained blood sample was 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 [21]. These blood samples were also used for detection of C Reactive Protein (CRP) as an acute phase reactant of the inflammatory response.

2.4. Identification of C. pneumoniae

The presence of *C. pneumoniae* — specific IgG and IgA antibodies in plasma was determined by indirect immunofluorescence test using Euroimmun BIOCHIP slide kit (commercial kit Germany). An IgG titer in serum of 1:100, and an IgA titer of 1:100 were judged to be positive and were interpreted as a current or earlier *C. pneumoniae* infection.