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Cytomegalovirus Infection and Atherosclerosis in Candidate of Coronary Artery Bypass Graft

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Background: Although there is enough evidence that infectious agents such as *Chlamydia pneumonia* and *Helicobacter pylori* may play a pathogenic role in atherosclerosis, this role for cytomegalovirus (CMV) is yet controversial.
Objectives: The aim of the present study was to detect CMV-DNA in atherosclerotic plaques in patients who underwent coronary artery bypass graft (CABG).
Patients and Methods: In this case-control study, candidates for CABG (cases) and patients with valvular or congenital malformation but without atherosclerotic plaques (controls) were studied from 2012 to 2013 at Golestan hospital, Ahvaz, IR Iran. Demographic and laboratory data were collected. Atherosclerotic and histological samples were obtained from visible plaques and from aorta by the surgeon. All the samples were examined for the presence of CMV-DNA by polymerase chain reaction (PCR) method using a commercial kit (SinaClon, Tehran, IR Iran).
Results: The mean ages in case and control groups were 60.8 ± 6.8 and 57.5 ± 11.5 years, respectively, with no significant difference (P = 0.09). Thirty patients (54.5%) in case and 32 (58.2%) in control groups were male with no significant difference (P = 0.7). CMV-DNA was present in 8 (14.5%) of the cases and 2 (4%) of the controls. CMV-DNA was associated with higher risk of atherosclerosis (OR: 7.7, 95% CI = 1.1-51.4, P = 0.03). Of the total normal aortic samples (55 in cases and 55 in controls), there was no individual with simultaneous positive CMV-DNA among aortic atherosclerotic and normal tissue samples.
Conclusions: The presence of CMV-DNA in aortic plaques is associated with increased risk of atherosclerosis. CMV infection may be considered as an independent risk factor for this event.

Keywords: Arteriosclerosis; Coronary Vessels; Cytomegalovirus; DNA

1. Background

Since the beginning of the 20th century, there have been some attempts to understand the interactions between cardiovascular conditions and infectious processes (1). To our knowledge, experimental animal models date back to 1933 (2), but during 1970-1980 many studies were conducted and published, which tried to simulate atherosclerosis using dietary changes, mechanical alteration on vessels, or incubating animals with certain pathogens; a number of studies sought to explain the differences of patients with atherosclerosis and healthy individuals which could not be attributed to traditionally accepted risk factors; a third group of these studies took particular interests over inflammatory cells and pathways involved in atherosclerosis and naturally the first links were made with infections in general (2). Our ever growing understanding of the atherosclerosis has not changed one thing; the point that medical care professionals still observe associations between some pathogens and atherosclerosis (1, 3-6).

After the recognition of a pathogen part in gastritis, which is *Helicobacter pylori*, (7) and the subsequent introduction of proper antimicrobial therapy to deal with recurrent gastritis (8, 9), a number of scientists and clinicians have aspired to make a similar break-through in the realm of cardiovascular research (10-13). However, much has changed since Benditt, who suggested an inflammatory process triggered by chemicals or viruses as a possible cause of atherosclerosis (14). Although there is enough evidence that infectious agents such as *Chlamydia pneumonia* (CP) and *H. pylori* may play a pathogenic role in atherosclerosis as the main underlying disease for cardiovascular or cerebrovascular events in the presence of obesity, hyperlipidemia, diabetes, hypertension and cigarette smoking, this role for cytomegalovirus (CMV) is yet controversial (15-20).

2. Objectives

The main objective of the present study was to show a

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possible association between CMV and atherosclerotic plaques in coronary arteries in patients who underwent coronary artery bypass graft (CABG) surgery.

3. Patients and Methods

3.1. Subjects

A cohort of 120 patients who were scheduled to undergo cardiac surgery participated in this case-control study from August 2012 to June 2013, at Golestan hospital, a teaching hospital affiliated to Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran. Ten patients were excluded from the study on the basis of the exclusion criteria. Fifty five consecutive patients with significant coronary arteries stenosis and aortic arch atherosclerotic plaque determined by cardiac catheterization, who were to undergo CABG, were assigned as the case group. The other group with the same size as the control group included individuals who needed cardiac surgery due to reasons other than atherosclerosis, such as congenital or valvular heart diseases without coronary artery disease (CAD). In the control group, patients who had undergone cardiac catheterization had no significant coronary artery stenosis and no atherosclerotic plaques in aortic arch. The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. The purpose of the study was explained to the participants and written consents were obtained.

3.2. Sampling

Samples of atherosclerotic plaques and normal tissues were taken from the aortic arch by surgeons during CABG and cardiac surgery. Normal tissues samples were obtained from the canola circulation site. A total of 165 samples including 110 normal aortic tissue samples (from 55 cases and 55 controls) and 55 atherosclerotic aortic samples (from 55 cases) were obtained.

3.3. Exclusion Criteria

Patients with congenital or valvular heart disease were not included in the CABG group, neither were those who underwent off-pump CABG. Patients with congenital or valvular heart diseases were also excluded from the study if coronary artery plaques or aortic arch plaque were detected on their cardiac or aorta catheterizations.

3.4. Measurements and Definitions

For all of the participants, demographic information and laboratory data including fasting serum sugar, triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were recorded. Blood pressures were measured by one trained staff. All the samples were stored in special tubes and kept at a constant -70°C until examination in Pasteur Laboratory (Ahvaz, IR Iran), using polymerase chain reaction (PCR) detection kits (SinaClon Co., Tehran, IR Iran). A total serum cholesterol level of higher than 200 mg/dL or serum triglyceride (TG) level higher than 150 mg/dL was defined as hyperlipidemia.

3.5. Statistical Analyses

Two-tailed student's t-test and chi-square were used to compare qualitative and quantitative variables, respectively, as indicated. Levene's test indicated that variances of variables were normally distributed. Fisher's exact test was employed when appropriate. Binary logistic regression was employed to assess the variables' ability to predict the presence of coronary arteries plaques. All the analyses were performed using SPSS software version 18 (SPSS Corp., Chicago, Ill, US). $P < 0.05$ was considered significant. Descriptive values were reported either as proportions and percentages for the categorical variables or as mean ± standard (SD) deviation for the continuous ones.

4. Results

The mean ages in case and control groups were 60.8 ± 6.8 and 57.5 ± 11.5 years, respectively, with no significant difference ($P = 0.09$). Thirty patients (54.5%) in case and 32 (58.2%) in control groups were male with no significant difference ($P = 0.7$). The two groups had no significant differences regarding the prevalence of diabetes mellitus (DM), blood hypertension (HTN), smoking, family history of CAD, and serum TG and HDL levels. Patients of the control group were younger than the case group, although that was statistically non-significant. Eight patients (14.5%) among CABG candidates had CMV-DNA in the tissue samples of the atherosclerotic plaque. Two patients (4%) in the control group had CMV-DNA in their aortic tissue samples. Regarding laboratory findings and demographic information, the two groups had significant differences in the prevalence of dyslipidemia, serum LDL levels, and the presence of CMV-DNA in tissue samples. Of the CABG group, 70.9% had a history of dyslipidemia in comparison with 49.1% of the control group.

There were no statistically significant differences between the two groups with regards to members' genders ($P > 0.05$). We did not observe any significant differences regarding age among the case and control groups; although we supposed, given their corresponding medical conditions, the control group might have been younger than the CABG candidates. There was no significant multi-co-linearity, so we used binary logistic regression to determine the odds ratio for each variable's ability to predict whether a given individual belongs to the CABG or the control group (Table 1). Binary logistic regression showed that presence of dyslipidemia, CMV-DNA in tissue sample and serum LDL levels were independent predictors of atherosclerotic plaques in patients who were candidates of CABG in our medical center (Table 1). There is a peculiar point about LDL serum levels which is that this variable has a lower level among CABG candidates than control patients. Omnibus tests of model coefficients

Table 1. Binary Logistic Regression and a Model for Predicting Atherosclerotic Plaques in Aorta in Candidates of Coronary Artery Bypass Graft [a,b]

Variables	Cases (n = 55) [c]	Controls (n = 55)	OR (95% CI)	P Value [d]
HTN	38 (69.1)	31 (56.4)	1.08 (0.40-2.93)	0.87
DM	24 (43.6)	21 (38.2)	0.66 (0.24-1.78)	0.41
Dyslipidemia	39 (70.9)	27 (49.1)	7.39 (2.23-24.54)	0.001
Family history of CAD	21 (38.2)	29 (52.7)	0.05 (0.20-1.28)	0.14
Smoking	14 (25.5)	18 (32.7)	0.65 (0.21-2.03)	0.46
Serum LDL	118.73 ± 31.97	131.87 ± 33	1.02 (1.01-1.04)	0.01
Serum HDL	40.3 ± 12.82	39.1 ± 8.24	0.98 (0.94-1.03)	0. 52
Serum TG level	179.31 ± 76.54	178 ± 67.77	1.00 (0.99-1.01)	0.57
Positive CMV-PCR test	8 (14.54)	2 (4)	7.72 (1.16-51.4)	0.03

[a] Abbreviations: OR, odds ratio; CI, confidence interval; HTN, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; CMV, cytomegalovirus; PCR, polymerase chain reaction.
[b] Data are presented as mean ± SD or No. (%).
[c] Data are provided for coronary artery bypass graft (CABG) patients.
[d] Statistically significant (P < 0.05).

Table 2. Comparison of the Prevalence of Cardiovascular Disease Risk Factors between cytomegalovirus -Positive and Negative Patients Who Underwent Coronary Artery Bypass Graft [a,b]

Variables	Positive CMV (n = 8)	Negative CMV (n = 47)	OR (95% CI) [c]	P Value [d]
HTN	6 (75)	32 (68.1)	0.29-0.43	1.00
DM	5 (62.5)	19 (40.4)	0.16-0.60	0.27
Dyslipidemia	5 (62.5)	34 (72.3)	0.45-0.25	0.67
Family history of CAD	4 (50)	17 (36.2)	0.24-0.51	0.46
Smoking	6 (75)	32 (68.1)	0.34-0.33	1.00
Serum LDL	114.75 ± 41.16	119.40 ± 30.63	20.06-29.37	0.70
Serum HDL	33.50 ± 8.48	41.47 ± 13.14	1.71-17.65	0.10
Serum TG level	194.38 ± 78.59	176.74 ± 76.74	76.69-41.43	0.55

[a] Abbreviations: CMV, cytomegalovirus; OR, odds ratio; CI, confidence interval; HTN, hypertension; DM, diabetes mellitus, CAD, coronary artery disease; LDL, low-density lipoprotein, HDL, high-density lipoprotein, TG, triglyceride.
[b] Data are presented as mean ± SD or No. (%).
[c] Data are provided for upper-lower normal limit.
[d] Statistically significant (P < 0.05).

also indicated that the latter variables, in combination, were significant predictors (χ^2 = 25.06, P = 0.009). The Cox and Snell R square was 0.204. Calculated odds ratios for the presence of CMV-DNA in aorta atherosclerotic plaques, dyslipidemia and serum LDL levels, expressed as OR (CI of 95%, lower limit-upper limit), were 7.7 (CI 95%, 1.16-51.40), 7.39 (CI 95%, 2.23-24.54), and 1.02 (CI 95% 1.01-1.04), respectively. Only these variables had P < 0.05.

A comparison of the risk factors for CAD among the individuals who underwent CABG, between those with positive CMV-DNA and those with negative CMV-DNA showed no statistically significant difference regarding the risk factors we investigated (Table 2). Of the total of 110 normal aorta samples examined for CMV DNA, two were positive in the control group; these samples were obtained from individuals admitted for non-CAD indications of cardiac surgery. We did not find any individuals who had simultaneous positive CMV-DNA samples of aorta atherosclerotic plaque and normal aorta sample.

5. Discussion

The present study showed that the presence of CMV-DNA was associated with a higher risk of atherosclerosis in the aortic arch (14.5% vs. 4%, P = 0.03). The findings of our study were compatible with those of similar studies in Iran (1, 4); all of them suggested a possible role of CMV in atherosclerosis. In the study of Izadi et al. (1), 105 patients who underwent CABG were assessed regarding circulatory IgG and IgM anti-CMV antibodies, their demographic information, and the presence of CMV-DNA in samples obtained from their coronary arteries, and left internal mammillary arteries. The authors suggested that if patients were categorized based on a history of

acute coronary syndrome (ACS), a significant difference would exist between the prevalence of positive CMV-DNA PCR tests among the two groups (1). They calculated an odds ratio of 4.21 for positive CMV-PCR test, when comparing cases of ACS against other candidates of CABG (1).

Since co-morbid coronary atherosclerosis has detrimental prognostic effects on individuals undergoing cardiac surgery, we had to perform cardiac and aorta catheterization on patients in the control group to rule out these diagnoses. These individuals did not have any visible plaques on their angiography, and biopsies performed on their aorta were positive for CMV-DNA PCR in two individuals. There was a significant difference between the prevalence of CMV-DNA in aorta samples of the two groups (OR: 7.7, CI 95%: 1.16-51.4, P = 0.03). Another study compared 113 patients diagnosed with CAD, determined by cardiac catheterization, and 44 other patients who had patent coronary arteries, detected by the same method (4). Authors observed a significant difference (P = 0.01) between serum anti-CMV IgG antibodies among the two groups.

It is a bit problematic to compare our findings with the results of this study, because these findings were not accompanied by histological evidence of CMV (4), plus the fact that circulating anti-CMV antibodies persist for a long time (20). DuRose et al. (21) in their seminal work on the interactions of human CMV and endothelium in an in vitro parallel-plate flow chamber demonstrated that varying degrees of laminar shear stress played a role in the susceptibility of endothelial cells to the entry of CMV (21). Detailed discussion of their findings was beyond the scope of our study; still, they proposed a number of mechanisms, through which CMV could mediate its role in atherosclerosis; permanent damage or endothelial cell death, alterations in cellular phenotype to more atherosclerosis-prone forms, alterations in chemokines in circulation, and human body immune system reaction to CMV, both in general and particularly by actions of CMV-specific T cells (21, 22).

The absence of CMV-DNA in normal tissue aortic samples of patients with positive CMV samples from aorta atherosclerotic plaques, when considered in combination with the existing findings (21), suggests a preference of CMV for atherosclerotic plaque. However, the two samples that were positive in the control group may be more at risk of atherosclerosis in the future. As demonstrated in the results, the comparison of CABG candidates based on presence or absence of CMV-DNA in their aorta atherosclerotic plaque samples showed no significant differences of the risk factors prevalence among members of these sub-groups; this finding suggests that contrary to our initial theory, CMV contributes to atherosclerosis independent of other atherosclerosis risk factors.

We obtain identical samples in the control group to assess the presence of CMV-DNA in the control group, which was the strength of this study. Another strength of this study was obtaining CMV using PCR method in two groups of patients and controls which distinguishes this study from the previous ones. We did not measure the titers of CMV antibodies, so we were not able to compare findings of other authors with ours in this regard. Small sample size caused small numbers of individuals in a number of groups. Lack of nationwide data on the prevalence of CMV antibodies is a general limitation for studies of this kind. The findings of this study and similar works support the possibility of involvement of infectious agents in atherosclerotic process; further investigations are warranted. There was no possibility of a biopsy of normal coronary artery; therefore, for better comparison, we compared biopsies from aorta in case and control groups, of which sampling can be available by operation. The findings of the present study explained that the presence of CMV-DNA in aortic plaques is associated with increased risk of atherosclerosis. CMV infection determined by PCR-DNA detection may be considered as an independent risk factor for this event.

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Authors' Contributions

Study concept and design: Habib Heybar and Seyed Mohammad Alavi. Search in literatures: Habib Heybar, Seyed Mohammad Alavi and Mehdi Farashahi Nejad. Drafting of the manuscript: Habib Heybar. Critical revision of the manuscript for important intellectual content: Habib Heybar, Seyed Mohammad Alavi and Mehdi Farashahi Nejad. Advising: Seyed Mohammad Alavi. Statistical analyses: Mahmood Latifi.

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