ASSOCIATION OF PRIOR INFECTION WITH CHLAMYDIA AND CEREBROVASCULAR DISEASE

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SUMMARY

Purpose: Chlamydia pneumoniae is a human respiratory pathogen that causes acute respiratory disease and ~10% of community-acquired pneumonia. The infections are geographically widespread. In addition to respiratory disease, seroepidemiologic studies have shown an association of this organism with atherosclerotic proces and cerebrovascular disease. The role of preceding chlamydial infection as a risk factor for stroke was investigasted.
Material and Methods: We studied 68 consecutive patients under 65 years of age with cerebrovascular disease; the mean age was 52.08 ± 5.74 years (yrs) (range 39 yrs to 64 yrs; 28 female and 40 male) and 29 randomly selected age-matched healthy subjects (control); the mean age was 49.76 ± 13.11 years (yrs) (range 41 yrs to 62 yrs; 12 female and 17 male). Specific antibodies to C pneumoniae and C trachomatis in serum were measured by the microimmunofluorescence with the method of Wang and Grayston in all the subjects. Quantitative variables were analyzed with Mann-Whitney test.
Results: The mean values of antibodies titers in patients (mean IgG antibodies to C pneumoniae in patients, 1.382 ± 0.256 vs. control, 1.152 ± 0.334 [p=.012], mean IgG antibodies to C trachomatis in patients, 0.043 ± 0.019 vs control, 0.031 ± 0.015 [p=.026]) were significantly higher compared with the controls. Although the mean IgM antibodies titers to C pneumoniae and C trachomatis are lower and IgA to C pneumoniae higher in patients than the controls, this differences were not found to be significant (mean IgM antibodies to C pneumoniae in patients, 0.502 ± 0.188 vs. control, 0.559 ± 0.149 [p=.087], mean IgM antibodies to C trachomatis in patients, 0.451 ± 0.348 vs. control, 0.683 ± 0.578 [p=.140], mean IgA antibodies to C pneumoniae in patients, 1.008 ± 0.246 vs. control, 0.971 ± 0.237 [p=.563]).
Conclusion: We conclude that chronic infection with chlamydiae is associated with an increased risk of cerebrovascular disease and believe that patients who have high values of IgG antibodies titers should be warned for a probable cerebrovascular disease and other risk factors like smoking, alcohol, diet, etc.
Key Words: Cerebrovascular disease, atherosclerosis, infection, and risk factors.

KLAMİDİA VE SEREBROVASKÜLER HASTALIKLAR ARASINDAKİ İLİŞKİ

Giriş ve Amaç: Klamidia pnömoni solunum sisteminde akut infeksiyonlara sebep olan bir patojendir. Ayrıca, seroepidemiyolojik çalışmalar bu organizmanın aterosklerotik olaylar ve serebrovasküler hastalıklarla (SVH) da ilişkili olduğunu göstermiştir. Bu çalışmada, klamidial infeksiyonların SVH'da risk faktörü olarak rollerinin araştırılmasını planladık.
Gereç ve Yöntem: Çalışmaya 65 yaş altında 68 SVH'lı hasta ve 29 sağlıklı kontrol grubu alındı. Hasta yaş ortalaması 52.08 ± 5.74; 28 kadın, 40 erkek ve sağlıklı kontrol grubunun yaş ortalaması 49.76 ± 13.11; 12 kadın ve 17 erkek idi. Hasta ve kontrol grubunun serumunda Wang ve Grayston'ın tanımladığı mikroimmunofloresans yöntemle C pnömoni ve C trakhomatis'e karşı spesifik antikor ölçüldü. Elde edilen değerler ortalama ve standart sapma olarak hesaplandı. Veriler Mann-Whitney U testi kullanılarak karşılaştırıldı.
Bulgular: SVH'lı hastaların serumunda C pnömoni'ye karşı ortalama IgG antikor titresi 1.382 ± 0.256, kontrollerde 1.152 ± 0.334 [p=.012], C trakhomatis'e karşı IgG antikor titresi 0.043 ± 0.019, kontrollerde 0.031 ± 0.015 [p=.026] olduğu tespit edildi. SVH'lı olgular kontrol grubuyla karşılaştırıldığında önemli derecede IgG antikor düzeyi yüksekliği saptandı. C pnömoni ve C trakhomatis'e karşı IgM düzeyleri SVH'larda kontrollerdekinden düşük (C pnömoni'ye karşı SVH'larda ortalama IgM 0.502 ± 0.188; kontrol, 0.559 ± 0.149 [p=.087], C trakhomatis'e karşı SVH'larda ortalama IgM 0.451 ± 0.348; kontrol, 0.683 ± 0.578 [p=.140]), C pnömoni'ye karşı IgA düzeyleri SVH'larda kontrollerdekinden yüksek (C pnömoni karşı SVH'larda ortalama IgA 1.008 ± 0.246; kontrol, 0.971 ± 0.237 [p=.563]) bulundu. Fakat istatistiksel olarak anlamlı değildi.
Sonuç: Klamidialara karşı serum IgG antikor düzeylerinin, SVH grubunda tek başına anlamlı bir risk parametresi oluşturabileceğini belirledik. Yüksek IgG değerlerinde sahip bireylerin belirlenerek diğer risk faktörleri açısından (sigara, alkol, diyet vs.) uyarılmaları gerektiğine inanıyoruz.
Anahtar Sözcükler: Serebrovasküler hastalık, aterosklerozis, İnfeksiyon ve risk faktörü.

INTRODUCTION

Stroke places a tremendous burden on health resources throughout the world. Improved detection and modification of risk factors could reduce the impact of this disease. Modifiable several factors, including arterial hypertension, cardiac diseases, hyperlipidaemia, high fibrinogen

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concentrations, diabetes mellitus, alcohol abuse, and smoking are associated with an increases risk of atherosclerotic process and cerebrovascular disease (CVD) (11-3). Chlamydia pneumoniae is a human respiratory pathogen that causes acute respiratory disease and ~10% of community-acquired pneumonia. The infections are geographically widespread (4-6). A relation may also exist between various infection and cerebrovascular disease. Recently, seroepidemiologic studies have shown an association of chlamydial infection with atherosclerotic process and CVD (4-11). The aim of the present study was to investigate the role of preceding chlamydial infection as a risk factor for stroke. The study was restricted to patients younger than 65 years, in whom other risk factors were missing or were less prominent.

MATERIAL AND METHODS

We studied 68 consecutive patients under 65 years of age, 42 with cerebral infarction and 26 with cerebral hemorrhage, from November 1998 to June 2001; the mean age was 52.08±5.74 years (yrs) (range 39 yrs to 64 yrs; 28 female and 40 male) and 29 randomly selected age-matched healthy subjects (control); the mean age was 49.76±13.11 years (yrs) (range 41 yrs to 62 yrs; 12 female and 17 male). The demographic characteristics of patients and control subjects are presented in **Table 1**. Both patients and control subjects were from the same region, Eastern Anatolia. The neurological diagnosis was confirmed by history, clinical findings, and computerized cerebral axial tomography. Blood samples for chlamydial serology were usually taken within 3 day after admission to the hospital and stored at -80°C until analysis. All other laboratory tests were done in the clinical routine, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as markers of acute infection. A standardized interview was performed with patients and control subjects to evaluation risk factors for vascular disease (diabetes, migraine, history of high blood pressure, oral contraception, and smoking habits) and symptoms of infection before admission (fever, headache, cough, hoarseness, sore throat, chills, myalgia, sweating, bronchitis, pharyngitis, arthritis and gynecological or urological disturbances). Criteria for exclusion, for both the CVD patients and the controls, were diabetes, migraine, oral contraception, history of high blood pressure, elevated cholesterol or triglycerides in serum, inflammatory or autoimmunovascular disease, and smoking habits.

Table 1: The demographic characteristics of patients and control subjects.

Sex	Patients		Control Group	
	Number (%)	Age (Yrs)	Number (%)	Age (Yrs)
Female	28 (41,17)	53,26±8,08	17 (58,62)	47,29±11,83
Male	40 (58,82)	49,92±10,66	12 (41,37)	51,36±10,86
Total	68 (100)	52,08±8,71	29 (100)	49,76±11,21

Table 2: Mean values of some parameters in the Patients and Control Group.

Parameters	Patients	Controls	P
IgG antibodies titers to C pneumoniae	1.382±0.256	1.152±0.334	<0.05
IgA antibodies titers to C pneumoniae	1.008±0.246	0.971±0.237	>0.05
IgM antibodies titers to C pneumoniae	0.502±0.188	0.559±0.149	>0.05
IgG antibodies titers to C trachomatis	0.043±0.019	0.031±0.015	<0.05
IgM antibodies titers to C trachomatis	0.451±0.348	0.683±0.578	>0.05

Chlamydial studies were performed in a blinded fashion; sera from both patients and control subjects were included in all test series. Specific IgG, IgA, and IgM to C pneumoniae and C thrachomatis in serum were measured by the microimmunofluorescence with the method of Wang and Grayston in all the subjects (12). Circulating immune complexes were isolated by polyethylene glycol precipitation according to the method of Schutzer et al. (13).

Data are expressed as mean ± standard deviation and were computed with SPSS (Statistical Package for the Social Sciences). Differences between the group' means were analyzed with Mann-Whitney test. Results were considered to be statistically and significantly different when confidence limits exceeded 95% (P<0.05).

RESULTS

Table 2 presents the mean values of antibody titers to C pneumoniae and C trachomatis in patients and control subjects. The mean values of antibody titers in patients (mean IgG antibodies to C pneumoniae in patients, 1.382±0.256 vs. control, 1.152±0.334 [p=.012], mean IgG antibodies to C trachomatis in patients, 0.043±0.019 vs. control, 0.031±0.015 [p=.026]) were significantly higher compared with the controls. Although the mean

IgM antibody titers to C pneumoniae and C trachomatis are lower and IgA to C pneumoniae higher in patients than the controls, this differences were not found to be significant (mean IgM antibodies to C pneumoniae in patients, 0.502±0.188 vs. control, 0.559±0.149 [p=.087], mean IgM antibodies to C trachomatis in patients, 0.451±0.348 vs. control, 0.683±0.578 [p=.140], mean IgA antibodies to C pneumoniae in patients, 1.008±0.246 vs. control, 0.971±0.237 [p=.563]).

DISCUSSION

Several studies suggested that infections were a risk factor for stroke, respiratory infections being the most common (4,7-16). In this study, elevated IgG antibody titers against C pneumoniae and C trachomatis in serum were significantly higher in the patients with recent CVD than in the control subjects, similar to that found in the previous studies (4,9-11). In contrast to the current study, Elkind et al. (10) reported that elevated IgA antibody titers against C pneumoniae were independently associated with the risk factors of CVD. We found IgA antibody titers against C pneumoniae higher in patients than the controls, but these differences were not found to be significant, probably because of a large proportion of older, inactive infection in our patient group. The serological pattern of increased IgA titers and specific IgG-containing immune complexes has been suggested to indicate chronic persistence of active infection, while IgG titers in the absence of IgA titers may be a serological marker of an older, inactive infection (17-19). There was no confounding by commonly recognized vascular risk factors, because of excluding others risk factors. Therefore, there may be a risk enhancement for stroke by C pneumoniae infection, as has already been definitely suggested for coronary artery disease and carotid atherosclerosis (6-8,20).

The current study has several limitations. First, the presence or absence of chlamydial antibody is not a perfect measure of past infection. Following an acute infection with chlamydia, antibody levels usually drop over a period of months to years, and may become undetectable in some people (21).

A second limitation of the study is that the presence of antibody probably does not simple distinguish subjects ever infected from those never infected. Therefore, if misclassification of prior infection based on current antibody status were equal in the case and control groups, as would seem likely, the effect would be to attenuate the estimated association between prior infection and disease.

It is known from previously published population in northwestern countries that the seroprevalence for positive IgG titers against C pneumoniae is high among adults, suggesting that most adults are infected one to several times during their life (22,23). In accordance, the present study shows that positive IgG titers are significantly higher in the patients than the control subjects. The high seroprevalence in both groups argues against the possibility of a selection bias in one of the groups, which may occur in a small case-control study such as the present one, although patients and control subjects were recruited from the same geographic area and the same time period.

It is possible that the specific immune complexes in circulating blood and raised C pneumoniae titers results from immunologic processes triggered by cerebrovascular damage rather than being associated with the generation of vascular occlusion. To exclude this possibility, prospective cohort studies are needed in which patients with elevated titers are investigated for subsequent vascular occlusive disease. Although such these studies are limited, raised IgA and the presence of immune complexes were shown to be associated with an increased risk of symptomatic coronary artery disease within the subsequent 6 months in the prospective Helsinki Heart Study (24) and Fagerberg et al. (10) reported that seropositivity for C pneumoniae was associated with an increased risk for future cardiovascular disease and, in particular, stroke. Further prospective epidemiological studies of the effect of this infection on stroke risk are warranted.

Infection is known to cause alternations in lipid values (25). Information on the association between high-density lipoprotein concentration and CVD is contradictory. Syrjanen et al. (5) reported that patients who had bacterial infection had lower high-density lipoprotein concentrations than those without such a history. These may be proposed as a possible additional risk factor for atherosclerosis and CVD.

The mechanism underlying vascular occlusion in C pneumoniae infection were not evaluated in current study. C pneumoniae has been shown the multiply in alveolar macrophages and in endothelial cells in culture (26). The presence of

circulating immune complexes in previous studies suggests that C pneumoniae actually gains access to the circulation in humans, possibly by invading cells in the vessel wall (4,9-11). C pneumoniae in the wall of atherosclerotic but not normal extra cerebral arteries in humans has been demonstrated by immunocytochemical stain and polymerase chain reaction (20, 27, 28). In addition to invasion and destruction of vessel wall cells, C pneumoniae and its lipopolysaccharide cell wall component are believed to induce tumor necrosis factor, interleukin-2, and tissue factor, which contribute to a procoagulant state (29-31). Direct atherosclerotic occlusion of cerebral vessels, therefore, is only one of several possible mechanisms for stroke. It is speculated that C pneumoniae infection also enhances the risk of CVD.

The current study supports previous evidence that infection is an important risk factor for CVD (4, 5, 9-11, 32, 33). The merit of screening patients for elevated C pneumoniae IgA and IgG titers and specific immune complexes in the primary and secondary prevention of stroke depends on whether treatment strategies such as antibiotic therapy or platelet inhibitors may reduce the number of subsequent ischemic episodes. We conclude that chronic infection with chlamydiae is associated with an increased risk of cerebrovascular disease and believe that patients who have high values of IgG antibodies titers should be warned for a probable cerebrovascular disease and other risk factors like smoking, alcohol, diet, etc.

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