M. Izadi, M.D.

CYTOMEGALOVIRUS LOCALIZATION IN ATHEROSCLEROTIC PLAQUES IS ASSOCIATED WITH ACUTE CORONARY SYNDROMES: REPORT OF 105 PATIENTS

[a]Morteza Izadi, M.D.; [b]Mozhgan Fazel, M.D.; [c]Seyed Hassan Saadat, M.D.; [a,d]Mohammad Hassan Nasseri, M.D.; [a,e]Mojtaba Ghasemi, M.D.; [f]Hossein Dabiri, M.D.; [a,d]Reza Safi Aryan, M.D.; [a]Ali Akbar Esfahani, M.D.; [a,g]Ali Ahmadi, Ph.D.; [a,e]Davood Kazemi-Saleh, M.D.; [a,d]Mohammad Hassan Kalantar-Motamed, M.D.; [h]Saeed Taheri, M.D.

[a]Health Research Center, Department of Infectious Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran; [b]International Travel Medicine Center of Iran, Baqiyatallah University of Medical Sciences, Tehran, Iran; [c]Behavioral Sciences Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran; [d]Department of Surgery, Baqiyatallah University of Medical Sciences, Tehran, Iran; [e]Department of Cardiology, Baqiyatallah University of Medical Sciences, Tehran, Iran; [f]Department of Microbiology, Shahid Beheshti University of Medical Science, Tehran, Iran; [g]Department of Laboratory Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran; [h]Dr. Taheri Medical Research Group, Tehran, Iran

Abstract

It has been shown that cytomegalovirus (CMV) is present in coronary atherosclerotic plaques, but the clinical relevance of this presence remains to be elucidated. In this study we sought to examine CMV infection in atherosclerosis patients defined by different methods and to identify the clinical significance of CMV replication in the atherosclerotic plaques. The study included 105 consecutive patients who were admitted to our department and underwent coronary artery bypass grafting (CABG) surgical interventions. Coronary atherosclerotic specimens as well as 53 specimens from the mamillary artery of these same patients were analyzed. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) methods were used for evaluations. The CMV PCR test result was positive for 28 (26.7%) of patients with coronary artery atherosclerosis. After adjusting for other risk factors, coronary artery disease patients with a history of acute coronary syndrome were more likely to be positive for CMV PCR test (P = 0.027; odds ratio: 4.2; 95% CI: 1.18-15.0). They were also more likely to have a positive family history for cardiovascular diseases (CVD). This study confirms previous evidence about the replication of CMV virus in the atherosclerotic plaques of coronary arteries and brings clinical significance to this observation by showing a higher prevalence of acute coronary syndromes in those patients with CMV-infected plaques. Our study also suggests a familial vulnerability to CMV replication in the coronary artery walls.

Introduction

The first evidence of a potential link between infective agents and atherosclerosis was found in bacterial infections and dates back to 1891, when Huchard suggested an association between childhood infections and the development of atherosclerosis in his article "Infectious diseases of childhood as potential cause of inflammation." Shortly after, Weisel (1906), Klotz (1906), and Osler (1908) reported relationships between atherosclerosis and infective agents including streptococci, typhoid, scarlet fever, measles, and acute infections.[1]

After measles, Marek's disease virus (MDV), a herpes-type DNA virus that is a well-demonstrated cause of T-cell type lymphomas, was the first viral agent to be associated with the development of atherosclerosis in the 1940s.[2] Fabricant et al.[3] also indicated that atherosclerosis appears only in MDV-infected chickens, which were fed with regular diets, but not in non-infected chickens that were fed with cholesterol-rich diets. Moreover, infected animals were much more likely to have visible atherosclerotic lesions compared to uninfected animals.[4]

Cytomegalovirus is one of the viruses accused of inducing endothelial injury, which has one of the highest prevalence rates in human populations. This virus causes a wide spectrum of disorders in human beings, ranging from a slightly symptomatic mononucleosis-like syndrome to life-threatening disseminated disease that occurs mostly in immunodeficient patients. CMV infection is also associated with severe birth defects when it occurs in pregnant mothers. Although it has been shown that CMV infection is associated with atherosclerosis, the exact pathogenesis of CMV-induced atherogenesis has not been well defined. Several studies have investigated the potential latency and replication sites of CMV to determine whether and how CMV infection can lead to atherosclerosis. Moreover, there is no mention as to whether or not CMV replication in the arterial walls can result in inauspicious outcomes.

In the current study, we sought to examine the prevalence of CMV antibody positivity rate in patients with atherosclerotic lesions. We also sought to use PCR methods to define the existence of CMV virus in these lesions. Finally, we wanted to determine whether CMV infection as a whole (antibody positivity alone)

is associated with atherosclerosis or if existence of the CMV virus detected by PCR methods is an independent predictor for atherosclerosis. Moreover, we tried to identify the clinical significance of CMV replication in the atherosclerotic plaques.

Methods and Material

Our study included 105 consecutive patients who were admitted to Baqiyatallah University of Medical Sciences hospitals between 2008 and 2010 with various manifestations of ischemic vascular disease and who underwent CABG surgery. In addition, 53 specimens from biopsies of macroscopically healthy regions of the left internal mamillary artery were collected from these patients at the National Forensic Medicine Department. Data on demographics, smoking habits, lipid profiles, and medical histories were recorded for all subjects. Acute coronary syndrome was defined as myocardial infarction and/or unstable angina. A positive family history was defined when a positive history was reported on the first and second family members including parents, siblings, offspring, grandparents, uncles, and aunts. This study was approved by the University Research Review Board (URRB) and the Ethics Committee of Baqiyatallah University of Medical Sciences. All subjects provided written informed consent to participate in the study and were assured that their personal information will remain anonymous and confidential. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the *International Journal of Cardiology*.

Tissue samples were dissected in the operating room and stored under sterile conditions. Artery segments were placed in microcentrifuge tubes without using binding buffer. Transport vials were sealed in the operating room and opened only in the laminar airflow safety cabinet at the microbiology laboratory. All of the specimens were kept at -20 degrees until processing. For preparation of genomic DNA and PCR, DNA was extracted from endarterectomy specimens by using the QIAamp DNA Mini-Kit (Qiagen, Inc., Valencia, CA, USA). The DNA absorbed in the QIAamp spin column was eluted with 55 µL of Tris-EDTA solution and then subjected to the PCR.

PCR was carried out for CMV using primers selected from the gB region of the CMV genome. The forward and reverse primers were 5′-CGG TGG AGA TAC TGC TGA GGT C-3′ and 5′- CAA GGT GCT GCG TGA TAT GAA G-3′ respectively. The reaction mixture of the PCR contained a total volume of 50 µL, including 75 mM Tris-HCL (pH 9), 1.5 mM MgCl2, 50 mM KCl, 20 mM of (NH4)2SO4, 50 µM of each one of the deoxynucleoside triphosphates, 20 pM of primers gB1and gB2, and 1 µg of DNA obtained from tissue. The reaction mixture was first incubated at 94° C for 3 mixtures, followed by 40 cycles at 94° C for 30 seconds, 55° C for 30 seconds, 72° C for 30 seconds, and finally for 3 minutes at 72° C. The PCR products were subjected to electrophoresis on a 2% agarose gel, and 257-bp amplicons were visualized by ultraviolet light after ethidium bromide staining. Each PCR assay included a positive control with HCMV AD169 DNA and a negative control containing no template (only distilled water). Serological evaluation of CMV IgG and IgM was performed using ELISA.

Statistical analysis

Data was analyzed using SPSS software version 17.0 (SPSS Corp., Chicago, IL, USA). Chi-square test, Fisher's exact test, and Kruskal-Wallis test were used where appropriate. Logistic regression models were used to evaluate independent associations of various factors with acute coronary syndromes. All statistical analyses were performed at the 0.05 significance level.

Parameters	Result
Mean age ± SD (yr)	58.2 ± 10.6
Male gender (%)	73 (69.5)
Mean weight ± SD (kg)	76.7 ± 10.6
Mean BMI ± SD (kg/m2)	28.1 ± 4.0
IgG CMV (%)	90 (85.7)
IgM CMV (%)	4 (3.8)
PCR CMV (%)	28 (26.7)
Biochemical examinations	
Triglyceride (mean ± SD	195.3 ± 110.4)
Fasting blood glucose (mean ± SD)	150.9 ± 67.2
Fibrinogen (mean ± SD)	203.8 ± 54.8
Cholesterol total (mean ± SD)	196.7 ± 189.7
LDL cholesterol(mean ± SD)	97.1 ± 38.5
HDL cholesterol (mean ± SD)	42.2 ± 11.1
CRP (mean ± SD)	1.7 ± 0.46
Medical history	
Hypertension (%)	57 (54.3)
Smoking (%)	26 (24.8)
Acute coronary syndromes (%)	62 (59)
Diabetes mellitus (%)	54 (51.4)

Table 1. Characteristics of the study participants.
SD: standard deviation; CMV: cytomegalovirus; BMI: body mass index; CRP: C-reactive protein; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

Results

Characteristics of the study participants are summarized in Table 1. Data of all 105 patients and their biopsy specimen were entered into analysis. CMV PCR test results were positive for 28 (26.7%) patients with coronary artery atherosclerosis, serologic test results showed only 4 (3.8%) positive cases for CMV IgM but 90 (85.7%) for CMV IgG tests, and 28 (26.7%) patients had a history of unstable angina or myocardial infarction. Coronary artery disease patients with a history of acute coronary syndrome were more likely to be positive for CMV PCR test ($P = 0.05$; Table 2). In order to evaluate a potential independent impact of CMV replication in the coronary artery wall on the incidence of unstable angina and/or myocardial infarction, we entered our data into a multivariable logistic regression model enrolling all factors that may affect these events, including age, gender, BMI, history of diabetes mellitus, triglyceride level, LDL level, and fasting blood glucose level. This model demonstrated that PCR-positive test for CMV is the only factor that independently increases the rate of unstable angina and myocardial infarction (Table 3).

We also reanalyzed data to find out whether CMV replication in the atherosclerotic plaques has any predictors. For this purpose, we correlated demographic and medical history of the patients (age, gender, weight, BMI, biochemical examinations, history of hypertension, smoking, and diabetes mellitus) with their CMV PCR test results. We found no difference between the two patient groups regarding any of the parameters.

Parameters	History of acute coronary syndrome (ACS)		
	ACS	No ACS	*P* value
Mean age ± SD (yr)	59.9 ± 9.2	60.8 ± 9.9	0.658
Male gender (%)	23 (69.7)	50 (69.4)	1.0
Mean weight ± SD (Kg)	75.5 ± 8.7	76.2 ± 11.0	0.75
Mean BMI ± SD (Kg/m2)	26.9 ± 2.7	27.9 ± 3.6	0.141
CMV IgG (%)	30 (90.9)	60 (83.3)	0.38
CMV IgM (%)	1 (3)	3 (4.2)	1.0
CMV PCR (%)	13 (39.4)	15 (20.8)	0.05
Biochemical examinations			
Triglyceride (mean ± SD)	220.4 ± 121.7	178.3 ± 101.9	0.114
Fasting blood glucose (mean ± SD)	148.9 ± 57.9	154.5 ± 75.3	0.406
Fibrinogen (mean ± SD)	194.9 ± 57.1	206.7 ± 54	0.406
Cholesterol total (mean ± SD)	190.1 ± 36.9	171.3 ± 41.5	0.046
LDL cholesterol (mean ± SD)	108.6 ± 34.1	93.9 ± 39.9	0.107
HDL cholesterol (mean ± SD)	42.2 ± 11.2	42.8 ± 11.4	0.817
CRP (mean ± SD)	1.8 ± 0.4	1.8 ± 0.4	0.944
Medical history			
Hypertension (%)	22 (66.7)	35 (48.6)	0.096
Smoking (%)	11 (33.3)	15 (20.8)	0.223
Diabetes mellitus (%)	20 (60.6)	34 (47.2)	0.216

Table 2. Comparison of the study parameters in participants with or without acute coronary syndrome.
SD: standard deviation; BMI: body mass index; CRP: C-reactive protein.

					95% Confidence Interval for Exp (B)	
Variables *	B	Std. Error	Sig.	Exp (B)	Lower Bound	Upper Bound
Age	0.041	0.030	0.177	1.042	0.982	1.106
Gender (male)	0.421	0.662	0.525	1.524	0.416	5.583
BMI	0.028	0.087	0.746	1.028	0.868	1.218
Diabetes mellitus history	0.965	0.751	0.199	2.625	0.603	11.435
Triglyceride	-0.003	0.003	0.388	0.997	0.991	1.004
LDL cholesterol	-0.013	0.008	0.084	0.987	0.973	1.002
FBS	0.010	0.006	0.064	1.010	0.999	1.022
Positive CMV PCR test	1.437	0.649	0.027	4.206	1.178	15.022

Table 3. Multivariable logistic regression model for evaluating independent association between unstable angina and myocardial infarction and other factors.
BMI: body mass index; FBS: fasting blood sugar. **The reference category is positive history for an acute coronary syndrome episode.*

We also correlated CMV PCR test results with family history for CVD. We found that patients with a positive CMV test result performed on their atherosclerotic plaques and evaluated by PCR are significantly more likely to have a positive family history for CVD in their first- and second-degree family members than those who had a negative CMV test result (9/28 versus 8/77, or 32.1% versus 10.4%, respectively; $P = 0.014$). Rate of IgG seropositivity among patients with a positive family history for CVD was comparable to that in other patients: 16/17 (94%) versus 74/88 (84%), respectively; $P = 0.456$). CMV IgM was only found in 4 (3.8%) of the atherosclerotic patients. We also correlated CMV IgM test result with the study parameters; we found that patients with CMV IgM antibody positivity in their serological tests are significantly more likely to have higher levels of triglyceride (197 ± 113 vs. 145 ± 8, respectively; $P < 0.001$). Then we reanalyzed data for coronary arterial atherosclerotic lesions and compared them to that of 53 mamillary artery specimens. None of the specimens from the mamillary artery was positive for CMV when it was evaluated by the PCR ($P < 0.0001$).

Discussion

The potential impact of viral pathogens on inducing endothelial injury — resulting in the exposure of underlying smooth muscle cells and development of atherosclerosis — has been studied massively, and CMV was the most commonly implicated agent investigated. Despite all the studies, however,

the role of CMV in atherosclerosis remains obscure. There are several reports indicating a potential role for CMV replication in the coronary arterial wall and atherosclerotic plaque formation in humans, while several other studies have doubted this hypothesis based on their own observations, which will be discussed shortly. Our study evaluated this association in a population of patients with CVD who have undergone CABG.

The prevalence of antibodies to CMV infection has been associated with atherosclerosis[5] and is common in the general population, with evidence of past infection in approximately 15% of adolescents, 50% of adults by age 35, and 70% of patients older than 75 years.[6] The rates in our study of Iranian CVD patients were also high, with 87% IgG positivity for CMV in adults ages 75 years and younger and 70% for those older than 75 years (the latter group included only seven patients). The high prevalence of CMV infection in the general population and in CVD patients coupled with the high rate of mortality from CVD in almost all parts of the world emphasizes the relevance of any potential relationship between CMV infection and CVD. In a prospective cohort of 134 age-matched pairs of male patients who underwent vascular surgery versus patients with no evidence of atherosclerosis, investigators found that patients in the surgery group are significantly more likely to have CMV antibodies than in the controls.[7] On the other hand, investigators also followed 46 pairs of patients, one of each having undergone vascular surgery and the other having had no surgery, all with symptoms of atherosclerosis. The latter cohort showed no difference between patients with respect to CMV antibodies. Those investigators concluded that elevated levels of CMV antibodies might be associated with CMV-mediated vascular injury and subsequent atherosclerosis. In our study, we found no association between CMV antibodies and acute coronary syndromes, but anti-CMV IgM was associated with hypertriglyceridemia — although, given the limited number of CMV IgM positive patients (4 cases), this study would need to be replicated to confirm any observations.

In autopsies of young people dying of trauma, viral pathogens from the herpes family have been found in various layers of the vessel wall, including endothelial and smooth muscle cells.[8, 9] This observation suggests that these viral agents exist in the arterial wall of young patients with no symptoms of atherosclerosis.[8,9] However, there is no consensus on the relevance of this colonization and its potential impact on the development of atherosclerosis. Several studies have investigated associations between CMV localization in the arterial walls and atherosclerosis formation; some of them were only observational studies showing a high rate of CMV DNA positivity in the atherosclerotic lesions of arteries.[10-15] Despite finding some higher proportion of atherosclerotic lesions with CMV infection, they failed to reach significance level, maybe due to limited sample size or improper testing.[16, 17] However, after using more sensitive techniques including PCR and a large sample size, researchers found a highly significant relationship between atherosclerotic patients and CMV DNA detection compared to patients with non-significant arterial disease,[18-20] although diverse results have also been reported.[21, 22] In our study, CMV DNA was detected in 27% of the atherosclerosis specimens from patients who had undergone CABG. However, none of the specimens from normal mamillary arteries were positive for CMV DNA. The same finding was reported by Ibrahim et al., who reported an exclusive detection of CMV DNA in coronary and carotid lesions versus in the mammary artery.[11] Nevertheless, another study found a large proportion of mammary artery specimens that were positive for CMV DNA.[23]

The association between CMV infection and acute coronary syndromes has also been investigated. Kol et al. investigated the presence of CMV in atherectomy specimens from patients with stable versus unstable angina using southern blotting and hybridization with a specific probe for detecting the CMV major immediate-early (MIE) gene.[24] They found no specimen with a positive hybridization signal and concluded that in patients with unstable angina, replication of CMV in coronary atherosclerotic plaques is not a major cause of plaque instability. However, Liu et al.[25] investigated the presence of CMV in the coronary plaques of 23 patients with coronary syndromes and compared them with 17 control patients using immunohistochemical techniques; they found a significantly higher rate of infection in the acute coronary syndrome group ($P = 0.01$). In our study, using a more sensitive PCR method, we found the same results as the latter study in which patients who had positive PCR results for CMV DNA were significantly more likely to have a history of unstable angina or myocardial infarction. This finding is of utmost relevance: not only does it show a high rate of CMV infection present in atherosclerotic plaques, but it also confirms through clinical evidence a higher risk of acute coronary syndromes for CMV replication in atherosclerotic plaques. This in turn should encourage us to find preventive strategies toward a potential favorable effect of using antiviral agents to prevent ominous heart events.

A novel finding of this study is the association between a positive CMV DNA detection in the atherosclerotic plaques and a positive family history for CVD. As mentioned above, autopsy analysis of young people who died from trauma revealed positive CMV DNA in their coronary artery specimens, with no clinical evidence of CVD. Some investigators suggested that this finding implies a role for CMV infection in initiating the atherosclerosis process in the coronary arteries. However, we suggest that the higher rate of a positive family history of CVD for patients with CMV-positive atherosclerosis implies that their arterial walls have a higher sensitivity to CMV replication. This implication is also very relevant because, if proven, one may assume that such family members should begin preventive antiviral treatment.

CMV infection has also been associated with arterial hypertension,[26] diabetes mellitus-mediated atherosclerosis,[27] and a positive test for proliferative signals including CRP.[13] In our current study, we did not find any relationship between CMV IgM, CMV IgG, and CMV PCR positivity and having arterial hypertension or higher systolic and/or diastolic blood pressure (data not shown). Moreover, no association was found with regard to diabetes mellitus and any of the CMV tests. The same observation was found when CMV test results were correlated with CRP.

Conclusion

This study confirms previous evidence about the replication of CMV virus in the atherosclerotic plaques of coronary arteries, and it brings clinical significance to this observation by showing a higher prevalence of acute coronary syndromes in the CMV-infected plaques. Moreover, patients with a positive PCR result for CMV in their atherosclerotic plaques were more likely to have a positive family history for CVD. This suggests a familial vulnerability to CMV replication in the coronary artery walls.

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