Markers of Chronic Infection and Inflammation

Are They Important in Cases with Chronic Coronary Heart Disease

H. Asuman KAFTAN, MD, Osman KAFTAN,[1] MD
and Mustafa KILIÇ, MD

SUMMARY

The human cytomegalovirus plays a causal role in atherosclerosis etiology, but it is discussed as controversial.

We conducted a case control study to investigate whether previous infection with cytomegalovirus is associated with coronary heart disease and markers of systemic inflammation, because systemic inflammation may play a role in atherosclerosis too. We also studied the correlation between anti-cytomegalovirus antibody titer and coronary artery disease.

The study involved 150 cases (45 females, mean age ± SD is 58.73 ± 7.68 years) with a documented coronary heart disease and 160 healthy volunteers (50 females, mean age ± SD is 57.82 ± 7.68, $p > 0.05$). Cytomegalovirus serology was performed to determine the presence of specific IgG antibodies and titers of the anti-cytomegalovirus IgG antibodies. In addition, C-Reactive protein levels were determined for each case. The prevalance of specific antibodies to cytomegalovirus was 57.30% for the patients and 56% for the controls ($p = 0.39$). But higher levels of anti-cytomegalovirus IgG antibody titer (> 1/800) were seen in the patient group (28.6% versus 10%, $p = 0.0000$). Mean value of C-reactive protein was higher in the patient group (2.99 ± 0.92 mg/*l* versus 1.79 ± 0.51 mg/*l*, $p = 0.0000$), and there was a linar correlation with the high antibody titers and the level of C-reactive protein ($r = 0.35$, $p = 0.0000$)

These findings support that not the seropositivity of the population but rather the titer of anti-cytomegalovirus antibody and the levels of C-reactive protein could predict patients with a high risk of coronary heart disease. (Jpn Heart J 1999; 40: 275–280)

Key words: Coronary heart disease, Infection, Cytomegalovirus, C-reactive protein

THE infectious theory of atherosclerosis was suggested by several scientists in the past.[1)] Systematic studies have linked antibodies to cytomegalovirus (CMV) and atherosclerosis for many years. Pioneering examples include the studies from Melnick's group.[2,3)] High titers of CMV antibodies were more frequent

From Department of Cardiology, Faculty of Medicine, University of Pamukkale, Denizli, Turkey, and [1]Department of Internal Medicine, Faculty of Medicine, University of Fatih, Ankara, Turkey.

Address for correspondence: H. Asuman Kaftan, MD, Delikliçinar Mah. 890 Sok., No: 5/3, Denizli 20100, Turkey.

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in patients undergoing surgery for atherosclerotic disease (70%) than in matched control cases (43%).[3,4] Risk in communities (ARIC) study documented a significant difference between carotid artery intimal thickening (determined by B-mode ultrasound) and the level of anti-CMV antibodies.[5] Both antigens and nucleic acid sequences of CMV were detected in smooth muscle cells from the atherosclerotic lesions obtained by coronary atherectomy.[7] Smooth muscle cells that were grown from such lesions expressed IE 84 (one of the immediate early proteins of the virus that binds and inhibits p53). These effects may enhance the proliferation of smooth muscle cells or inhibit apoptosis. Either of which may contribute to restenosis.[7,8] Nieto et al.[9] demonstrated a graded relation between the odds of intimal medial thickening and the levels of CMV antibodies that remained significant after adjustment for the main cardiovascular risk factors.

However, some studies have shown an association between CMV and atherosclerosis, while others have not. The purpose of this investigation was to resolve these apparently conflicting results. Specifically, we examined the severity of CMV infection as reflected by the level of anti-CMV antibody titers and the levels of C-reactive protein (CRP), that determines whether infection with CMV or levels of CRP appears to predispose to the development of atherosclerosis and whether there is a correlation between the antibody levels and one of the inflammatory markers-CRP.

Methods

We enrolled 150 cases aged 38–71 (45 females, mean age ± SD is 58.73 ± 7.68 years) with a coronary stenosis greater than 50% and 160 controls (blood donors without angina pectoris) who were matched for sex and age (39–70 years) (50 females, mean age ± SD is 57.82 ± 8.13 years) in the study. All of the patients had an angiographic documentation of a coronary stenosis ≥ 50%, and all without recent or remote myocardial infarction. All of the patients were at the chronic stage of their myocardial infarction and had their myocardial infarctions at least three years ago. The control group was choosen from healthy volunteers not having any cardiac or inflammatory disease. All underwent total blood count, resting electrocardiography and an exercise stress testing to exclude any inflammatory or ischemic cardiac disease. The exclusion criteria were: recent or past myocardial infarction, stable or unstable angina pectoris, an immunologic disease and an infectious or an inflammatory disease in the last 6 months. For each subject, blood samples were thawed and assayed for both anti-CMV IgG and CRP. Anti-CMV IgG antibodies were measured with an enzyme linked immunosorbent assay (ELISA) kit. Positive and negative controls were used in every plate. The positive samples were further diluted in order to determine the

highest positive titer. CRP was also assayed with a high sensitivity ELISA.

Statistics: Statistical analysis of the frequency counts was done by chi-square test or Fisher exact test for small samples; means were compared with the 2-sample *t* test. Multivariate regression analysis was done to determine the relation between the variables and the existence of coronary heart disease.

Results

Of the 150 patients 86 (57.3%) and of the 160 healthy control cases 84 (56%) were seropositive ($p = 0.39$) for the anti-CMV IgG antibodies (Table). In the patient group 43 seropositive patients had an antibody titer of ≥ 1:800 (43 of 150 patients with documented coronary artery disease, 28.6%), whereas in the control group only 16 volunteers had a high titer (16 of 160 patients, 10%, $p = 0.000$). The patient group had higher CRP levels (2.99 ± 0.92 mg/*l*) than the control subjects (1.79 ± 0.51 mg/*l*, $p = 0.000$) (Table). The CRP level and the anti-CMV IgG antibody titer showed a linear relation with the existence of coronary heart disease ($r = 0.63$, $p = 0.0000$ for the CRP) ($r = 0.24$, $p = 0.00$ for the titer). CRP also showed a linear relation with the titer ($r = 0.35$, $p = 0.000$). There was no statistical difference between the groups or in each group with respect to age, sex, and risk factors including smoking, high cholesterol level, diabetes mellitus, and hypertension, tested by multivariate linear regression analysis.

Discussion

The results of our study demonstrated a similar prevalance of CMV seropositivity in patients with coronary artery disease and in an age and sex matched control group like in several other studies.[8,10,12] Rothenbacher *et al.*[10] found that the serologic evidence of previous infection with CMV was not a major risk factor for coronary artery disease. However, in our study anti-CMV IgG antibody titers were higher in patients with coronary artery disease (28.6% versus 10%, $p = 0.000$). Increased but within normal limits of CRP levels were

Table. Anti-CMV Antibody Titer and CRP Levels of Studied Cases

Variables	Patient group ($n = 150$)	Control group ($n = 160$)	p
Anti-CMV antibody			
(+)	57.3%	56%	0.39
(−)	52.7%	54%	0.39
(+) titer ≥ 1:800	28.6%	10%	0.000
CRP level (mg/*l*)	2.99 ± 0.92	1.79 ± 0.51	0.000

found in the patient group and it was associated with the presence of high titers of anti-CMV IgG antibody.

A large body of experimental evidence shows that human vascular wall cells, including smooth muscle cells and endothelium can be infected by CMV.[13,14] CMV can also infect human endothelial cells, and alter functions related to atherosclerosis, such as the procoagulant balance of endothelium.[15–17] *In vitro* CMV infection can augment macrophage production of messenger RNA's encoding the cytokines, interleukin-1β, tumor necrosis factor $-\alpha$, and macrophage colony stimulating factor, and alter cytokine production by endothelial cells. Infection of endothelial cells with CMV can induce the expression of endothelial leucocyte adhesion molecules, such as intercellular adhesion molecule-1.[18–22] By augmenting the expression of such leucocyte adhesion molecules, bacterial and viral infection could influence this crucial aspect of atherogenesis. The host response to infectious agents usually involves a change in the program of hepatic protein synthesis. The cytokine interleukin-6 may mediate much of this switch from production of "housekeeping" proteins such as albumin to greater synthesis of proteins collectively known as "acute phase reactants", some of which may influence atherogenesis. Augmented production of fibrinogen and plasminogen activator inhibitor during the acute phase response could promote thrombosis. Increased production of serum amyloid A protein can alter the potential function of HDL in cholesterol export from atheromatous lesions, and in coronary risk.[18] The virus has the capacity to increase accumulation of oxidized low density lipoprotein cholesterol in smooth muscle cells contained within atherosclerotic lesions. High antibody titers have been described before in atherosclerotic patients.[9,23] However we demonstrated that anti-CMV antibody titer can distinguish between the general seropositive population and those who are at a higher risk for coronary artery disease. The presence of normal but relatively high levels of CRP in our patient group may be a nonspecific phenomenon reflecting cytokine mediated hepatic production triggered by most forms of inflammation, infection or tissue injury. In fact in the acute phase of coronary artery disease CRP was found to be elevated independent of the presence of infection, due to primary inflammation of the coronary endothelium named coronary arteritis,[24,25] but the levels in acute cases were found to be higher than the levels found in our cases, that is to say chronic stable coronary artery disease cases. Also CRP is a marker for inflammation that appears to predict cardiovascular events among apparently healthy men. For example, in the prospective Physicians' Health Study (PHS), high plasma concentration of CRP was associated with a two fold increase in risk of stroke, a 3-fold increase in risk of myocardial infarction and a 4-fold increase in risk of developing peripheral vascular disease.[26,27] Finally CRP has been associated with increased risks of fatal coronary events among high risk

male smokers,[28] incident coronary disease among elderly,[29] and recurrent coronary events among those with known coronary disease.[30,31]

The results of the present study demonstrated that not the seropositivity of the population but the level of the anti-CMV IgG antibody titer could be associated with coronary artery disease, and with the scanning of titers for the anti-CMV IgG antibody for the high risk patients, we could predict future cardiac events and the levels of CRP is not a potent determinant of chronic stable coronary disease but may partly provide evidence about the presence of this disease.

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