Original Article

Association between chlamydia pneumoniae infection and carotid atherosclerotic plaques

Fereshteh Ashtari*, Alia Saberi**, Vahid Shayegannejad***, Alireza Khosravi****, Roya Sherkat*****, Elham Khosravi******

Abstract

BACKGROUND: Several studies have suggested an association between Chlamydia pneumonia infection and atherosclerosis. This study was designed to investigate the association between this organism and atherosclerotic plaque formation in right and left common carotid arteries (CCAs) and extracranial portions of internal carotid arteries (ICAs).

METHODS: Antibodies to Chlamydia pneumoniae (IgA and IgG) were measured and compared in 42 patients who had plaque in at least one CCA or ICA (detected by duplex ultrasound) and 82 patients without any plaque in these arteries. Cp.IgG and Cp.IgA titers over 1.10 ISR were defined to be positive.

RESULTS: We found that 6.1% of control subjects and 16.7% of cases were Cp.IgA seropositive. The difference between these two groups was prominent but was not statistically significant (P = 0.104). 4.2% of females without atherosclerotic plaque and 31.6% of females with plaque were Cp.IgA seropositive. This difference is statistically significant (P = 0.005). There was no significant difference in seropositivity of Cp.IgG between case and control subjects or in male and female groups with or without plaque.

CONCLUSIONS: Cp.IgA is a predictor of atherosclerosis in women, but Cp.IgG has no predictive value for plaque formation in either gender.

KEY WORDS: Atherosclerotic plaque, Chlamydia pneumoniae, serum antibody.

Cerebrovascular events that include cerebral infarction due to thrombosis or emboli and intracranial hemorrhage are common causes of morbidity and mortality. Atherosclerosis is one of the precipitating factors of ischemic stroke that is caused by some risk factors such as hypertension, hyperlipidemia, diabetes mellitus, and smoking [1], but these conventional risk factors do not fully explain all atherosclerotic vascular disease, so new risk factors were suggested. Recently, increasing interest has focused on the putative causal role of chronic infections such as Chlamydia pneumonia, coxsackievirus and cytomegalovirus [2-7]. Chlamydia pneumonia is an obligatory intracellular microorganism that has been suggested as a contributing factor in pathogenesis of atherosclerosis by means of various mechanisms [8-10]. So with assessing the markers of this infection as the predictors of atherosclerosis, plaque formation may be prevented by some antibiotics [11]. Chlamydia

*Associated Professor, Department of Pediatrics, Isfahan University of Medical Sciences, Isfahan, Iran. e-mail: f_ashtari@med.mui.ac.ir (Corresponding Author)
**Clinical Resident, Department of Neurology, Isfahan University of Medical Sciences, Isfahan, Iran.
***Associated Professor, Department of Neurology, Isfahan University of Medical Sciences, Isfahan, Iran.
****Associated Professor, Cardiovascular Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
*****Associated professor, Department of immunology and Infectious Disease, Isfahan University of Medical Sciences, Isfahan, Iran.
******BSc in Laboratory Science, Cardiovascular Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

pneumonia has been detected in carotid artery specimens with prevalence ranging from 0% to 80% [2]. Considering these controversies in this theme, and also the significance of the subject in prophylactic modalities of atherosclerosis, this study was designed to investigate the influence of chronic active Chlamydia pneumoniae infection on atherosclerotic plaque formation and to predict the role of its markers for occurrence of atherosclerosis and cerebrovascular events.

Methods

This case-control study was done between Sept. 2005 and Feb.2006 in the department of neurology, section of duplex ultrasonography in Alzahra Hospital, affiliated with the Isfahan University of Medical Sciences in Iran. Cases and control subjects were chosen randomly from the referred patients who underwent duplex ultrasonography of cervical arteries. Duplex sonography was performed by two neurologists using 5.5 MH transducer of ATL duplex ultrasound. After performing duplex ultrasonography on extracranial portions of carotid arteries, all cases with atherosclerotic plaque in external carotid and vertebral arteries were excluded, and the remaining patients were divided into two groups: 42 patients with atherosclerotic plaque in at least one internal carotid artery or common carotid artery as case subjects; and 82 patients without any plaque in aforementioned arteries as control subjects. These two groups were adjusted for age, hypertension, diabetes mellitus, hyperlipidemia and smoking. Patients with recent pulmonary infection (radiological or clinical) and ischemic heart diseases were not included in the study. The patients with atherosclerotic plaque were divided in 3 groups according to the severity of carotid stenosis [12] and Cp.IgG and Cp.IgA titers were compared in them. The Ethical Review Committee of Isfahan University of Medical Sciences approved the study protocol. The nature of the study was explained to the patients or their family and his /her written consent was obtained.

Serological Testing

From every case 2 ml of fasting blood was obtained and centrifuged within two hours after cooling in 4^{0c}. Then, its serum was separated and stored in -20^{0c} temperature for a maximum of one week, then in -70^{0c} temperature until analysis time. Sera were tested for IgG and IgA against Chlamydia pneumoniae (Cp.IgG and Cp.IgA) by one investigator using the ELISA method by Trinity biotech Capita kit, made in Ireland, by Trinity biotech manufacturer. Cp.IgG and Cp.IgA titers were presumed as negative when they were equal or lower than 0.9 ISR (Immune Status Ratio), borderline between 0.91 and 1.09 ISR, and positive when equal to or higher than 1.1 ISR. The laboratory technician who performed all the tests was unaware of the study hypothesis.

Analysis of Data

We used t-student test for comparing the means of antibody titers between case and control groups. For analysis of qualified data, we used X^2 (chi-square) tests. All analysis was performed using statistical software SPSS, 11th edition.

Results

There were 42 cases that had atherosclerotic plaque in at least one of their right or left common carotid arteries and/or internal carotid arteries and 82 controls that did not have any plaque in those arteries. The mean ages in case and control groups were 68.71 years (SD = 11.48) and 66.01 years (SD = 10.97), respectively. There was no statistically significant difference between these groups (P = 0.204) (table 1). 19 females and 23 males were in the case group and 48 females and 34 males in the control group, and there was no statistically significant difference between these groups (P = 0.185) (table 1). These two groups were adjusted for hypertension, diabetes mellitus, hyperlipidemia and smoking (table 1). The difference of Cp.IgA seropositivity between case and control subjects (table 2) was high but not statistically prominent (OR = 3.08, %95CI: 0.914 to 10.383). There was no significant difference in

Cp.IgG seropositivity between case and control subjects (OR = 1.585, %95CI: 0.653 to 3.848) (table 2). Although the means of Cp.IgA and Cp.IgG titers in case subjects were higher than those in control subjects (table 3), the differences were not significant statistically (P = 0.103 and 0.064, respectively). Statistical analysis was done in men and women separately. In females Cp.IgA was positive in 4.2% of controls and 31.6% of cases (table 2) and the difference was significant (P = 0.005). But Cp.IgG seropositivity didn't differ between females without plaque and those with plaque in previously mentioned arteries, and the difference was not significant (table 3). In females the means of Cp.IgA titer were 0.48 (SD = 0.22) in

the case group and 0.83 (SD = 0.45) in the control group, and there was a significant difference between them (P = 0). The means of Cp.IgG titers in female case and control groups had no significant difference. (table 3) In males there was no significant difference between case and control subjects in seropositivity of Cp.IgA and Cp.IgG and the means of the titers of these antibodies (tables 2 and 3). The means of titers of Cp.IgA and Cp.IgG were compared in patients with plaque in three grades of stenosis severity and the differences among these three groups were insignificant for both antibodies (table 4). These 3 groups had been adjusted for age, sex, hypertension, diabetes mellitus, hyperlipidemia and smoking (table 5).

Table 1. Comparing the mean age and prevalence of risk factors in cases with atherosclerotic plaque and control subjects without plaque.

	Mean age	SD of age	HTN	DM	HLP	Smoking	Total
Plaque	68.71	11.48	26	15	9	12	42
			62%	36%	21%	28%	
No plaque	66.01	10.97	52	38	19	22	82
			63%	46%	23%	27%	

Table 2. Comparing the prevalence of Cp.IgG and Cp.IgA seropositivity in cases with atherosclerotic plaque and control subjects without plaque.

	Male			female			Total		
	plaque 23	no plaque 34	p.value	plaque 19	no plaque 48	p.value	plaque 42	no plaque 82	p.value
CP.IgA	4.3%	8.8%	0.641	31.6%	4.2%	0.005	16.7%	6.1%	0.104
No.	1	3		6	2		7	5	
CP.IgG	26.1%	14.7%	0.322	26.3%	20.8%	0.747	26.2%	18.3%	0.354
No.	6	5		5	10		11	15	

Table 3. Comparing the means of CP.IgG and CP.IgA titers in cases with atherosclerotic plaque and control subjects without plaque.

		plaque	SD	no plaque	SD	p.value
total	CP.IgA	0.6338	0.41	0.5199	0.33	0.103
	CP.IgG	0.8100	0.60	0.6134	0.52	0.064
female	CP.IgA	0.8339	0.45	0.4899	0.22	000·0
	CP.IgG	0.9191	0.77	0.6230	0.56	0.092
male	CP.IgA	0.4691	0..28	0.5606	0.44	0. 386
	CP.IgG	0.7200	0.40	0.6003	0.46	0.321

Table 4. Comparing the means of Cp.IgG and Cp.IgA titers in patients with atherosclerotic plaque with 3 grades of stenosis severity.

	< 50% n=23	SD	50-75% n=14	SD	> 75% n=5	SD	p.value
Cp.IgG	0.6243	0.37	0.6807	0.50	0.5460	0.37	0.818
Cp.IgA	0.8104	0.63	0.8001	0.60	0.8360	0.54	0.994

Table 5. Comparing the mean age, sex, and prevalence of risk factors in patients with atherosclerotic plaque with 3 grades of stenosis severity.

Severity	Mean age	SD	Sex		HTN	DM	HLP	Smoking	Total
			F	m					
< 50% n=23	65.69	13.17	10 43.5%	13 56.5%	15 65.2%	8 34.7%	5 21.7%	7 30.4%	23
50-75% n=14	71.07	8.53	7 50%	7 50%	8 57.1%	5 35.7%	3 21.4%	4 28.5%	14
> 75% n=5	76.00	4.89	2 40%	3 60%	3 60%	2 40%	1 20%	1 20%	5
	P= 0.122		P= 0.899						42

Discussion

In the current study the prevalence of seropositivity of Cp.IgA was higher in patients with atherosclerotic plaque, and especially in females was statistically prominent; so this infection may be considered as a risk factor for atherosclerosis plaque formation in the carotid arteries, and one of its markers (Cp.IgA) may be a predictor of atherosclerosis in these vessels in women. In one study that assessed only Cp.IgA, this marker was introduced as a predictor of atherosclerosis and cerebrovascular events [13]. But the prevalence of CP.IgG seropositivity in case and control groups of our study was not significantly different and the means of Cp.IgG titers in the two groups were not significantly different. Also, in male and female groups there was no significant difference in case and control groups. Another study that assessed only CP.IgG indicated that it does not have a role in the prediction of atherosclerosis [14]. It is noteworthy that one sixth of our cases were Cp.IgA seropositive in comparison with one sixteenth of the control subjects, but one fourth of cases and one fifth

of control subjects were Cp.IgG seropositive with atherosclerotic plaque. This hallmarks the prediction value of Cp.IgA in comparison with Cp.IgG. The present results are consistent with data obtained in other studies that indicated chronic active infection has a role in atherosclerosis and Cp.IgA is a predictor of plaque formation [15-17]. Here it is necessary to explain the difference between serology of acute, chronic and active infections. In acute infection, IgM rises and in chronic inactive infection, IgG rises, but in chronic active infection IgA and immune complexes containing specific IgG are high [18]. So, according to our results, chronic active infection has a greater role in atherosclerosis than chronic inactive infection. Although the association of Cp.IgA and atherosclerotic plaques was detected, this finding does not establish whether infection plays a causal role in disease or whether the organism simply existed with the lesion as an innocent bystander. So it may be useful to follow up the cases with Cp.IgA seropositivity for atherosclerosis and ischemic cerebrovascular events. Such studies have been done by Trance

168   Journal of Research in Medical Sciences July & August 2007; Vol 12, No 4.

www.mui.ac.ir

D. et al. in 2003 and Eagle et al. in 2005 and it was concluded that parallel to the rise of anti-bodies, the risk of ischemic stroke due to atherosclerosis increases although there is not any prominent association [6,19]. In similar studies that were done on cardiovascular events and in 6 years follow-up, there was an increase in cardiovascular events in seropositive patients for Chlamydia pneumoniae [20]. But in another study the frequency of myocardial infarction was lower in CP.IgA positive patients [21]. On the other hand, future studies should be done to assess the efficacy of antibiotics in preventing atherosclerosis. Some studies have shown a significant difference in CP.IgG titer in patients with plaque compared to subjects without plaque [22,23], which does not agree with our results. In another study by Anzini et al. in 2004, the prevalence of seropositivity of both Cp.IgA and Cp.IgG were higher in patients with thrombotic ischemic stroke due to atherosclerosis [24]. In some studies done by Gerdes V.E. et al. and Ngeh J. et al. in 2003, neither Cp.IgA nor Cp.IgG were associated with atherosclerosis or cerebrovascular events [21,25].

About the mechanism of Chlamydia pneumonia infection effect on atherosclerotic plaque formation there are multiple theories. This microorganism tends to proliferate in vascular endothelial cells such as alveolar macrophages and its presence in the wall of atherosclerotic cerebral vessels (not in normal vessels) was proved by RCR and immunohistochemical methods. On the other hand, the infection can deteriorate the plaque by T-cell activation and inflammatory response and destabilize the initial cap at plaque [2,26]. In addition to direct involvement of the vessel wall the lipopolysaccharide components of the Chlamydial cell wall induce the tumor necrosis factor (TNF), Interleukin (IL2) and tissue factor (TF) [1,26-29], and these are due to change in metabolism and function of endothelial cell, monocytes and macrophages because of infection [9,30]. Another theory indicates that the antigenic mimicry between heavy chain of myosin filaments and outer membrane of Chlamydia pneumoniae induces immunological reaction [31]

and bacterial infection can initiate, progress and destabilize the plaque [32]. There is the question of why the differences of Cp.IgA seropositivity between case and control groups were significant in females but not in males. As we mentioned above, some theories argue that autoimmune responses mediated by infectious agents have major roles in atherosclerotic plaque formation. As we know, autoimmunity reactions and disorders are more prevalent in women [33] and this may explain the difference between men and women in atherogenesis due to infection. One other explanation for this difference may be under the concept of "metabolic syndrome" (MetS). This is a new subject with definable criteria in men and women that is highly atherogenic condition [34,35]. Also, it is established that metabolic syndrome is associated with high inflammatory status. However, MetS traits or MetS by itself have a stronger effect on atherosclerosis and ischemic events among women than men [34,36]. The third reason for highlighting this difference is the information about smoking that is not reliable in men. Smoking has very low prevalence among females in Iranian culture. So by limiting the study to females group and omitting this confounding factor the results are more reliable. Also, in this study we compared the means of Cp.IgG and Cp.IgA titers in patients with different grades of cervical carotid stenosis. There wasn't any significant difference in the means of titers of antibodies among 3 groups. So it may be concluded that Chlamydia pneumoniae infection has no role in progression of atherosclerosis according to our study, but it would be better to do this assessment with larger groups for a more accurate conclusion.

Conclusion

This study focused on the role of chronic active infection in atherosclerosis. Based on these results and the results of similar studies, we can predict atherosclerosis with screening of serum Cp.IgA, especially in women, and may use the antibiotics with other medications for primary and secondary prevention of atherosclerosis.

Journal of Research in Medical Sciences July & August 2007; Vol 12, No 4.

169

References

1. Biller J, Love BB. **Vascular diseases of the nervous system: Ischemic cerebrovascular diseases**. In: Bradley WE, Daroff RB, Fenichel GM, Jankovic J, editors. *Neurology in clinical practice*. 4 ed. Boston: Butterworth Heinemann; 2004. 1219.

2. Jackson LA, Grayston JT. **Chlamydia pneumoniae**. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and Practice of Infectious Disease*. 5 ed. Philadelphia: Churchill Livingstone; 2000. 2011-2012.

3. McDonald K, Rector TS, Braulin EA, Kubo SH, Olivari MT. **Association of coronary artery disease in cardiac transplant recipients with cytomegalovirus infection**. *Am J Cardiol* 1989; 64(5):359-362.

4. Monno R, Di Biase M, Costi A, de Nicolo T, Correale M, Bolognese P et al. **[Chlamydia pneumoniae, atherosclerosis, and coronary disease]**. *Ital Heart J Suppl* 2003; 4(5):383-397.

5. Nikoskelainen J, Kalliomaki JL, Lapinleimu K, Stenvik M, Halonen PE. **Coxsackie B virus antibodies in myocardial infarction**. *Acta Med Scand* 1983; 214(1):29-32.

6. Tanne D, Haim M, Boyko V, Goldbourt U, Reshef T, Adler Y et al. **Prospective study of Chlamydia pneumoniae IgG and IgA seropositivity and risk of incident ischemic stroke**. *Cerebrovasc Dis* 2003; 16(2):166-170.

7. Woods JD, Nimmo MJ, Mackay-Scollay EM. **Acute transmural myocardial infarction associated with active Coxsackie virus B infection**. *Am Heart J* 1975; 89(3):283-287.

8. LaBiche R, Koziol D, Quinn TC, Gaydos C, Azhar S, Ketron G et al. **Presence of Chlamydia pneumoniae in human symptomatic and asymptomatic carotid atherosclerotic plaque**. *Stroke* 2001; 32(4):855-860.

9. 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):719-734.

10. Virok D, Kis Z, Karai L, Intzedy L, Burian K, Szabo A et al. **Chlamydia pneumoniae in atherosclerotic middle cerebral artery**. *Stroke* 2001; 32(9):1973-1976.

11. Sander D, Winbeck K, Klingelhofer J, Etgen T, Conrad B. **Reduced progression of early carotid atherosclerosis after antibiotic treatment and Chlamydia pneumoniae seropositivity**. *Circulation* 2002; 106(19):2428-2433.

12. Babikian VL, Tegeler CH. **Ultrasound imaging of the cerebral vasculature**. In: Bradley WE, Daroff RB, Fenichel GM, Jankovic J, editors. *Neurology in clinical practice*. 4 ed. Boston: Butterworth Heinemann; 2004. 651.

13. Madre JG, Garcia JL, Gonzalez RC, Montero JM, Paniagua EB, Escribano JR et al. **Association between seropositivity to Chlamydia pneumoniae and acute ischaemic stroke**. *Eur J Neurol* 2002; 9(3):303-306.

14. Medrano V, Castano MD, Andreu M, Molto JM, Sanchez-Perez RM, Hernandez-Lorido R et al. **[The relation between carotid ultrasound changes and seropositivity to Chlamydia pneumoniae in patients with acute stroke]**. *Rev Neurol* 2000; 31(10):932-936.

15. Cook PJ, Honeybourne D, Lip GY, Beevers DG, Wise R, Davies P. **Chlamydia pneumoniae antibody titers are significantly associated with acute stroke and transient cerebral ischemia: the West Birmingham Stroke Project**. *Stroke* 1998; 29(2):404-410.

16. Elkind MS, Lin IF, Grayston JT, Sacco RL. **Chlamydia pneumoniae and the risk of first ischemic stroke : The Northern Manhattan Stroke Study**. *Stroke* 2000; 31(7):1521-1525.

17. Wolf SC, Mayer O, Jurgens S, Vonthein R, Schultze G, Risler T et al. **Chlamydia pneumoniae IgA seropositivity is associated with increased risk for atherosclerotic vascular disease, myocardial infarction and stroke in dialysis patients**. *Clin Nephrol* 2003; 59(4):273-279.

18. Wimmer ML, Sandmann-Strupp R, Saikku P, Haberl RL. **Association of chlamydial infection with cerebrovascular disease**. *Stroke* 1996; 27(12):2207-2210.

19. Corrado E, Rizzo M, Tantillo R, Muratori I, Bonura F, Vitale G et al. **Markers of inflammation and infection influence the outcome of patients with baseline asymptomatic carotid lesions: a 5-year follow-up study**. *Stroke* 2006; 37(2):482-486.

20. Saikku P, Leinonen M, Tenkanen L, Linnanmaki E, Ekman MR, Manninen V et al. **Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study**. *Ann Intern Med* 1992; 116(4):273-278.

21. Gerdes VE, Verkooyen RP, Kwa VI, de Groot E, van Gorp EC, Ten Cate H et al. **Chlamydial LPS antibodies, intima-media thickness and ischemic events in patients with established atherosclerosis**. *Atherosclerosis* 2003; 167(1):65-71.

22. Colpan ME, Attar A, Colpan A, Karahan C, Seckin S, Sargon MF et al. **Chlamydia pneumoniae infection related atherosclerotic clinical variables on carotid stenosis**. *J Clin Neurosci* 2004; 11(4):389-394.

23. Kawamoto R, Kajiwara T, Oka Y, Takagi Y. **An association between an antibody against Chlamydia pneumoniae and ischemic stroke in elderly Japanese**. *Intern Med* 2003; 42(7):571-575.

24. Anzini A, Cassone A, Rasura M, Ciervo A, Beccia M, Di Lisi F et al. **Chlamydia pneumoniae infection in young stroke patients: a case--control study**. *Eur J Neurol* 2004; 11(5):321-327.

25. Ngeh J, Gupta S, Goodbourn C, Panayiotou B, McElligott G. **Chlamydia pneumoniae in elderly patients with stroke (C-PEPS): a case-control study on the seroprevalence of Chlamydia pneumoniae in elderly patients with acute cerebrovascular disease**. *Cerebrovasc Dis* 2003; 15(1-2):11-16.

26. Fryer RH, Schwobe EP, Woods ML, Rodgers GM. **Chlamydia species infect human vascular endothelial cells and induce procoagulant activity**. *J Investig Med* 1997; 45(4):168-174.

27. Lehr HA, Sagban TA, Kirkpatrick CJ. **[Atherosclerosis--progression by nonspecific activation of the immune system]**. *Med Klin (Munich)* 2002; 97(4):229-235.

28. Tannenbaum SH, Finko R, Cines DB. **Antibody and immune complexes induce tissue factor production by human endothelial cells**. *J Immunol* 1986; 137(5):1532-1537.

29. Valtonen VV. **Infection as a risk factor for infarction and atherosclerosis**. *Ann Med* 1991; 23(5):539-543.

30. Rupp J, Berger M, Reiling N, Gieffers J, Lindschau C, Haller H et al. **Cox-2 inhibition abrogates Chlamydia pneumoniae-induced PGE2 and MMP-1 expression**. *Biochem Biophys Res Commun* 2004; 320(3):738-744.

31. Kazmierski R, Kozubski W. **[Effect of Chlamydia pneumoniae infection on carotid atherosclerosis development]**. *Neurol Neurochir Pol* 2002; 36(1):131-142.

32. Muhlestein JB. **Bacterial infections and atherosclerosis**. *J Investig Med* 1998; 46(8):396-402.

33. Haynes BF, Fauci AS. **Disorders of immune system, connective tissue, and joints**. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Lonco DL, Jameson JL, editors. *Harrison principles of internal medicine*. **15** ed. **New York**: **McGraw-Hill**; 2001. 1805-2018 .

34. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. **Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study**. *Arterioscler Thromb Vasc Biol* 2005; 25(5):1045-1050.

35. Saito I, Mori M, Shibata H, Hirose H, Tsujioka M, Kawabe H. **Prevalence of metabolic syndrome in young men in Japan**. *J Atheroscler Thromb* 2007; 14(1):27-30.

36. Kawamoto R, Tomita H, Inoue A, Ohtsuka N, Kamitani A. **Metabolic syndrome may be a risk factor for early carotid atherosclerosis in women but not in men**. *J Atheroscler Thromb* 2007; 14(1):36-43.