

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

Fatma Sirmatel,¹ Munife Neyal Muftuoglu,² Nursan Tahtaci,³
Abdurrahman Neyal,⁴ Ocal Sirmatel,⁵ Binnur Bulbul²

¹Gaziantep University, School of Medicine, Infectious Diseases Dept., Gaziantep- Turkey

²Gaziantep University, School of Medicine, Neurology Dept., Gaziantep- Turkey

³Gaziantep University, School of Medicine, Anesthesiology Dept., Gaziantep- Turkey

⁴Gaziantep State Hospital, Neurology Clinic, Gaziantep- Turkey

⁵Harran University, School of Medicine, Radiology Dept., Sanliurfa- Turkey

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 Deutschland) 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.

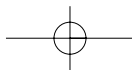
Results

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 antibodies.

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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.

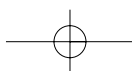
MATERIAL AND METHOD

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 sagittal 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 Deutschland 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 second 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,

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 Bilateral	7 9	Not done
Neuroimaging Findings *	Hemispheric Basal gang./ thalamus Brainstem Internal caps.	18 5 8 2	24 21 12 – Not done
CRP	High Normal	5 21	3 23 Not done
Specific IgG	+ – %	16 10 61.5%	19 7 73.8%
Specific IgA ^{&}	+ – %	9 17 47.3%	7 19 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

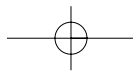
3. Seropositivity for *C. pneumoniae* of both patient groups (Stroke and SBI groups) for Cp IgA antibodies.

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 (TIA) 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%).

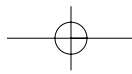
Serologic results for CRP and specific antibodies IgG and IgA to *Chlamydia pneumoniae* for each group have been shown in Table 1.

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).

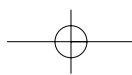
Specific *C. pneumoniae* IgG antibodies were found in 73.8% of stroke group and in 61.5% of SBI group. There wasn't any significant difference between two groups. When both patient groups had been considered together, specific *C. pneumoniae* seropositivity had been found as 67.3%. Although, it was higher 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. pneumoniae* 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.

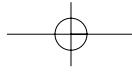
In the present study, we couldn't confirm a relation of *C. pneumoniae* seropositivity with stroke neither in SBI nor in stroke patients. However, our patient groups were relatively small. Additionally, we did not follow the patients up to determine the prognosis of cases with positive serology of specific 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.

REFERENCES

1. Stegmayr B, Asplund K. Measuring stroke in the population based stroke registry. *Neuroepidemiology* 1992; 11: 204–13.
2. Bova IY, Bornstein NM, Korezyn AD. Acute infection as a risk factor for ischemic stroke *Stroke* 1996; 27: 2204–206.
3. Shor A, Kou CC, Palton DL. Detection of *Chlamydia pneumoniae* in coronary arterial fatty streaks and atheromatous plaques. *S Afr Med J* 1992; 82: 158–61.
4. La Biche, Koziol D, Quinn TC and et al. Presence of *Chlamydiae pneumoniae* in human symptomatic and asymptomatic carotid atherosclerotic plaque. *Stroke* 2001; 32(4): 855–40.



5. Grau A, Buggle F. Infection, atherosclerosis and acute ischemic cerebrovascular disease. *Rev Neurol* 1999; 29(9): 847–51.
6. Valassina M, Cusi MG, Corsaro D, Cellesi C. Chlamydiae pneumoniae viability in atherosclerotic tissue: true or false *JID* 2000; 182: 1577–79.
7. Chiu B. Multiple infections in carotid atherosclerotic plaques *Am Heart J* 1999; 138(Suppl 2): 5534–36.
8. Mattila KJ, Valtonen VV, Nieminen MS, Asikainen S. Role of infection as a risk factor for atherosclerosis, myocardial infarction, and stroke. *Clin Infect Dis* 1998; 26(3): 719–34.
9. Glader CA, Stegmayr B, Boman J and et al. Chlamydia pneumoniae antibodies and high lipoprotein levels do not predict ischemic cerebral infarctions. *Stroke* 1999; 30: 2013–2018.
10. Saikku P. Chlamydia pneumoniae and atherosclerosis an update. *Scand J Infect Dis Suppl.* 1997; 104: 53–56.
11. Longstreth WT Jr., Bernick C, Manolio T, et al. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: The cardiovascular Health Study. *Arch Neurol* 1998; 55: 1217–25.
12. Modrego Pardo PJ, Labrador Fuster T, et al. Silent brain infarctions in patients with coronary heart disease. A Spanish population survey. *J Neurol* 1998; 245: 93–7.
13. Nakagawa T, Sekizawa K, Nakajoh K, et al. Silent cerebral infarction: a potential risk for pneumonia in the elderly. *J Intern Med* 2000; 247: 255–9.
14. Price TR, Manolio TA, Kronmal RA et al. Silent brain infarction on magnetic resonance imaging and neurological abnormalities in community-dwelling older adults. The cardiovascular Health Study. CHS Collaborative Research Group. *Stroke* 1997; 28: 1158–64.
15. Shintani S, Shiigai T, Arinami T. Silent lacunar infarction on magnetic resonance imaging (MRI): risk factors. *J Neurol Sci* 1998; 160: 82–6.
16. Sugiyama T, Lee JD, Shimizu H, et al. Influence of treated blood pressure on progression of silent cerebral infarction. *J Hypertens* 1999; 17: 679–84.
17. Kario K, Matsuo T, Kobayashi H, et al. ‘Silent’ cerebral infarction is associated with hypercoagulability, endothelial cell damage, and high Lp (a) levels in elderly Japanese. *Arterioscler Thromb Vasc Biol* 1996; 16: 734–41.
18. Van Zagen M, Boiten J, Kessels F, et al. Significant progression of white matter lesions and small deep (lacunar) infarcts in patients with stroke. *Arch Neurol* 1996; 53: 650–5.
19. Watanabe N, Imai Y, Nagai K, et al. Nocturnal blood pressure and silent cerebrovascular lesions in elderly Japanese. *Stroke* 1996; 27: 1319–27.
20. Yamamoto Y, Akiguchi I, Oiwa K, et al. Adverse effect of nighttime blood pressure on the outcome of lacunar infarct patients. *Stroke* 1998; 29: 570–6.
21. Davis PH, Clarke WR, Bendixen BH, et al. Silent cerebral infarction in patients enrolled in the TOAST Study. *Neurology* 1996; 46: 942–8.
22. Zito M, Muscari A, Marini E, et al. Silent lacunar infarcts in elderly patients with chronic non valvular atrial fibrillation. *Aging* 1996; 8: 341–6.
23. Masuda J, Nabika T, Notsu Y. Silent stroke: pathogenesis, genetic factors and clinical implications as a risk factor. *Curr Opin Neurol* 2001; 14(1): 77–82.
24. Grayston JT, Mordhorst CH, Bruu AL, Vene J, Wang SP. Country wide epidemics of Chlamydiae pneumoniae strain TWAR in Scandinavia 1981–1983. *J Infect Dis* 1989; 159: 1111–14.
25. Wang SP, Grayston JT. Micro-immunofluorescence serological studies with the TWAR organism. In: Oriel JD, Ridgway G, Schachlter J, Taylor Robinson D, Ward M. eds. *Chlamydial infections*, Cambridge UK, Cambridge University Press 1986; 329–32.
26. Shor A, Phillis JI. Chlamydia pneumoniae and atherosclerosis *JAMA* 1999; 282: 2071–73.
27. Kuo CC, Shor A, Campell LA, Fukushi H, Palton D, Grayston JT. Demonstration of Chlamydiae pneumoniae in atherosclerotic lesions of coronary arteries *J Infect Dis* 1993; 167: 841–49.
28. Fagerberg B, Gnarp J, Gnarp H, Agewall S, Wikstrand J. Chlamydia pneumoniae but not cytomegalovirus antibodies are associated with future risk of stroke and cardiovascular disease: a prospective study in middle-aged to elderly men with treated hypertension. *Stroke* 1999; 30: 299–305
29. Wimer MLJ, Sandman SR, Saikku P, Haberl RL. Association of chlamydial infection with cerebrovascular disease. *Stroke* 1996; 27: 2207–210.



30. Cook PJ, Honeybourne D, Gregory YH. Chlamydia pneumoniae antibody titers are significantly associated with acute stroke and transient cerebral ischemia The West Birmingham Stroke Project. Stroke 1998; 29(2): 2404-10.

Correspondence Address: Dr. Fatma Sirmatel
Gaziantep University, School of Medicine,
Infectious Diseases Dept.
Kolejtepe 27090
GAZIANTEP-TURKIYE
Phone and fax: 0+90 342 335 74 60
E-mail: sirmatel@gantep.edu.tr

