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The Association Between *Chlamydia pneumoniae* Infection and Ischemic Stroke

Peyman Eini [1]; Fariba Keramat [1,*]; Nasim Farajpoor [2]

[1]Department of Infectious Diseases, Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran
[2]School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

[*]*Corresponding author*: Fariba Keramat, Department of Infectious Diseases, Brucellosis Research Center, Hamadan University of Medical Sciences, P. O. BOX: 65168, Hamadan, IR Iran. Tel: +98-9181113281, Fax: +98-8138269808, E-mail: faribakeramat@yahoo.com

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Background: *Chlamydia pneumoniae* has been linked with increased risk of cardiovascular diseases; however, data on stroke and cerebrovascular accidents are sparse.
Objectives: The aim of this study was to determine the association between *C. pneumoniae* infection and ischemic stroke.
Patients and Methods: In a case-control study, 141 patients, admitted with ischemic stroke, were compared with gender and age-matched control subjects (n = 141). Using an enzyme-linked immunosorbent assay kit, the presence of *C. pneumoniae* IgG and IgA in the patients' sera was determined. The data were analyzed by SPSS software (version 15) and were compared between the two groups using T-test and chi square test.
Results: The mean ages of the case and control groups were 68.97 ± 12.29 and 66.95 ± 6.68 years old, respectively. The difference between these two groups was not statistically significant (P = 0.102). The seroprevalence of *C. pneumoniae*-specific IgG were 78.7% in the patients with stroke and 52.5% in the control group. The difference between the two groups was statistically significant (P = 0.0001). The seroprevalence of *C. pneumoniae*-specific IgA were 41.1% in the stroke and 15.6% in the control group. The difference between the two groups was statistically significant (P = 0.0001).
Conclusions: The results supported the hypothesis that serological evidence of *C. pneumoniae* infection may be associated with an increased risk of ischemic stroke and cerebrovascular accident.

Keywords:Chlamydia pneumoniae; Cerebrovascular Accident; Stroke

1. Background

Chlamydia pneumoniae was first described in 1986 (1). It is one of the most common agents involved in respiratory tract infections including pneumonia, bronchitis, pharyngitis, and sinusitis. Approximately, 5-10% of all cases of adult pneumonia and bronchitis are caused by *C. pneumoniae* (2, 3). Atherosclerosis is a multifactorial disease. The various explanations of the pathogenic process include chronic infection with certain pathogens. The presence of *C. pneumoniae* has recently been demonstrated in atherosclerotic lesions of coronary, aorta, carotid and cerebral arteries. Conventional stroke risk factors include hypertension, cigarette smoking, diabetes mellitus and hyperlipidemia, which do not fully explain the incidence of atherosclerotic vascular diseases (4-6). Serologic evidence of infection with *C. pneumoniae* has been associated with cardiovascular diseases; however, its relationship with stroke risk remains uncertain. In addition, inflammation has been postulated to play an important role in initiation and development of atherosclerosis. Retrospective and cross-sectional studies have suggested that both bacterial and viral infections may be risk factors for atherosclerosis, ischemic stroke and acute coronary events (7-11). Some studies do not confirm the association between seropositivity to *C. pneumoniae* and increased risk of cardiovascular diseases in populations with stroke (12). Although, the role of *C. pneumoniae* infection has been seriously considered in patients with myocardial infarction as a new atherothrombotic risk factor, but in acute ischemic stroke there are few data to support the eventual association (13).

2. Objectives

The aim of this study was to determine the association between *C. pneumoniae* infection and ischemic stroke.

3. Patients and Methods

A case-control study was conducted on patients with ischemic stroke, admitted to the Department of Neurology in Farshchian Hospital, Hamadan, west of Iran, from March 2008 to March 2009. Stroke was defined according to the recommended standard definition of World Health Organization as a focal or global neurological impairment of sudden onset, lasting 24 hours of presumed

vascular origin (13). Moreover, all patients with ischemic stroke were diagnosed by brain computed tomography scan (CT scan) or brain magnetic resonance imaging (MRI). Totally, 141 patients with ischemic stroke were enrolled in this study and compared to 141 control subjects who were selected randomly from volunteer blood donors. The control group did not have a history of pulmonary or cardiovascular disorders, and there was no evidence of active cardiac, vascular or pulmonary disease. The two groups were also matched by age, gender and place of residence. Written informed consents were obtained from all the participants. Moreover, blood samples were drawn from the control group and the patients with stroke over a period of maximum of 48 hours from the onset of stroke. All the sera were separated via centrifugation and stored at -20°C until analysis; then, they were tested by one investigator. Samples were tested using *C. pneumoniae* IgG and IgA enzyme-linked immunosorbent assay method (Medac Diagnostika *C. pneumoniae* IgG and IgA ELISAs; Hersteller Manufacture Fabricant, Hamburg, Germany). All the data were analyzed statistically with SPSS 15 software using T-test, Fisher's exact and chi square tests. The P Values below 0.05 were considered statistically significant.

4. Results

Totally, 141 patients with ischemic stroke (81 males and 60 females) with mean age of 68.97 ± 12.99 (range: 30-95) years old were enrolled in the study and were compared with 141 control subjects (81 males and 60 females) with mean age of 66.95 ± 6.68. Of the study population, 188 (66.7%) lived in urban areas and the rest were from rural areas. In both groups, there was no significant difference in regards to age, sex and place of residence. The left hemisphere of the brain of 76 (52.5%) cases was the most affected hemisphere, whereas involvement of both right and left hemispheres was reported in one patient (0.7%). The brain areas involved in the patients, revealed by imaging (brain CT scan or MRI), were reported as the following: parietal lobe in 43 cases (30.5%), which was the most common site of involvement; temporal lobe in 36 cases (25.5%); simultaneous involvement of parietal and temporal lobes in 21 (14.9%); frontal lobe in 12 (8.5%); occipital lobe in 10 (7.1%); simultaneous involvement of parietal and occipital lobes in 10 (7.1%); brain stem in 4 (2.8%); simultaneous involvement of parietal, temporal and occipital in 2 (1.4%). According to Canadian Neurological Scale, 54 patients (38.3%) had severe, 68 (48.2%) had moderate, and 19 (13.5%) had mild stroke severity (14). Anti-*C. pneumoniae* IgG was detected in 185 (65.6%) subjects of the study population. Table 1 shows that 111 patients with thrombotic stroke (78.8%) had positive serology for anti-*C. pneumoniae* IgG; however, in the control group, 74 (52.5%) subjects were positive for anti-*C. pneumoniae* IgG. Therefore, anti-*C. pneumoniae* IgG level in the patients with stroke was significantly higher than the control group (P = 0.0001). Table 2 shows that anti-*C. pneumoniae* IgA was detected in 80 (28.4%) subjects of the study population and the rest of them had negative results. In addition, 58 (41.1%) patients with thrombotic stroke and 22 (15.6%) subjects in the control group were positive for anti-*C. pneumoniae* IgA. In other words, the positive rate of anti-*Chlamydia* IgA in patients with thrombotic stroke was significantly higher than the control group (P = 0.0001).

Table 1. Comparison of IgG Antibody Against *Chlamydia pneumoniae* Between Stroke and Control Groups

IgG Antibody	Case Group, No. (%)	Control Group, No. (%)
Positive	111 (78.7)	74 (52.5)
Negative	30 (21.3)	67 (47.5)
Value	0.0001 [a]	21.51 [b]

[a] P value.
[b] Chi square; degree of freedom = 1.

Table 2. Comparison of IgA Antibody Against *Chlamydia pneumonia* Between Stroke and Control Groups

IgA Antibody	Case Group, No. (%)	Control Group, No. (%)
Positive	58 (41.1)	22 (15.6)
Negative	83 (58.9)	119 (84.4)
Value	0.0001 [a]	22.61 [b]

[a] P value.
[b] Chi-Square; degree of freedom = 1.

5. Discussion

The present study investigated the relationship between *C. pneumoniae* infection and ischemic stroke. In this case-control study, 141 patients were enrolled and compared with 141 control subjects. The mean ages of the patients and controls were 68.97 and 66.95 years old, respectively. Therefore, there was no significant difference between the two groups regarding age. Though, different classes of antibodies are produced in *C. pneumoniae* infection, each of which has a different clinical implication. For example, IgA antibody presence is short-term and represents acute infection, whereas, IgG antibody lasts several months and indicates chronic infection (2).

In the present study, anti-*C. pneumoniae* IgG was detected in 111 patients with thrombotic stroke (78.8%); however, in the control group, 74 (52.5%) patients had positive antibody titer. Anti-*C. pneumoniae* IgG in patients with stroke was significantly higher than the control group. In Ghachkar's study, 44 patients with acute stroke and 44 controls were studied. Seven patients (15.9%) had specific IgG against *C. pneumoniae*, but none of the controls had such antibody, which was similar to this study (15). In Japan, 40 patients with stroke and 85 healthy individuals were studied by Kawamoto and colleagues. They reported that the level of anti-*C. pneumoniae* IgG in patients with stroke was significantly higher than that

of the control group (16). This was similar to the results of our study. However, other studies were unable to find out a relationship between thrombotic stroke and Chlamydial infection (16, 17). In Ashtari's research, 81 patients with stroke and 43 healthy controls were studied and there was no significant difference between the two groups (17). In Medrano's study, there was no relationship between IgG antibodies against *C. pneumoniae* and cerebrovascular accidents (18). In another study in Cameroon, 64% of the patients with ischemic stroke had IgG against *C. pneumoniae*, compared to 55% positivity in the control group, the difference of which was not statistically significant (7).

In this study, anti-*C. pneumoniae* IgA in patients with thrombotic stroke was present in 58 (41.1%) patients, and 22 (15.6%) subjects in the control group had a positive antibody titers; in other words, the positive rate of anti-*Chlamydia* IgA in patients with thrombotic stroke was significantly higher than the control group (P = 0.0001). In Elkind's study on 89 patients with first ischemic stroke and 89 healthy controls, increased levels of IgA antibodies against *C. pneumoniae* was associated with increased risk of ischemic stroke (19). In another case-control study, 246 patients with ischemic stroke and 474 controls were studied, which reported that anti-*C. pneumoniae* IgA level in patients was higher than the controls, while this was not observed for IgG levels (8). In the study of Wolf and Mayer, positive levels of anti-*C. pneumoniae* IgA were associated with increased stroke risk in dialysis patients (20). Njamnshi and colleagues reported that 78% of the patients had IgA against *C. pneumoniae*, the rate of which was 42% in the control group and the difference was statistically significant (7). These results were similar to the present study. It should be noted that the IgA positivity rates in patients from the above study were higher than those of this study. The presence of IgA antibody in *C. pneumoniae* infection is short-term and represents acute infection, whereas IgG antibody lasts several months and indicates chronic infection (2). Therefore, in our study, the patients with thrombotic stroke were infected by *C. pneumoniae* and had acute infections. In another study at Whipps Cross Hospital in London on 100 patients with stroke and 87 controls, conducted to detect the differences between IgG and IgA antibodies in two groups, significant differences were not observed (21). Furthermore, in Tanne's research, IgG and IgA antibodies to *C. pneumoniae* between two groups were not significantly different (22). In Ashtari's study, 13.6% of patients with stroke and 9.3% of controls had positive levels of anti-*Chlamydia* IgA, but the difference was not significant (17). The rate of positive anti-*C. pneumoniae* IgA in the case group in our study was higher than that of the mentioned study.

Inflammation has been postulated to play an important role in initiation and development of atherosclerotic diseases. Microorganisms could directly invade the vascular endothelial cells and sometimes indirectly influence atherosclerosis progression by stimulation of inflammatory cytokines and tissue growth factors in the arterial endothelium (23). Therefore, elevated titers against *C. pneumoniae* in sera from such patients point to an exacerbation in a chronic infection, as does a change in the nature of immune complexes containing chlamydial lipopolysaccharide LPS. The presence of antibodies to *C. pneumoniae* proteins in immune complexes suggests an intimate association of the pathogen with the vascular system (24). For the first time in 1988, Saikku and colleagues in a case-control study showed that patients who have recently had myocardial infarctions, had considerable amounts of IgA and IgG antibody titers against *C. pneumoniae* compared to control individuals. Following these findings, the researchers suggested that myocardial infarctions might be associated with exacerbation of chronic *Chlamydia* infection (25). In another study, Thom and colleagues reported that patients who had heart angiography with rising titer of IgG against *C. pneumoniae* as an indicator of chronic or previous infection, were twice as likely to be diagnosed with coronary artery disease (26). Several comments on various mechanisms causing stroke by *C. pneumoniae* have been posted. *C. pneumoniae,* in addition to alveolar macrophage proliferation, has the tendency to replicate in vascular endothelial cells in the culture environment. Its presence in the arterial wall of atherosclerotic cranial, detected by immunohistochemical and PCR methods, has been proven (27, 28). *C. pneumoniae* can also activate via T cell inflammatory response and cause deterioration of atherosclerosis (29).

In conclusion, this study showed an association between ischemic stroke syndrome and *C. pneumoniae* infection. *C. pneumoniae* infection may increase the susceptibility to stroke by inducing a systemic inflammatory response that can lead to a hypercoagulable state, destabilization of preexisting atherosclerotic plaques, and local thrombosis. Therefore, it should be strongly supported to further explore the role of *C. pneumoniae* infection in the etiology of ischemic stroke.

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Authors' Contributions

Peyman Eini, Fariba Keramat, Nasim Farajpoor were involved in the study concept and design, drafting of the manuscript, critical revision of the manuscript, and

study supervision. Nasim Farajpoor was also in charge of acquisition, analysis and interpretation of data.

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References

1. Grayston JT, Kuo CC, Wang SP, Altman J. A new Chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med.* 1986;**315**(3):161–8.
2. Hahn DL, Anttila T, Saikku P. Association of Chlamydia pneumoniae IgA antibodies with recently symptomatic asthma. *Epidemiol Infect.* 1996;**117**(3):513–7.
3. Numazaki K, Ikebe T, Chiba S. Detection of serum antibodies against Chlamydia pneumoniae by ELISA. *FEMS Immunol Med Microbiol.* 1996;**14**(2-3):179–83.
4. 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):1973–6.
5. Valassina M, Migliorini L, Sansoni A, Sani G, Corsaro D, Cusi MG, et al. Search for Chlamydia pneumoniae genes and their expression in atherosclerotic plaques of carotid arteries. *J Med Microbiol.* 2001;**50**(3):228–32.
6. Vink A, Poppen M, Schoneveld AH, Roholl PJ, de Kleijn DP, Borst C, et al. Distribution of Chlamydia pneumoniae in the human arterial system and its relation to the local amount of atherosclerosis within the individual. *Circulation.* 2001;**103**(12):1613–7.
7. Njamnshi AK, Blackett KN, Mbuagbaw JN, Gumedze F, Gupta S, Wiysonge CS. Chronic Chlamydia pneumoniae infection and stroke in Cameroon: a case-control study. *Stroke.* 2006;**37**(3):796–9.
8. Elkind MS, Tondella ML, Feikin DR, Fields BS, Homma S, Di Tullio MR. Seropositivity to Chlamydia pneumoniae is associated with risk of first ischemic stroke. *Stroke.* 2006;**37**(3):790–5.
9. Kol A, Libby P. The mechanisms by which infectious agents may contribute to atherosclerosis and its clinical manifestations. *Trends Cardiovasc Med.* 1998;**8**(5):191–9.
10. 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):321–7.
11. Haider AW, Wilson PW, Larson MG, Evans JC, Michelson EL, Wolf PA, et al. The association of seropositivity to Helicobacter pylori, Chlamydia pneumoniae, and cytomegalovirus with risk of cardiovascular disease: a prospective study. *J Am Coll Cardiol.* 2002;**40**(8):1408–13.
12. 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):303–6.
13. World Health Organization . *The World Health Organization stepwise approach to stroke surveillance.* 2005.
14. Cote R, Hachinski VC, Shurvell BL, Norris JW, Wolfson C. The Canadian Neurological Scale: a preliminary study in acute stroke. *Stroke.* 1986;**17**(4):731–7.
15. Ghachkar L, Malek-Hosseini M, Ghareghozlooei K, Esfandyari N. [Relationship between Chlamydia pneumoniae infection with acute stroke]. *Pejoohandeh.* 2002;**7**(1):53–7.
16. 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):571–5.
17. Ashtari F, Shayegannejad V, Saberi A, Karkheyran F, Khosravi E. [Chlamydia pneumoniae infection and ischemic stroke]. *J Isfahan Med Sch.* 2007;**24**(83):8–14.
18. 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):932–6.
19. 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):1521–5.
20. 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):273–9.
21. Ngeh J, Goodbourn C. Chlamydia pneumoniae, Mycoplasma pneumoniae, and Legionella pneumophila in elderly patients with stroke (C-PEPS, M-PEPS, L-PEPS): a case-control study on the infectious burden of atypical respiratory pathogens in elderly patients with acute cerebrovascular disease. *Stroke.* 2005;**36**(2):259–65.
22. 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):166–70.
23. Fong IW. Infections and their role in atherosclerotic vascular disease. *J Am Dent Assoc.* 2002;**133 Suppl**:7S–13S.
24. Saikku P. Epidemiologic association of Chlamydia pneumoniae and atherosclerosis: the initial serologic observation and more. *J Infect Dis.* 2000;**181 Suppl 3**:S411–3.
25. Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet.* 1988;**2**(8618):983–6.
26. Thom DH, Wang SP, Grayston JT, Siscovick DS, Stewart DK, Kronmal RA, et al. Chlamydia pneumoniae strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arterioscler Thromb.* 1991;**11**(3):547–51.
27. 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):168–74.
28. Campbell LA, O'Brien ER, Cappuccio AL, Kuo CC, Wang SP, Stewart D, et al. Detection of Chlamydia pneumoniae TWAR in human coronary atherectomy tissues. *J Infect Dis.* 1995;**172**(2):585–8.
29. Muhlestein JB. Bacterial infections and atherosclerosis. *J Investig Med.* 1998;**46**(8):396–402.