Novel Inflammatory Biomarkers and Their Correlation to *Chlamydia pneumoniae* Titres in Acute Ischemic Stroke

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Background: Young stroke patients constitute 15%-30% of all stroke patients in India as against 3.0%-8.5% reported from the West. The mechanisms for stroke in the young may include unconventional risk factors such as infections. We aimed to investigate the role (if any) of *Chlamydia pneumoniae* antibodies in young patients with acute ischemic stroke (AIS). Several proinflammatory cytokines and biomarkers are released early after the onset of brain ischemia. We assessed the role of heat shock protein (hsp) 65, neopterin, and myeloperoxidase upregulation after AIS in predicting stroke severity. We also assessed relationship of upregulated inflammatory biomarkers with *C pneumoniae* antibody titres (IgG, IgA, and IgM). *Methods:* Eighty acute stroke patients and healthy age- and sex-matched controls were recruited. Blood samples were drawn within 1 week from the onset of stroke. Detection of IgA, IgG, and IgM antibodies to *C pneumoniae* was done with a validated microimmunofluorescence technique from 5 mL of serum in all subjects. Inflammatory biomarkers such as neopterin, myeloperoxidase and hsp 65 were estimated with sandwich enzyme linked immunosorbent assay (ELISA) method. *Results:* hsp 65 and neopterin were significantly elevated in all stroke patients with respect to healthy controls (odds ratio [OR], 4.9; 95% confidence interval [CI], 23.5-67.8; $P = .001$ and OR, 4.4; 95% CI, 2.08-9.4; $P = .04$, respectively). Eighty-one percent of cases were seropositive for IgA versus 32% of controls ($P = .003$), and IgG was positive in 52.7% versus 17.3% of controls ($P = .05$). Myeloperoxidase levels were similar in patients and controls. Correlation and multiple regression indicated a high level of predictability and sensitivity of hsp 65 to IgA. *C. pneumoniae* antibody titres when all other variables were constant ($F[4,90] = -6.8$, $P = .001$). Patients with high NIHSS scores (>15) had elevated levels of hsp 65 (mean, 13.2 ng/mL) suggesting correlation with stroke severity. *Conclusions:* The study demonstrated high levels of hsp 65 and neopterin levels in AIS correlated to significantly elevated IgA titres of *C pneumoniae*. Elevated levels of hsp 65 were associated with stroke severity. **Key Words:** Acute ischemia—inflammatory biomarkers—C pneumoniae—stroke severity.

Introduction

Stroke in young, including stroke in children and young adults (<45 years), is an important cause of morbidity and mortality throughout the world, especially in developing countries.[1] Young stroke patients constitute 15%-30% of all stroke patients in India, vis

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a vis 3.0%-8.5% of all stroke patients reported from the West.[2]

Serologic evidence of past infection with *Chlamydia pneumoniae* has been found in epidemiologic studies to be associated with risk for atherosclerosis and cardiac disease, although prospective cohort studies have not always confirmed this association.[3] Because of 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). The link between *C pneumoniae* and cerebrovascular disease has been investigated in a number of seroepidemiologic and antibiotic intervention studies.[4] However, the role of *C pneumoniae* infection in acute stroke as a cause/effect/trigger remains controversial.

Because infectious diseases in general are more common in India, compared with Western countries, we aimed to investigate the role (if any) of *C pneumoniae* antibodies in patients with AIS. Recent reports from South India suggest positive association of *C pneumoniae* infection with the occurrence of AIS. Our previous study also confirmed the same.[5,6] The present study was undertaken to investigate the possible role of *C pneumoniae* in young stroke patients.

The role of inflammatory markers in ischemic cascade after AIS has been widely studied. All stages of the atherosclerotic plaque—initiation, growth, and complications—might be considered to be an inflammatory response to injury. Potential targets, which could help to identify and monitor the ongoing inflammatory[7,8] process are C-reactive protein (CRP), interleukins (IL-1,6), tumor necrosis factor-α, neopterin, myeloperoxidase, heat shock protein (hsp) 65 and others. There has been some literature published on the predictor ability of CRP for both prognosis and occurrence of AIS.[9,10] Although many blood markers have been investigated to date, no single blood marker has been proven to be clinically useful to predict stroke severity and outcomes. We investigated the role of hsp 65, neopterin, and myeloperoxidase in predicting the severity of AIS and their correlation to the titres of *C pneumoniae*.

Methods

The criteria for inclusion were patients in the age group of 18-45 years, presenting with AIS within a week of onset. Complete medical history of hypertension, smoking, diabetes mellitus, hypercholesterolemia, ischemic heart disease, stroke, or TIA was recorded. Neurologic evaluation included recording of National Institute of Health Stroke Scale (NIHSS). noncontrast computed tomography head was performed in all, and if needed magnetic resonance imaging/computed tomography angiography/magnetic resonance angiography was performed according to the clinical requirement. Controls selected for the previously mentioned analysis were healthy age- and sex-matched subjects, who did not have any history of cerebrovascular disease. Blood samples were drawn within 1 week from the onset of stroke. In each case, 5 mL of serum was obtained by centrifugation (699 g for 15 minutes at +4°C) and then stored at 70°C until analysis. Detection of IgA, IgG, and IgM antibodies to *C pneumoniae* was done with a validated microimmunofluorescence technique. The cutoff point for seropositivity was 1/16 for IgA and 1/64 for IgG. Inflammatory biomarkers such as hsp 65, neopterin, and myeloperoxidase were estimated using commercially available high sensitivity quantitative sandwich enzyme immunoassays according to the manufacturer's instructions (R & D Systems, Minneapolis, MN). This study was cleared by the institute review board, and written informed consent was obtained from all patients.

Statistical Methods

Analysis of data was carried out using SPSS statistical analysis software (SPSS Inc, Chicago, IL). For continuous variables such as age and NIHSS scores, descriptive statistics was calculated and reported as means ± standard deviation. Normality of distribution of continuous variables was verified using the parametric tests.

Results

This study was a case–control design in which 80 AIS patients were recruited with the same number of healthy age-matched controls. The mean age in the study group was 43.6 ± 6.7 years and 43.2 ± 7.3 years in controls (male:female ratio = 2:1). Standard stroke care was administered to all patients. The risk factors found in stroke patients were hypertension (38%), diabetes mellitus (23.4%), hypercholesterolemia (29.4%), and smoking (22.3%; Table 1). hsp65 was found elevated significantly (mean of 30.4 ng/mL in 58 patients vs. none in controls, $P < .05$). Neopterin levels were found elevated in 50% (40 patients vs. 14 controls, with a mean of 19.26 μg/mL

Table 1. *Risk factors in study and control groups*

Risk factors	Patients, %	Controls, %
Hypertension	38	11
Hypercholesterolemia	29.4	18
Diabetes mellitus	23.4	15
Smoking	22.35	12
Alcohol	22.3	16
h/o cardiac disease	21.1	8.5
Atrial fibrillation	1.17	0
Migraine	7.05	1.5
Family h/o stroke	20.14	12.2

Abbreviation: h/o, history of.