Role of herpes simplex virus-1, cytomegalovirus and Epstein-barr virus in atherosclerosis

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Abstract: Infectious agents such as herpes viruses may be implicated in the inflammatory atherosclerotic process. The aim of this study was to assess the levels of IgG antibody specific for Herpes simplex virus-1 (HSV-1), Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) among patients with atherosclerotic vascular diseases and to examine the relation between the levels of these antibodies and lipid profile, high-sensitive C-reactive protein (hsCRP) in these patients. Seventy five patients [20 with acute coronary artery disease (ACAD), 20 with chronic coronary artery disease (CCAD), 20 with cerebral stroke and 15 with peripheral arterial disease (PAD)] along with 15 healthy individuals as a control group. The studied individuals were subjected to complete history taking, thorough physical examination, and assessment of the blood glucose level, lipid profile, creatine kinase (CK), hsCRP by nephlemetry and virus-specific IgG antibodies by enzyme immunoassay (EIA).

Results showed that the levels of cholesterol, triglycerides, LDL-c and hsCRP were significantly higher, while HDL-c was significantly lower among patients compared to that of the controls. A significantly ($P<0.05$) higher perecentage of patients had CMV-specific IgG as compared to the controls. Higher percentage of patients had HSV- and EBV-specific IgG antibodies, however, there was no significant difference between the 2 groups. Individuals who had CMV-specific IgG were more liable to have vascular disease compared to those without (OR=4.10, CI= 1.07-15.75). The levels of CMV- and EBV-specific IgG antibodies were significantly ($P<0.01$ and < 0.05 respectively) elevated among patients with atherosclerotic vascular diseases when compared to those of the controls. There was no significant correlation between the levels of virus-specific IgG and lipid profile or hsCRP.

In conclusion, the level of CMV- and EBV- specific antibodies are elevated among vascular disease patients and the presence of CMV-specific IgG is associated with development of the disease. Serum lipids and hsCRP were increased among the studied patients; however, no significant correlation was detected between antiviral IgG levels and lipid profile or hsCRP.

Keywords: Atherosclerosis, coronary artery disease, cytomegalovirus, Epstein-Barr virus, herpes simplex virus, peripheral artery disease.

INTRODUCTION

Atherosclerosis is a major health problem worldwide; more people die of the complications of atherosclerosis than of any other cause (Cullen *et al.*, 2005). Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries. It is the primary cause of coronary artery disease (CAD), stroke and peripheral arterial disease (PAD) (Lusis, 2000).

Coronary artery disease (CAD) due to atherosclerosis is the most common cause of angina pectoris. Acute coronary syndrome considers both unstable angina and acute myocardial infraction (Schlant and Alexander, 2001). Acute stroke was defined as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 h with no apparent cause other than that of vascular origin (Mario and Zrink, 2005). Cereberovascular stroke includes transient ischaemic attacks, ischaemic infarction and hemorrhage (Charles, 2001). PAD is caused by atherosclerotic occlusion of the arteries of the legs (Hiatt, 2001).

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Risk factors of atherosclerosis include hypertension, diabetes mellitus, active smoking, hyperlipidemia, and a positive family history (Blum *et al.*, 2003). The inability of traditional risk factors such as hypercholesterolemia, hypertension, and smoking to explain the incidence of atherosclerosis in about 50% of the cases prompted a search for additional putative risk factors involved in the development of the disease (Kis *et al.* 2001). Several studies have suggested that an association exists between certain microorganisms and the development of atherosclerosis (Watt *et al.*, 2003 and Schlitt *et al.*, 2005). Chronic infectious diseases that are accompanied by low-grade inflammation have been suspected to take an active part in the initiation and progression of atherosclerosis (Kunes *et al.*, 2005 and Oshima *et al.* 2005). Microbes have been proposed as inciting agents of tissue injury and inflammation, both of which underlie the pathogenesis of atherosclerosis (Vercellotti, 2001).

Inflammatory processes play an important role in atherosclerosis, and increasing evidence implies that microbial pathogens and pro-inflammatory cytokines are involved in the development and activation of atherosclerotic lesions (Olofsson *et al.* 2005). Not only

specific microorganisms but also the infectious burden, defined as the number of pathogens to which a patient is exposed, has been associated with atherosclerosis (Tsirpanlis *et al.* 2005). *In vitro,* these agents promote a proinflammatory and a procoagulant phenotype in vascular cells. Viruses augment cell accumulation through alterations of apoptosis. Infectious agents may play a role in pathogenesis of atherosclerosis by triggering an autoimmune response due to microbial molecular mimicry (Vercellotti, 2001).

The role of herpes viruses in atherosclerotic vascular disease is not well studied in our locality. Therefore, this study was performed to estimate HSV1, CMV and EBV infections among patients with different atherosclerotic vascular diseases and to study their relation with other risk factors of atherosclerosis especially hsCRP and lipid profile.

PATIENTS AND METHODS

This study was carried out at King Abdulazez University Hospital, Jeddah, KSA. The samples were collected from the intensive care unit (ICU), and the General Surgery and Cardiology departments.

I) The studied subjects

This study involved 75 patients (49 males and 26 females), their age ranged between 33-85 years. In addition, 15 apparently healthy age- and gender-matched subjects were involved in this study as a control group (9 males and 6 females). The studied patients included 20 patients with ACAD (13 males and 7 females ranging in age from 42-70 years), 20 patients with CCAD (16 males and 4 females ranging in age from 42-70 years), 20 patients with cerebrovascular diseases (12 males and 8 females ranging in age from 34-85 years) and 15 patients with PAD (8 males and 7 females) ranging in age from 33-82 years. Each patient was subjected to full history taking and thorough clinical examination, ECG, coronary angiography (for patients with CAD), CT scan (for patients with cerebral stroke) and doppler (for patients with PAD).

The following laboratory investigations were performed for all the studied patients and controls: complete urine analysis, assessment of fasting blood glucose, lipid profile and cardiac enzymes and quantitative measurements of hsCRP and HSV-1-, CMV- and EBV-specific IgG antibodies.

II) Blood sampling

Seven mililitres of blood were collected from each individual after fasting 12 hours under aseptic conditions by clean vacutainer tubes. The blood was left to clot at 37ºC and then centrifuged at 4000 rpm for 10 min. The separated serum was divided into two parts one part was used for immediate assessment of fasting blood sugar, lipid profile, cardiac enzymes and the other part was stored in aliquots at -70ºC for estimation of hsCRP and antiviral antibodies.

III) Laboratory methods

1) Biochemical tests for detection of fasting blood glucose, lipid profile and cardiac enzymes were done on Synchron CX5 autoanalyser using kits supplied by Beckman (Beckman Coulter, Inc. Fullerton, CA).
2) Estimation of high sensitive C-reactive protein (hsCRP):

Assessment of high sensitive C reactive protein (hsCRP) was done on ARRAY 360 using kit supplied by Beckman (Beckman Coulter, Inc.Fullerton, CA). The "high sensitivity" refers simply to the lower detection limit of the assay being used (Pepys & Hirschfield, 2003). Quantitative assessment of CRP was performed by rate nephelemetry. The method measures the rate of increase in light scattered from particles suspended in solution due to complexes formed during an antigen-antibody reaction. Antibody to human CRP is brought into contact with human CRP protein in a sample. The increase in light scattered from the antigen-antibody reaction is converted to a peak rate signal which is a function of the sample hsCRP concentration. Following calibration, the peak rate signal for a particular assay is automatically converted to concentration units by the analyzer (Thomas *et al.*, 2003).

3) **Estimation of aniviral IgG antibodies**

Assessment was performed by EIA according to Manufactuer's instructions:

a) HSV1-specific IgG was measured using HSV1 ELISA kit (Arvin and Prober, 1995) and EBV-specific IgG was measured using EBV ELISA kit (Bauer, 2001) supplied by Genzyme Virotech GmbH, (Lowenplatz, Russelsheim, Germany). The antibody in the serum formed an immune complex with the antigen coated on the microtiter plate. Unbound immunoglobulins were removed by washing process. The enzyme conjugate attaches to this complex. Unbound conjugate was removed by washing processes. After adding the substrate solution (TMB), a blue color is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution was added
b) CMV-specific IgG was measured using bioelisa CMV IgG kit (Landini and Lazzarroto (1999) supplied by Biokit, SA (Barcelona, Spain). The test was performed by incubating diluted test specimen in a microplate well coated with CMV antigen. Antibodies to CMV, if present in the specimen, would combine with the antigen attached to the well. The well was then washed to remove residual test specimen, and

enzyme-labelled antibodies to human IgG were added. After another washing to eliminate unbound material, an enzyme substrate solution containing a chromogen was added. Development of blue color would occur if the sample contains anti-Cytomegalovirus IgG. The blue color changes to yellow after blocking the reaction with sulphuric acid. The intensity of the color was proportional to the amount of anti-CMV IgG in the test specimens. The concentration of antibodies in the sample was then estimated by means of a calibration curve.

STATISTICAL ANALYSIS

Data were collected, tabulated and statistically analysed using SPSS version 11.1 statistical package (SPSS Inc, Chicago, IL, USA) at 5% level of significance. Qualitative data were tested by Chi-square (X) test used to compare proportion and Odds ratio (OR) along with 95% confidence interval (CI) to measure risk of having vascular disease and previous viral infection. Quantitative data were tested for normality by Klomogovov-Smirnov test of normality. Student's t test was used for comparison of two groups and ANOVA test was used for comparison of the means of more than two groups followed by LSD post-hoc test of normally distributed data. Mann-Whitrey test) was used for comparison of two groups and Kruskal-Wallis test was used for comparison of more than two groups of non-normally distributed quantitative data. Pearson correlation coefficient test was used to test correlation between continuous variables (Indryan and Sarmukadda, 2001).

RESULTS

Table 1 shows the age, gender and the levels of fasting blood glucose, lipid profile, CK and hsCRP among the studied vascular disease patient groups and controls. Patient groups had significantly higher levels of FBS, cholesterol, LDL-c, CK and hsCRP. On the other hand, HDL-c was significantly lower in patients as compared to the controls. Patients with ACAD had significantly ($p<0.05$) higher level of CK-MB isoenzyme than that of the controls. Comparison of all the studied patients and controls is demonstrated in table 2. Patients had significantly higher levels of FBS, cholesterol**,** triglycerides, LDL-c, CK and hsCRP and significantly ($p<0.001$) lower HDL-c as compared to that of the controls.

Table 3 shows no significant difference between the studied groups regarding the percentage of HSV1-, CMV- and EBV-specific IgG antibodies. Table 4 shows that persons with positive CMV-specific IgG were 4 times more liable to have atherosclerotic vascular disease compared to those without (OR = 4.1, 95% CI = 1.07-15.75, $p< 0.05$).

Table 5 shows that CMV- and EBV-specific IgG serum levels were significantly ($p<0.01$ and $p<0.05$ respectively) higher among vascular disease patients compared to the control group. However, HSV-specific IgG showed no significant difference.

Table 1: Age, gender and laboratory characteristics of the studied patients and controls.

The studied parameter	Vascular disease patients				Controls (n=15)	Test of significance	P value
	ACD (n=20)	CCD (n=20)	Stroke (n=20)	PAD (n=15)			
	No %	No %	No %	No %	No %		
Age (years)	60.8 ± 9.4	51.0 ± 6.2	60.8 ± 12.6	59.6 ± 12.0	46.3 ± 15.4	F = 5.7	P<0.001
Gender: Males Females	 13 65% 7 35 %	 16 80% 4 20 %	 12 60% 8 40 %	 8 53.3% 7 46.7%	 9 60% 6 40 %	X = 3.3	P>0.05
FBS (mg/dl)	89.4 ± 9.4	88.5 ± 11.3	94.5 ± 11.8	94.4 ± 8.7	74.5 ± 6.9	F = 16.3	P<0.001
Cholesterol (mg/dl)	196.3 ± 67.5	210.9 ± 40.1	204.8±42.9	200.4±42.1	136.5±25.3	F = 6.6	P<0.01
Triglycerides (mg/ml)	210.2±156.1	135.2±46.5	136.6±29.2	109.3± 21.2	90.1± 38.2	F = 6.3	P<0.01
HDL-c (mg/dl)	40.9 ± 14.7	33.5 ± 6.9	40.2±3.2	39.7 ± 2.7	48.9 ± 4.8	F = 7.7	P<0.01
LDL-c (mg/dl)	122.4 ± 44.9	150.9 ± 38.3	141.8±39.2	139.7±39.7	71.5± 22.9	F = 11.2	P<0.001
CK (IU/L)	389.9±323.8	107.6 ± 48.3	299.9±361.5	272.5±331.9	67.1±36.6	H = 13.9	P<0.01
CK-MB (IU/L)	37.2 ± 24.8	-	-	-	17.1 ± 4.9	U = 2.5	P<0.05
hsCRP (mg/dl)	2.96 ± 3.90	1.10 ± 0.50	1.76 ± 1.55	1.57 ± 0.67	0.33 ± 0.07	H = 9.2	P<0.05

F = ANOVA, X2 = Chi-square, H = Kruskal-Wallis, U = Mann-Whitney

-The age of the studied patients and controls showed a significant difference, however, no difference was detected regarding gender.

-There were significant differences in the levels of FBS, cholesterol, triglycerides, LDL-c, CK and hsCRP among the studied groups.

-There was a significant difference in the level of CK-MB between ACAD patients and controls.

Table 2: Comparison of the laboratory findings of the studied vascular disease patients and controls

The studied parameter	Vascular disease patients (n=75)	Controls (n=15)	Test of significance	P value
FBS (mg/dl)	115.8 ± 62.7	74.5 ± 6.9	t = 5.5	P<0.001
Cholesterol (mg/dl)	203.4 ± 49.2	136.5 ± 25.3	t = 5.1	P<0.001
Triglycerides (mg/ml)	144.2 ± 93.9	90.1 ± 83.3	t = 2.2	P<0.05
HDL-c (mg/dl)	38.5 ± 8.9	48.9 ± 4.8	t = 4.3	P<0.001
LDL-c (mg/dl)	139.3 ± 40.9	71.5 ± 22.9	t = 8.9	P<0.001
CK (IU/L)	267.1 ± 305.2	67.1 ± 36.6	U = 3.6	P<0.001
hsCRP (mg/dl)	1.86 ± 2.28	0.33 ± 0.07	U =5.8	P<0.001

t = Student's t test, U = Mann-Whitney
Vascular disease patients had significantly higher levels of FBS, cholesterol, triglycerides, LDL-c, CK and hsCRP and significantly lower HDL-c level compared to that of the controls.

Table 3: Virus-specific IgG seropositivity among the studied patients and controls

The studied parameter	Vascular disease patients				Controls (n=15)	X2	P value
	ACD (n=20)	CCD (n=20)	Stroke (n=20)	PAD (n=15)			
	No %	No %	No %	No %	No %		
HSV	20 100	18 90	20 100	14 93.9	14 93	3.66	P>0.05
CMV	12 60	8 40	9 45	9 60	3 20	7.14	P>0.05
EBV	20 100	20 100	18 90	12 80	14 93.3	7.5	P>0.05

X2 = Chi-square
There was no significant difference between the different studied groups regarding virus-specific IgG seropositivity.

Table 4: Distribution of the positive and negative HSV, CMV and EBV individuals among patients and controls

The studied herpes virus	HSV		CMV		EBV	
	+ve (n=86)	-ve (n=4)	+ve (n=41)	-ve (n=49)	+ve (n=84)	-ve (n=6)
Patients:						
No	72	3	38	37	70	5
%	83.7	75	92.7	75.5	83.3	83.3
Controls						
No	14	1	3	12	14	1
%	16.3	25	7.3	24.5	16.7	16.7
OR	1.71		4.1		-	
%CI	0.16-7.70		1.07-15.75		-	
P value	>0.05		<0.05		>0.05	

OR = Odds ratio CI = Confidence interval
Patients who had CMV-specific IgG were 4 times more liable to have vascular disease as compared with those not having these antibodies (OR=4.10, 95% CI=1.07-15.75, p<0.05).

Table 6 shows that there was no significant correlation between the lipid profile, hsCRP and virus-specific IgG antibodies among the studied vascular disease patients. Moreover, there was no significant correlation between hsCRP and lipid profile or virus-specific IgG antibodies among the different studied patient groups or controls (table 7).

DISCUSSION

Atherosclerosis is a chronic inflammatory disease of arteries, associated with multiple genetic and environmental factors (Froberg *et al.,* 2001b). One of the candidate inflammatory triggers is infection (Rott *et al.,* 2003 and Reszka *et al.,* 2008). Chronic infectious diseases that are accompanied by low-grade inflammation have been suspected to take an active part in the initiation and progression of atherosclerosis (Kunes *et al.,* 2005 and Cheng *et al.,* 2009).

In this study, the levels of cholesterol, triglycerides and LDL-c were significantly higher, while HDL-c was significantly lower among patients with vascular disease compared to controls. Similar findings were reported in

Table 5: Virus-specific IgG levels among the studied patients and controls

The studied parameter	Vascular disease patients (n=75)	Controls (n=15)	t value	P value
HSV(VEU/ml)	21.8 ± 4.9	20 ±6.4	1.2	>0.05
CMV(U/ml)	0.96 ± 0.20	0.80 ± 0.17	2.9	< 0.01
EBV(VEU/ml)	13.2 ± 3.8	11.0 ± 3.6	2.1	< 0.05

Vascular disease patients showed significantly higher levels of CMV- and EBV-specific IG as compared to that of the controls, however, HSV-specific IgG was not significantly different between the 2 groups.

Table 6: Correlation between lipid profile, log-hsCRP and virus-specific IgG levels among vascular disease patients

Studied parameter	Cholesterol	Triglycerides	HDL-c	LDL-c	Log-hsCRP
Log-hsCRP	0.041	0.206	-0.016	0.061	-
HSV-IgG	0.231	0.058	0.050	0.194	0.024
CMV-IgG	0.003	-0.011	0.043	-0.030	0.100
EBV-IgG	-0.080	-0.009	-0.052	-0.009	0.111

Pearson correlation coefficient was used for correlating different variables.
There was no significant correlation between lipid profile, Log-hsCRP and herpes virus-specific IgG among the studied 75 patients with vascular disease.

Table 7: Correlation between log-hsCRP and lipid profile and virus-specific IgG levels among the studied patient groups

Studied parameters	Cholesterol	Triglycerides	HDL-c	LDL-c	HSV	CMV	EBV
ACAD	0.073	0.246	-0.153	0.181	-0.268	0.302	0.255
CCAD	0.226	-0.068	0.084	0.209	0.289	0.156	0.255
Stroke	0.073	-0.081	-0.327	-0.041	-0.181	-0.255	0.067
PAD	0.309	0.065	0.109	0.319	0.391	-0.071	0.061

Pearson correlation coefficient was used for correlating different variables.
There was no significant correlation between log-hsCRP and lipid profile or herpes virus-specific IgG among patient groups.

patients with CAD by Ferrario *et al* (2005), Olsson *et al* (2005), Pac *et al* (2005), and Shai *et al* (2005) and in patients with PAD (Muntner *et al.,* 2005). This finding may support the atherogenic role of lipids and indicate the importance of LDL-c in pathogenesis of atherosclerosis. It has been hypothesized that elevated levels of LDL-c result in injury to the endothelial cells of the artery and then initiate the inflammatory process. Low levels of HDL-c show a consistent relationship with the development of atherosclerosis. As triglyceride levels exceed the range of 90–100 mg/dL, there is a proportional increase in production of small, dense LDL particles. Small LDL particles are highly oxidizable, and their unregulated uptake and incorporation in macrophages enhance the release of inflammatory cytokines, resulting in elevated CRP. In addition, the triglyceride-rich very low-density lipoprotein and chylomicrons may induce endothelial vascular cell adhesion molecule 1 and activate other proinflammatory transcription factors, including NF-kappa B. Uptake of triglyceride-rich lipoproteins by macrophages may promote foam cell formation, up-regulate plasminogen activator inhibitor, and promote inflammation by facilitating neutrophil transendothelial migration (Ahmad *et al.,* 2001 and Pesonen *et al.,* 2008).

Increasing evidence has shown that atherogenesis is not only caused by hypercholesterolemia. Several risk factors, including bacterial and viral infection, are involved in the overall inflammatory reaction in the blood vessels (Wu and Wu, 2006). Beyond traditional cardiovascular risk factors, markers of inflammation and infections seem to significantly influence the occurrence of cerebrovascular and cardiovascular events (Corrado *et al.,* 2006).

In this study, hsCRP, an important inflammatory marker, was significantly higher among all the studied patients, indicating that an inflammatory reaction may play a role in pathogenesis of atherosclerosis in our patients. The level of CRP was reported to be significantly higher among patients with CAD (Ferrario *et al.,* 2005 and Shai *et al.,* 2005) and patients with PAD (Muntner *et al.,* 2005) when compared with controls. The level of hsCRP was considered by some investigators to be a 'golden marker' for cardiovascular disease. Apart from being an inflammatory marker, hsCRP has been shown to be a risk factor for CAD as it enhances LDL aggregation and the production of vascular cell adhesion molecules triggering the atherosclerotic process. Moreover, it stimulates matrix metaloproteinase expression which increases plaque

vulnerability (Williams *et al.,* 2004). Miyamoto *et al* (2006) concluded that localized up-regulation of innate immune markers early after infection, rather than systemic inflammation, contributes to pathogen-accelerated atherosclerosis.

In this study, higher percentage of patients with vascular diseases had herpes virus-specific IgG antibodies (especially CMV) compared to that of the controls. Moreover, presence of CMV-specific IgG was significantly associated with occurrence of atherosclerotic vascular disease. It was found that the prevalence of high CMV seropositivity was an independent predictor of CAD and that population with CAD had a high rate of CMV infection (Eryol *et al.,* 2005). A relation between pathological atherosclerotic process in CAD and CMV infection was reported by Corrado and Novo (2005) and Susloparov *et al* (2005). CMV was also reported to play an important role in atherosclerotic cerebral infarction (Hu *et al.,* 2001) and CMV-seropositive individuals were shown to have endothelial dysfunction that was independent of conventional risk factors (Grahame-Clarke *et al.,* 2003). CMV is thought to be the most possible etiological factor of atherosclerosis. CMV infection may relate to the pathogenesis and the artery itself may be the site of CMV latency (Chen *et al.,* 2003). Active CMV infection was found to be enhanced in atherosclerotic blood vessels compared to atherosclerosis-free vascular equivalents (Nerheim *et al.,* 2004).

On the other hand, lack of association between chronic infection with CMV, EBV or pathogen burden and endothelial function was observed, suggesting that these agents are not implicated as early etiologic triggers in the genesis of CAD (Andrie *et al.,* 2003, Khairy *et al.,* 2003 and Xenaki *et al.,* 2009). Rothenbacher *et* al (2005) suggested that seropositivity to CMV might not be a strong risk factor for recurrent cardiovascular events in patients with manifest CAD, and is not associated with levels of established inflammatory markers. However, a significant association between the presence of CMV DNA and EBV DNA in aortic walls and atherosclerosis was reported (Horvath *et al.,* 2000). Rodent models as well as *in vitro* studies suggested that CMV might enhance lesion formation in various ways, like augmentation of the oxLDL uptake, altering monocyte adhesion or increasing the production of pro-inflammatory cytokines (Stassen *et al.,* 2006). CMV infection was reported to increase the production of inflammatory cytokines in CAD (Sun *et al.,* 2005). Primed leukocytes were found to produce soluble factors with either anti-viral or pro-inflammatory activity due to production of interferons and several cytokines which sets up virus latency or elimination. However, the same cytokines act on infected and/or neighboring endothelial cells and initiate the cascade of inflammatory reactions in the vascular wall (Scheglovitova *et al.,* 2002).

Our findings show that the occurrence of HSV-and EBV-specific IgG was more frequently encountered among patients than controls (although it was not significant most probably due to the small number of the studied samples), suggesting that infection with these viruses may play a role in atherosclerotic vascular diseases. It was reported that the high incidence and kinds of herpesviruses were related to the high incidence of atherosclerosis (Shi and Tokunaga, 2002). Moreover, seropositivities to EBV and HSV1 and HSV2 were independently associated with the future risk of cardiovascular death (Rupprecht *et al.,* 2001 and Kotronias and Kapranos, 2005 and Muneuchi *et al.,* 2009).

Kotronias and Kapranos (2005) suggested that HSV seems to play a significant role in the initiation and progression of coronary atherosclerosis. Different mechanisms by which HSV may contribute to atherogenesis have been described. In vascular cells, HSV infection leads to lipid accumulation. HSV infection of endothelial cells attracts leukocytes with subsequent inflammatory damage; it activates procoagulant changes on endothelium with increased thrombin generation and platelet adhesion, and changes its interaction with extracellular matrix proteins (Visser and Vercellotti, 1993). It was found that HSV-1 could infect vascular endothelial cells, leading to endothelial cell dysfunction (Chirathaworn *et al.,* 2004).

The biological properties of some herpes viruses such as the ability of latent persistency in the host cells and the presence of viral DNA in atherosclerotic lesions, suggest the possible role of herpes viruses in the development of atherosclerosis (Horvath *et al.,* 2000). Kwon *et al* (2004) and Ibrahim *et al* (2005) detected viral DNA of HSV, EBV and CMV in biopsies from atherosclerotic plaques and concluded that herpes viral infections may have a role in atherosclerosis. If these agents cause persistent infection in the vessel wall, they can directly promote a proinflammatory, procoagulant, and proatherogenic environment (Leinonen and Saikku, 2002).

In this study, vascular disease patients had significantly higher levels of CMV- and EBV-specific IgG than that of the controls. This result may indicate that high level of antibodies may have a role in pathogenesis of atherosclerosis. High levels of CMV antibodies were significantly associated with incident CAD (Sorlie *et al.,* 2000). Handzha (2004) suggested that viral infection exerts an immune reactions and that autoimmune inflammation plays a role in the development of unstable angina. Lunardi *et al* (2005) proposed that immune response to particular CMV proteins might result in autoaggression through a mechanism of molecular mimicry of normally expressed endothelial cell surface molecules.

Leskov and Zatevakhin (2005) suggested that intracellular infections such as HSV and CMV may induce endothelial injury through both direct and indirect immune system-mediated effect. During CMV infection, antibodies against the virus can arise that are able to cross react with human HSP60 and cause apoptosis of non-stressed endothelial cells (Bason *et al.,* 2003). Rott *et al.,* (2003) suggested that T lymphocytes, clonally expanded in response to antigens presented by CMV infection, home to sites of vascular injury and locally release IL-6 which then triggers endothelial cells to release MCP-1, which recruits more monocytes and T-cells into the vessel wall and thereby exacerbates local inflammation, and thus atherogenesis (Froberg *et al.,* 2001b). Roberts and Cech (2005) indicated that persons previously exposed to CMV were 12 times more liable to have diabetes, which may accelerate atherosclerosis.

In conclusion, the levels of serum lipids, hsCRP and CMV- and EBV-specific IgG antibodies were higher among patients with atherosclerotic vascular diseases. Presence of CMV-specific IgG is significantly associated with development of vascular disease. However, no significant correlation was detected between IgG levels and lipid profile or hsCRP.

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