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NOTE

Chlamydia Pneumonia Seropositivity Correlates with Serum Fibrinogen and Lipoprotein a Levels: Any Role in Atherosclerosis?

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Abstract. The aim of the study is to determine the impact of Chlamydial seropositivity on atherosclerosis in a group of patient requiring coronary and/or carotid revascularization. A population of 30 diabetic patients (group 3) and 26 nondiabetic patients (group 2) with angiographically documented coronary and/or carotid artery disease were enrolled for the study. Volunteers from the relatives of hospital staff with no known disease (n=29; group 1) were included as the control group. Serum samples from the participants were assayed for cardiovascular risk factors including total serum cholesterol, triglyceride and lipoprotein levels, fibrinogen, Hb $A1_c$ levels and IgG titers for Chlamydia pneumonia (C. pneumonia). Chlamydial seropositivity was analysed further to determine a possible impact on atherogenesis. Serum LDL cholesterol levels revealed statistically significant difference between groups 1 and 2 (p=0.001). There was no difference between groups 2 and 3 regarding LDL cholesterol levels. There was no significant difference among the groups with respect to C. pneumonia seropositivity and the other atherosclerotic risk factors. Chlamydial seropositivity was found to be more frequent in males than in females (p=0.008). In the C. pneumonia seropositive group, serum fibrinogen and lipoprotein a levels were found to be significantly higher than the seronegative group (p=0.0001 and p=0.001, respectively). Other atherogenic risk factors were similar in the seropositive and negative groups. The causal role of Chlamydial infections in atherosclerotic plaque formation might be due to their influence on the serum fibrinogen and lipoprotein a levels.

Key words: Chlamydia pneumonia, Atherosclerosis, Fibrinogen, Lipoprotein a

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ATHEROSCLEROTIC cardiovascular disease is a major health problem causing nearly half of the deaths in most populations. Although significant efforts have been made to determine the etiology of this process, only epidemiologic associations with important risk factors have been found. Traditional atherosclerotic risk factors which include serum cholesterol, diabetes mellitus, hypertension, smoking, male gender and family history do not fully explain the incidence of atherosclerotic macrovascular diseases in most cases. Thus important risk factors have yet to be defined [1–4]. In searching for additional risk factors for macrovascular diseases, the potential role of infection in the development of atherosclerosis has recently been reevaluated [1, 3, 5]. Clinical data and animal models suggest that common chronic infections (including cytomegalovirus, herpes viruses, Helicobacter pylori, and dental sepsis) may contribute to the pathogenesis of atherosclerosis. The impact of these chronic infections on the development and prognosis of atherosclerotic vascular damage has yet to be established [1, 5–7].

Diabetic patients are an at-risk population for cardiovascular and thrombo-occlusive cerebral disease. Despite the fact that infectious diseases are more common in diabetic patients, there is not

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enough data available as to the prevalence of Chlamydial infections and their role in the development of cardiovascular disease in these subjects [6, 8]. The present study has been designed to determine the impact of Chlamydial seropositivity on atherosclerotic disease in a group of patients requiring coronary and/or carotid revascularization.

Materials and Method

A population of 30 diabetic patients and 26 non-diabetic patients with angiographically documented coronary and/or carotid artery disease requiring coronary or carotid revascularization, contacted during their periodic check-up between January 1997 and June 1999 at Hacettepe University hospital, were enrolled for the study. Healthy control group included the volunteers from the relatives of hospital staff with no known disease (n=29). All patients with angiographically documented coronary artery disease (CAD) were pretreated with aspirin, beta-adrenergic blocking agents, calcium channel blockers, nitrates and dipyridamole. Diabetic patients were managed with hypoglycemic drugs and/or insulin in a standard way in the outpatient clinic of endocrinology and metabolism. All the participants gave informed consent to participate in the study in accordance with the Helsinki declaration.

The diagnosis of diabetes mellitus was made according to the diagnostic criteria of the American Diabetes Association on diabetes mellitus [9]. Hypertension was defined as systolic blood pressure of $\geq$140 and diastolic blood pressure of $\geq$90 mmHg and/or history of antihypertensive drug treatment. Information regarding smoking history was obtained by interviews. Retinopathy was documented by standard fundus examination in all the diabetic patients by the same experienced ophthalmologist. Clinical neuropathy was defined by an abnormal neurological examination, plus abnormal nerve conduction in at least two peripheral nerves. Microalbuminuria was defined as urinary albumin excretion between 30–300 mg/day. Advanced nephropathy was defined by the presence of urinary albumin excretion more than 300 mg/day and a creatinine clearance of less than 70 ml/minute.

Blood specimens were collected after an overnight fasting of at least 10 hours.

Triglycerides and cholesterol were measured by commercial colorimetric assay (GPO-PAP and CHOP-PAP kit, respectively, Boehringer-Mannheim, Germany). HDL cholesterol in plasma was determined by a precipitation-based method with phos-

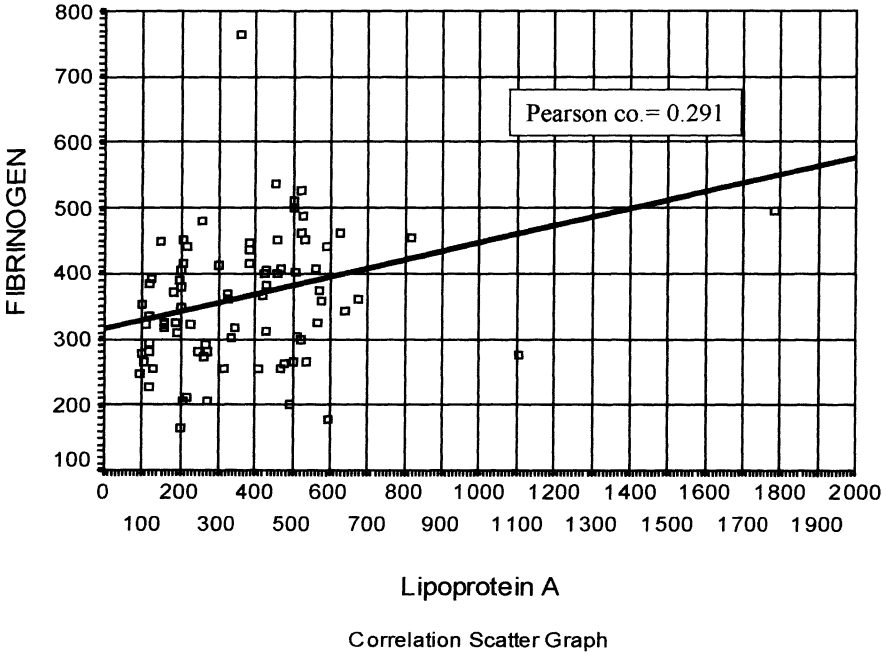

Fig. 1. Scatter graph of fibrinogen versus lipoprotein a, showing the linear correlation.

photungustic acid [10]. LDL cholesterol was calculated by Friedewald formula [11]. Plasma glucose determinations were obtained from venous sampling after 12 hours of overnight fasting, by glucose-oxidase method (Boehringer-Mannheim, Germany). Plasma fibrinogen determination was made by the clotting method of Clauss (STA compact analyser) [12]. Plasma C-reactive protein (CRP) concentrations were measured using an ELISA method (Boehringer-Mannheim kit). Lipoprotein a (Lip a) concentrations were measured using an ELISA method (Boehringer-Mannheim kit). The detection limit of this assay was 0.5 mg/dl. The intra- and interassay coefficients of variation were 5–12% and 2–6%, respectively. IgG antibody titers to C. pneumonia were assayed using indirect immunofluorescence technique (Euroimmun, Germany). IgG titers with dilutions 1/100 were considered as seropositive.

Statistical Analysis

Differences between patient groups were assessed for statistical significance using Student's *t* test, chi-square and ANOVA, where appropriate. Tukey's honest significance difference test was used as ANOVA post hoc tests. Bivariate correlations were assessed using Pearson and Spearman correlation coefficients where applicable. All results were expressed as means±SD unless otherwise indicated. Statistical analysis was conducted using the SPSS for Windows software package, Release 7.0. Statistical significance was considered when a two-tailed P value was 0.05 or below.

Results

The clinical characteristics of the healthy control group (group 1), nondiabetic (group 2) and diabetic patients (group 3) are given in Table 1. Mean age of the study population was 56.68 with a standard deviation of 8.87. There were a total of 54 women and 31 men enrolled in the study. C. pneumonia seropositivity was detected in 28 people, 21 out of 85 participants were hypertensive and 23 were smokers. There were 3 cases of peripheric neuropathy and 4 cases of retinopathy in the diabetic patient group.

There was no significant difference between the 3 groups by ANOVA regarding age ($p=0.055$), fibrinogen ($p=0.29$), CRP ($p=0.31$), HDL cholesterol levels ($p=0.06$) and Lip a levels ($p=0.98$). Comparison of the group means by ANOVA for serum LDL cholesterol levels revealed statistically significant difference between the groups ($p=0.002$). LDL cholesterol levels were lowest in group 1 (124.69±26.01) and post-tests revealed significant difference between groups 1 and 2 ($p=0.001$). There was no difference between groups 2 and 3 regarding LDL cholesterol levels. There was no significant difference among the groups with respect to C. pneumonia seropositivity frequency and prevalence

Table 1. Descriptive characteristics of the study population

	Healthy Controls	Nondiabetic patients with CAD	Diabetic patients with CAD
Number	29	26	30
Age (years)	52.0±6.2	57.4±6.15	60.6±10.95
Sex (M/F)	12/17	9/17	10/20
C. Pneumonia Seropositive	9/20	6/20	13/17
LDL Cholesterol (mg/dl)	124.7±26.0	157.08±39.2	144.83±33.17
Lipoprotein a (mg/dl)	370.8±351.9	361.3±156.0	357.9±188.8
Fibrinogen (mg/dl)	349.2±111.3	341.7±76.9	380.5±99.4
CRP (mg/dl)	5.6±2.1	4.8±2.6	5.4±1.9
Smoking (n)	11/29	5/26	7/30
Hypertension (n)	8/29	13/26	8/30
DM duration (years)	—	—	7.9±5.7
Hb A1c (%)	—	—	7.2±1.0
Microalbuminuria	—	—	28.0±4.6

CAD: Coronary artery disease; DM: diabetes mellitus; CRP: C-reactive protein.

of hypertension.

The study population was further analysed with respect to C. pneumonia serology. Seropositive and seronegative groups are compared with respect to various cardiovascular risk factors (Table 2). Chlamydial seropositivity was found to be more frequent in males than in females ($p=0.008$). In the C. pneumonia seropositive group, serum fibrinogen and Lip a levels were found to be significantly higher than the seronegative group ($p=0.0001$ and $p=0.001$, respectively). There was no difference between groups regarding CRP levels. Other atherogenic risk factors including cigarette smoking, hypertension, total cholesterol, LDL cholesterol and triglyceride concentrations were similar in the two groups. Among the diabetic patients there was no difference in the seropositive and negative subgroups with respect to diabetes duration, Hb A1c levels and the chronic complications of diabetes mellitus. C. pneumonia seropositivity in the diabetic patient group did not differ significantly than that of non-diabetic subjects ($p=0.15$).

When healthy controls were compared to the coronary artery disease population (group 2 plus group 3), there was no difference between the groups regarding frequencies of sex, C. pneumonia seropositivity, hypertension and cigarette smoking (chi-square; $p=0.64$, $p=1.00$, $p=0.79$ and $p=0.13$, respectively).

Our present data revealed a highly significant correlation between C. pneumonia seropositivity and serum fibrinogen and Lip a levels ($p<0.01$ and $p<0.01$). Also serum fibrinogen and Lip a levels correlated strongly with each other ($p=0.023$). Higher prevalence of C. pneumonia seropositivity in males exhibited a strong correlation between gender and serology at the level of $p=0.005$.

Discussion

Known risk factors for macrovascular disease do not explain all of the clinical and epidemiological features of atherosclerosis. Atherosclerosis is pathologically similar to a chronic inflammatory response [1, 13]. Recent studies have shown some evidence linking C. pneumonia, an obligate intracellular pathogen, with coronary heart disease [6, 13–15]. The mechanisms by which C. pneumonia might influence cardiovascular risk are unknown. One hypothesis is that chronic infection might indirectly contribute to atherosclerosis by increasing the concentration of acute phase reactants and inflammatory mediators, such as sialic acid, fibrinogen, lipoproteins, C-reactive protein and certain cytokines like interleukin 6 and tumor necrosis factor-α, or they may infect arteries directly and lead to endothelial

Table 2. Characteristics of the study population with regard to seropositivity to C. pneumonia

	Chlamydia Pneumonia seropositive	Chlamydia Pneumonia seronegative
Number	28	57
Male sex (n)	16	15
Female sex (n)	12	42
Healthy group (n)	8	21
Smokers (n)	7	16
CAD	19	37
Fibrinogen (mg/dl)	411.5±81.6	331.6±95.0
CRP (mg/dl)	6.4±1.3	5.1±2.1
LDL cholesterol (mg/dl)	141.3±38.0	141.8±34.1
Lipoprotein a (mg/dl)	512.2±318.0	290.2±161.8
Hypertension (n)	4	17
DM (n)	13	17
DM; Hb A1c (%)	7.1±1.2	7.2±0.8
DM Neuropathy	2	1
DM Retinopathy	3	1

CAD: coronary artery disease; DM: diabetes mellitus; CRP: C-reactive protein.

damage. For such an infection to occur, the bacteria should not only be present but must also be viable in the arterial wall [5, 13]. In the present study, C. pneumonia seropositivity was found to be strongly associated with high fibrinogen and Lip a levels which are well known atherogenic proteins. High plasma fibrinogen, which is an acute-phase reactant, is associated with disturbed microcirculation, plaque formation and thrombosis. It is known that fibrinogen increases with age, smoking and body mass index [16, 17]. Subtle chronic infections might elevate serum fibrinogen levels by increasing synthesis of acute phase proteins by the liver. Lip a, which is a modified form of low density cholesterol that contains apo B100 linked by a disulfide bridge to a highly polymorphic glycoprotein apolipoprotein a, is found to be correlated to C. pneumonia seropositivity [18]. Although it is difficult to establish any cause and effect relation between Lip a and chronic Chlamydial infection from the results of this study, further prospective studies should be carried out to clarify the influence of any factor on the serum levels of this lipoprotein, which is an important risk factor for atherogenesis. It was also reported previously by Dahlen and Stenlund that immune response to Lip a may be initiated and promoted by intracellular microbes such as C. pneumonia [19]. Infection of macrophages by C. pneumonia results in synthesis and expression of B7 molecule on macrophage cell membranes which is one of the most potent of costimulatory signals to induce T-cell activation against Lip a and/or LDL-engulfed macrophage or foam cells in the arterial wall. Normally, T-cells are activated and proliferate only when B7 molecules are expressed by the same cell which also presents the HLA antigen complex [19]. This study is in agreement with previous reports documenting the relation found between Lip a levels, certain HLA class II DR genotypes in males and C. pneumonia seropositivity [20–22].

Results of this study also suggest that diabetic patients with atherosclerosis do not have an increased incidence of C. pneumonia infection with regard to nondiabetic population and probably C. pneumonia is not a significant risk factor in the pathogenesis of accelerated atherosclerosis in these patients.

This study revealed no difference with regard to the incidence of C. pneumonia seropositivity between people with no documented cardiovascular disease and with those having atherosclerotic vessels. This would argue for the direct causal role of Chlamydial infections in the atherosclerotic process. Hence these infections might have auxiliary roles in this scenario by augmenting the present inflammatory cascade in the arterial wall. A causal relation between C. pneumonia and atherosclerotic plaque formation will have to be shown by further prospective studies in various populations which could add to their control and prevention.

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