Upsala J Med Sci 108: 205–212, 2003

Chlamydia Pneumoniae Seropositivity in Patients with Cerebral Ischemic Attack with or without Silent Brain Infarcts

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ABSTRACT

Purpose

We examined the seropositivity of specific antibodies IgG and IgA to Chlamydia pneumoniae in the patients with ischemic stroke and examined if it has a notability in stroke patients with or without silent brain infarcts.

Material and method

The clinical, laboratory and radiological findings of 26 cases with silent brain infarcts (SBI) without acute stroke and 26 cases with acute ischemic stroke without SBI (30 male, 22 female) were prospectively gathered. Risk factors were noted in all subjects. Control group was consisted of fifty-three healthy volunteer blood donors (40 male and 13 female). The presence of C. pneumoniae specific IgG antibody in serum samples was determined by indirect micro-immunofluorescence test according to the method of Wang and Grayston (Euroimmun GmbH in Deustchland) and of specific IgA antibody in serum samples was determined by indirect micro-immunofluorescence test with the manufactured kit Orgenium-Helsinki. The results were evaluated according to the groups and to the risk factors.

Results

There was not any correlation between risk factors and C. pneumoniae seropositivity. Seropositivity for specific IgG antibody for C. pneumoniae was observed as 73.8% in SBI, as 61.5% in stroke and as 56.3% in control groups. Seropositivity for specific IgA antibody for C. pneumoniae was observed in 7 out of 16 SBI cases (43.8%) SBI and in 9 out of 19 stroke cases (47.3%) with positive IgG antiobodies.

Received 11 March 2003
Accepted 3 June 2003

Key words: Chlamydia pneumoniae, stroke, seropositivity.

Conclusion

We could not confirm a relation of C.pneumoniae seropositivity neither with SBI nor with acute stroke.

INTRODUCTION

Infections and immunological mechanism have been suggested as risk factors in addition to the other well-known risk factors for stroke (1–7). Although specific causative relationship is yet unclear, some evidence for association of chronic infections with atherosclerosis, which is an accelerating factor for unstable angina, myocardial infarction, as well as for stroke, has been shown previously (7–9). Seroepidemiological studies and analyses of carotid plaques indicate a role of C. pneumoniae in ischemic stroke (2). Moreover, it has been reported that, Chlamydia pneumoniae (C. pneumoniae) may often be found in fragmented smooth muscle cells, with their accompanying organism, which were engulfed by macrophages (3). In the C. pneumoniae infections, its colonization directly in the vessel wall may result in the damage the vessel directly or indirectly due to immunologic cascades. Chronic infection may play a role in the initiation, progression, or destabilization of atherosclerotic plaques and might, also, influence the natural course of the preexisting plaque by the same mechanisms. Furthermore, it was suggested that chronic or acute chlamydial infection anywhere in the body could play a role in atherosclerotic plaque activation (10).

The studies on differentiation of infarcts from other white matter changes following the widespread usage of magnetic resonance imaging (MRI), brought about the detection of asymptomatic brain infarcts (Silent brain infarcts-SBI) (11–16). Older age, hypertension, nocturnal decrease in blood pressure and atrial fibrillation were suggested as the associated risk factors with SBI (15–22). But, it is not clear, yet, if SBI and ischemic stroke share similar risk factors (12, 23) and if C. pneumoniae infection has a role in SBI pathogenesis.

The aim of the present study was to evaluate the seropositivity of specific antibodies IgG to Chlamydia pneumoniae in the patients with cerebral ischemic attack and to examine if it has a notability in stroke patients with or without silent brain infarcts.

MATERIAL AND METHOD

Patient group

The clinical, laboratory and radiological findings of 26 cases with silent cerebral infarct without acute ischemic stroke (Stroke group) and 26 cases with acute ischemic stroke without SBI (SBI group) were prospectively gathered in a 6 months period. All cases were over age 45 years.

Collection of clinical and laboratory data

A semistructered questionnaire, that included sociodemographic parameters, present complaints, past medical history and known risk factors for cerebrovascular dis-

eases, had been completed for each patient. Complete blood count, blood glucose and urea nitrogen levels, serum levels of total cholesterol, high-density lipoprotein (HDL), triglycerides, a panel for coagulation and collagen, endocrine and hepatic diseases, C-reactive protein, electrocardiography (ECG), CT and/or MRI were completed in all of the cases. Blood lipids were studied by spectrophotometric methods in autoanalyzer. Carotid duplex ultrasonography has been done in a total of 48 and echocardiography (ECHO) in 32 cases.

Control group

53 cases (40 male and 13 female) (age range between 41–72, mean 61.9) were selected from healthy volunteer blood donors as control group. Control subjects were selected randomly and none of them had pulmonary, cardiovascular or cerebrovascular disorders.

Radiological examination

Computed tomography (CT) was performed without contrast with slice thickness of 10 mm for the supratentorial sections and 5 mm for the infratentorial section. Axial T1 and T2, flair, coronal T1 and T2 and sagital T1 sequences were completed for magnetic resonance imaging. SBIs were defined as focal hypodense lesions on CT and/or MRI evidence of focal lesions, hyperintense in T2 sequences with correlative hypointensity in T1 sequences that were not compatible with the events in the past medical history.

Serologic examination

The blood samples were kept in deep freeze at –20°C. All samples were thawed and studied in the same day.

The presence of C. pneumoniae specific antibodies IgG and IgA in serum samples were determined by indirect micro-immunofluorescence test according to the method of Wang and Grayston (manufactured kits Euroimmun GmbH in Deustchland for specific IgG antibody and Orgenium-Helsinki for specific IgA antibody) (24,25).

The IgG titer in serum samples of >1/100 was judged to be positive. In the seond step, we studied the IgA titers in the serum samples of 35 cases that had >1/100 seropositivity for specific IgG antibody for C. pneumoniae. The seropositivity of IgG antibody was interpreted as a current or earlier C. pneumoniae infection, and seropositivity for both IgG and IgA antibodies as a current or persistent C. pneumoniae infection.

Statistical evaluation

Statistical analyses were made for comparisons of;

1. The age, sex, associated systemic diseases, Duplex USG findings and presence of previous TIA and stroke history of both patient groups,

2. Seropositivity for C. pneumoniae of both patient groups (Stroke and SBI groups) and control group for Cp IgG and,

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Table 1: Clinical and serological data from patients with stroke and control

		Stroke Group	SBI Group	Control
Total subjects (male/female)		26 (12/14)	26 (12/14)	53 (30/13)
Age (mean)		41–72 (59.5)	45–85 (61.6)	40–72 (60.9)
Hypertension		15	13	8
Smoking		5	7	46
Hyperlipidemia		8	9	2
Diabetes mellitus		7	8	–
Congestive heart failure		0	1	–
Coronary artery disease		5	3	–
Atrial fibrillation		2	3	–
Heart valve diseases		2	1	–
Transient ischemic attack		5	8	–
Previous stroke		6	4	–
Multiple risk factors		9	15	–
Carotid Duplex USG	Unilateral	8	7	Not done
	Bilateral	5	9	
Neuroimaging Findings *	Hemispheric	18	24	Not done
	Basal gang./ thalamus	5	21	
	Brainstem	8	12	
	Internal caps.	2	–	
CRP	High	5	3	Not done
	Normal	21	23	
Specific IgG	+	16	19	30
	–	10	7	23
	%	61.5%	73.8%	56.3%
Specific IgA[&]	+	9	7	Not done
	–	17	19	
	%	47.3%	43.8%	

* Neuroimaging studies revealed more than one lesion in 6 cases in stroke group and in all cases in SBI group. [&] Specific IgA antibodies to C. pneumoniae has been studied in the cases that had seropositivity for specific IgG antibodies

3. Seropositivity for C. pneumoniae of both patient groups (Stroke and SBI groups) for Cp IgA antibodies.

Chi-square test and one-way analysis has been used for statistical analysis. The statistical significance was accepted as $p<0.05$.

RESULTS

Demographic characteristics and risk factors

Fifty-two patients (30 male, 22 female) and 53 healthy volunteer blood donors have been enrolled into the study. The ages of the patients were ranged between 45–92 years (mean 61.6 years) and ages of the control cases were between 40–72

years (mean 60.9 years). Sex, age and the most frequent risk factors have been shown in Table 1.

Male sex, diabetes mellitus and the presence of bilateral atherosclerotic lesions of carotid arteries in Doppler USG were more frequent in SBI patients. Presence of multiple risk factors, as well as, elder age and bilateral atherosclerotic lesions of carotid arteries in Doppler USG showed a tendency to have a positive correlation but it was not statistically significant ($p= 0.055$, $p= 0.06$, $p= 0.06$, respectively). Smoking, hypercholesterolaemia, hypertriglyceridemia, high hematocrit levels, echocardiographic and alectrocardiographic findings, presence of transient ischemic attacks (TİA) or stroke did not show any significant differences between the two groups ($p> 0.05$ for each item).

Transient ischemic attacks were noted in 8 of SBI group and 5 of acute ischemic stroke cases. 4 cases from SBI group and 6 cases from acute ischemic stroke reported previous strokes.

Neuroimaging findings

Radiological evaluation revealed hemispheric infarcts in 18, brain stem lesions in 8, basal ganglia and/or thalamic lesions in 5 and internal capsule lesion in 2 cases in stroke group. 6 cases had more than one lesion. Neuroimaging studies showed basal ganglia and/or thalamic lesions in 21, brainstem lesions in 12 and hemispheric lesions in 24 cases in SBI group. All cases had more than one lesion.

Serologic evaluation

C-reactive protein (CRP) found to be positive in 5 cases in stroke group and in 3 cases in SBI group, without any statistical significance ($p> 0.05$).

Specific antibody IgG to C. pneumoniae was observed in 73.8% of cases with silent brain infarct (SBI group), in 61.5% of acute stroke cases (Stroke group) and in 56.3% of controls. A seropositivity of 67.3% was found in patient group when both patient groups were taken together.

Specific antibody IgA to C. pneumoniae was found to be positive in 9 cases in stroke group (47.3%) and in 7 cases in SBI group (43.8%).

Serologic results for CRP and specific antibodies IgG and IgA to Chlamydia pneumoniae for each group have been shown in Table 1.

Seropositivity of specific antibody IgA to C. pneumoniae did not show a significant difference between stroke and SBI groups. C. pneumoniae seropositivity for specific antibodies IgG showed a tendency to be higher in silent brain infarct patients, without statistical significance.

DISCUSSION

There is growing evidence for a connection between the obligation of intracellular bacterium C.pneumoniae and atherosclerosis that may be a risk factor for subsequent coronary heart disease, myocardial infarction and stroke (10,26,27). Current

knowledge for the relation of C.pneumoniae and atherosclerosis comes from both observational and experimental studies (10, 26–30). It has been shown that, inoculation of C.pneumoniae in the endothelial cells of vessels, more efficiently, produces atherosclerotic like lesions or accelerates the process (26,27). In addition, seropositivity for C.pneumoniae was reported to be associated with an increased risk for further cardiovascular diseases and, also, for stroke (28–30).

Specific C. pneumoniae IgG antibodies were found in 73.8% of stroke group and in 61.5% of SBI group. There wasn't any significant difference between two groups. When both patient groups had been considered together, specific C. pneumoniae seropositivity had been found as 67.3%. Although, it was higher than the result of the control group in the same region (56.3%), any statistical differences could not be found. IgA antibodies last only for 3–5 days in the circulation and is a marker of recent or persistent infection, where as IgG titers remain elevated during a more prolonged period (months and years) (29). La Biche et al.(4) noted that seropositivity for anti-chlamydial IgG, IgA, and IgM anti-chlamydial antibodies did not correlate with identification of C pneumoniae in the aortic plaques which was atherosclerotic area..

TIA is accepted as a risk factor for further fatal or non-fatal strokes and C. pneumonia antibody has previously been shown in 32.4% of TIA cases (30). In the present study, TIA has been noted in a considerable number of patients. However, TIA was compatible in both groups without any significant difference. The tendency to presence of multiple risk factors, elder age and bilateral atherosclerotic lesions of carotid arteries gives the impression that SBI might be a part of widespread atherosclerotic disease.

In the present study, we couldn't confirm a relation of C. pneumoniae seropositivity with stroke neither in SBI nor in stroke patients. However, our patient groups were relatively small. Additionally, we did not follow the patients up to determine the prognosis of cases with positive serology of specific antibodies IgG to C. pneumoniae. It seems logic to examine C. pneumoniae seropositivity in stroke patients for if it has a role in at least in some subtypes of cerebrovascular accidents, especially in which a widespread atherosclerosis had been suggested for the underlying mechanism. Further studies with larger patient groups are expected to clarify the potential relationship of C. pneumoniae infection to stroke.

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