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ORIGINAL ARTICLE

Role of Chlamydia pneumoniae in cardiovascular disease

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ABSTRACT

Background: Cardiovascular disease (CAD) is the leading cause of death in developed countries. The cause is multifactorial. A substantial proportion of patients with CAD do not have traditional risk factors. Infectious diseases may play a role in these cases, or they may intensify the effect of the risk factors. The association of CAD and Chlamydia pneumoniae infection is firmly established, but causality is yet to be proven. We investigated their presence in carotid atherosclerotic plaques.

Materials and methods: One-hundred two atherosclerotic plaques in dead patients were studied. The highly sensitive polymerase chain reaction method was employed with primers specific for this agent. PCR targeting the 16S rRNA gene and a nested PCR targeting the ompA gene were performed to detect C pneumoniae DNA.

Results: The presence of Chlamydia DNA was detected in 22 (23.3%) samples. The following risk factors were found among these 23 C. pneumoniae-infected cases: low HDL in 8 (34.8%), hypertension in 5 (21.7%), diabetes mellitus in 4 (17.4%), smoking in 11 (47.8%) and family history of cardiovascular disease in 6 (26.1%).

Conclusion: The presence of Chlamydia DNA supports the hypothesis that this agent is associated with atherosclerosis.

Keywords: *Chlamydia pneumoniae, Atherosclerosis, PCR amplification, cardiovascular disease*.
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INTRODUCTION

Only half of coronary artery disease, and half of carotid plaque measured by ultrasound, can be explained by the usual risk factors: age, sex, hypertension, hyperlipidemia, smoking, and diabetes. It is likely that much of unexplained atherosclerosis is explained by genetic factors. A Swedish twin study showed that myocardial infarction is heritable. This suggests that few environmental factors that would make a major contribution to atherosclerosis remain to be dissolved. Recently, infections have been proposed (1).

Data obtained from several seroepidemological studies has given rise to the hypothesis that an infection can initiate or maintain the atherosclerotic process (2). Chlamydia pneumoniae is a common cause of a usually mild, community acquired pneumonia. This organism, however, can spread from the respiratory tract into other parts of the body and has been detected in up to 70% of athermanous lesions in blood vessels. Nevertheless, the exact mechanism of the contribution of C. pneumoniae to the pathogenesis of atherosclerosis remains unknown (3). C. pneumoniae has been associated with coronary and carotid artery disease

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in seroprevalence epidemiological studies, and in one prospective cohort study C. pneumoniae elementary bodies have been detected in the atherosclerotic plaques and fatty streaks of the aorta and coronary arteries of autopsy cases (4).

In the present study, the presence of C. pneumoniae was investigated by PCR in arterial plaque of dead subjects.

PATIENTS and METHODS

For this descriptive study, samples were obtained from 102 dead cases with infarction due to atherosclerosis. Basic demographic data and clinical information such sex, smoking status, family history of coronary artery diseases, diabetes mellitus, blood pressures and level of blood cholesterol (LDL & HDL) in sera were extracted from the medical files.

DNA was extracted from 50µl of homogenized tissues by proteinase K digestion (100µg/ml for 1 to 2 h at 65°C) followed by phenol-chloroform extraction and ethanol precipitation. The DNA was then resuspended in 50 µl of Tris-EDTA buffer. For PCR, 10 µl of DNA solution, was added per 50 µl of reaction mixture (5).

For PCR amplification the following steps were observed: PCR targeting the 16S rRNA gene and a nested PCR targeting the ompA gene were performed to detect C pneumoniae DNA. All amplification reactions were done in a volume of 50 µl containing 200 µM of four deoxynucleoside triphosphates. PCR primers tested were CPN90 5' GGT CTC AAC CCC ATC CGT GTC GG 3', CPN91 5' TGC GGA AAG CTG TAT TTC TAC AGT T 3', CP1 5' TTA CAA GCC TTG CCT GTA GG 3', CP2 5' GCG ATC CCA AAT GTT TAA GGC 3' (3).

Briefly PCR was performed using CPN90-CPN91 primer pair with a 0.25 µM concentration of each primer, 2.5 mM MgCl2 and 20 µl of the extracted DNA. Cycling protocol was 75 seconds at 95°C, followed by 60 cycles of denaturation at 94°C for 45 seconds, annealing beginning at 64°C and ending at 52°C for 45 seconds, and extension at 72°C for one minute. The annealing temperature was lowered 10°C every four cycles until 52°C and this temperature was kept until the end of the cycling process.

CP1-CP2 primers with nested pair CPC-CPD were used for the ompA nested PCR. The first round of amplification used 1.5 mM $MgCl_2$, 0.4µM of each primer and 20µl of the extracted DNA. Cycling consisted of nine minutes at 95°C for Taq polymerase activation, 20 cycles of one minute at 94°C, one minutes at 65°C (temperature was decreased 0.5°C for each cycle) and one minute at 72°C plus an additional 20 cycles of one minute at 94°C, one minute at 55°C and one minute at 72°C. The PCR products amplified by the outer primer pair were diluted 1:5 and 5 µl was added to a new PCR mixture containing 1 µM of each primer and 3mM of $MgCl_2$. Cycling protocol entailed nine minutes at 95°C for Taq DNA polymerase activation, 30 cycles of one minute at 94°C, one minute at 50°C and one minute at 72°C.

RESULTS

The study population included 75 males and 27 females with their age ranged between 20 and 79 years. All were died due to atherosclerosis. Totally, 100 cases had primary and two cases had secondary obstruction. The majority of obstructions (91%) were detected in left anterior descending artery (LAD).

Of 102 cases, Chlamydia DNA was detected in 23 (22.5%). The following risk factors were determined among these 23 C. pneumoniae-infected cases: low HDL in 8 (34.8%), hypertension in 5 (21.7%), diabetes mellitus in 4 (17.4%), smoking in 11 (47.8%) and family history of cardiovascular disease in 6 (26.1%).

DISCUSSION

Human atherogenesis appears to be multifactorial, thus, no single entity can fully explain the pathogenesis. There is little doubt that risk factors such as genetic predisposition, hypercholesterolemia, hypertension, smoking, and diabetes mellitus are major predisposing conditions for atherosclerosis. There is substantial evidence, albeit circumstantial, that infectious agents are associated with atherosclerosis, but their exact role in the pathogenesis of atherosclerosis is unknown. The most compelling evidence to date is the presence of infectious agents in the arterial wall, particularly in diseased vessels or within atherosclerotic plaques (6).

C. pneumoniae, an obligate intracellular gram-negative bacterium, has been associated with atherosclerotic cardiovascular disease by seroepidemiological studies, indicating a significantly higher prevalence of circulating *C.* pneumoniae antibody or immune complexes among persons with clinical or radiographic evidence of atherosclerotic disease. C pneumoniae has now been detected in atherosclerotic plaques in several different arterial sites (coronary arteries, aorta, and carotid arteries) and in early lesions (fatty streaks). The organism has been detected by electron microscopy, immunocytochemisty, direct immunofluorescence, and the PCR in coronary artery and carotid artery plaque specimens (2,6-8).

Bartels et al found that occluded aorta-coronary venous grafts harbor C pneumoniae (9). Using PCR and immunohistochemistry, C pneumoniae was detected in arterial biopsies from femoral, popliteal, and coronary arteries, as well as in the aorta, indicating that the organism is widespread in atherosclerosis of the vascular system (8,10). Some studies found that the percentage of arteries with immunoreactivity to C pneumoniae was associated with the average area stenosis.

On the other hand, Andreasen et al did not detect C pneumoniae in calcified or degenerative atherosclerotic aortic heart valve disease (11).

Furthermore, it is unclear whether C pneumoniae initiates the process of atherosclerosis, facilitates progression of existing plaques, or merely colonizes the lesions. Some study showed that the adventitia of atherosclerotic coronary arteries frequently contains C. pneumoniae that seems to be located within macrophages. These results might indicate a possible route for infected circulating macrophages to home into atherosclerotic lesions in the artery via vasa vasorum (12). Another study was to determine the presence of C. pneumoniae in coronary artery plaques, carotid artery plaques and old vein grafts that were harvested at the time of surgery. But it failed to find C. pneumoniae in any of the vascular tissue (13).

In our study C. pnemoniae was detected in 22 (23%) out of 102 tissue plaques from dead atherosclerosis patients. Since other risk factors have been found in the affected patients, it seems unlikely that infection will be the only or main cause of atherosclerosis. The role of these newly emerging risk factors and their relationship with traditional risk factors such as hypertension or lipids, remains unexplored. The uncertainty of their role and the types of infection or types of patients that should be treated must be explored in properly conducted, prospective studies. However, the findings to date are intriguing, and the hope that anti-infective therapy may reduce the burden of stroke is worth pursuing.

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