The association between CMV and ischemic heart disease in Kirkuk city

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Abstract

Background: Cytomegalovirus is a β-herpes-virus that infects from 40 to 100% of adults in all regions of the world and is endemic to this virus. Current, latent and recurrent infections could be associated with it. In addition, reactivation of the latent virus has been demonstrated in vascular cells.

Objective: The goal of this research was to investigate the seroprevalence of anti-CMV IgG antibody in coronary artery diseases.

Patients and methods: In the current study, two hundred and forty individuals which includes 120 cardiovascular patients (60 males and 60 females) and 120 healthy people as a control group (60 males and 60 females), the age of both groups are (25-86 years range) done in Kirkuk province hospital between and were collected to recognize IgG-cytomegalovirus antibody by utilizing enzyme linked immunosorbent assay.

Results: The mean age ± standard deviation was 53.11±14.606 for (27 cases) have positive CMV-IgG antibody whereas was about 60.08±16.821 for (213 cases) have negative CMV-IgG antibody with P-value = 0.04, statistically. There was a significant difference in age between CMV positive and negative cases. The outcomes appeared the occurrence of CMV-IgG antibody depending on patients residence was mainly urban 79 (65.9%) and the rest were rural 41 (34.1%). The current results also reviewed the incidence of Cytomegalovirus-IgG antibody in patients and healthy groups, among the atherosclerosis patients 26 (21.7%) have CMV-IgG antibody positive, but among the control groups only 1 (0.8%) have CMV-IgG antibody positive. P-value = 0.005, statistically, there was a significant relation between the presence of IgG CMV in cases and control.

Conclusion: In conclusion, the Cytomegalovirus infection is significantly associated with increased risks of coronary artery diseases.

Keywords: Human cytomegalovirus, coronary artery disease, enzyme linked immunosorbent assay

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Introduction

Human cytomegalovirus is a prototype member of the herpesviridae and cytomegalovirus family of the Betaherpesvirinae subfamily. Human herpes virus-5 (HHV-5), salivary gland virus, HCMV or CMV is the commonly known. Mature virion particle measures about 200 nm and is surrounded by a phospholipid surface external membrane envelope, which delimits the tegument that contains important viral replication proteins. Additionally, the virus has a capsid of icosahedral symmetry consisting of 162 capsomeres; This capsid is double-stranded and linear viral DNA genome containing 230 kbp with more than 190 coding areas. The outcome of the infection is mainly seen in large cells and the presence of typical halo-intracellular inclusions, this makes the 'Owl-Eyes' look gives. The virus enters the salivary gland, breast epithelia, prostate, endometrium, renal tubules, and other bodies like the bones marrow and lungs (1,2). The human population has high prevalence of cytomegaloviruses, ranging from 45 to 100 percent (3,4). CMV generally cause asymptomatic infection but it can detect and occasionally reactivate for lifelong persistence in the absence of sufficient cellular immunity to lead to disease (4).

The virus may occur in various secretions of a person infected, such as oropharynx discharge, vagina, semen, urine, semen, milk and blood components. CMV is transmitted through close contact among people through urine, oropharyngeal secretions, tears, semen and breast milk. Children can become infected by CMV from their mothers by intrauterine infection (congenital infection), through contact with infected genital secretions (perinatal infection) while passing through the birth canal, or postpartum by breastfeeding and parenteral (postnatally infected) transplantations of blood, organs or bone marrow transplant s (5,6).

Coronary cardiac atherosclerotic disease, also known as CHD, includes the coronary and other circulations. Atherosclerosis (AS) causes vascular stenosis and/or blockages and ultimately leads to myocardial ischemia, hypoxia and CHD with social loss and even death. The classic risk factors such as high blood lipids, blood pressure, obesity and the diabetes cause the CHD, and the etiology of atherosclerosis is not fully understood. Such traditional risk factors have been reduced successfully in recent years, but the incidence of AS remains high. 30-50% of AS patients do not have these classical risk factors, which mean that their pathogenesis includes certain unknown factors (7).

A variety of infectious pathogens are linked to the involvement of CAD. Many research studies have shown a link between earlier Chlamydia infections, herpes simplex virus, Helicopeter pylori (CMV), and hepatitis virus or respiratory tract infection and the existence of CAD. While other research did not demonstrate such a relationship (7,8,9,10,11).

Cytomegalovirus may play a significant role in atherosclerosis development and in the atherosclerotic plaques may detect Cytomegalovirus DNA. Seropositivity of cytomegalovirus IgG was related to the potential risk of stroke when other risk factors had been adjusted. There is increasing evidence that this virus plays a significant role in vascular pathologies, causing the vessel wall to become slowly yet persistently inflammatory (12).

HCMV replication in vascular ECs is key to the onset, transmission and onset of persistent viral infections and is the straight cause of endothelial dysfunction and apoptosis (13). The viral infection contributes to abnormal metabolism of the vascular ECs and their role. They can increase AS in the general population, by their ability to enter and replicate HCMV in different cell types such as EC, SMCs, fibroblasts and monocytes / macrophages, is an important factor in these conditions. The CMV
CMV can cause both direct and indirect atherosclerosis. The pathogenesis of atherosclerosis by the CMV gene products includes basic mechanisms. Endothelial cell CMV infection causes IE72 and IE84 gene products (15) that activate the COX-2 promoter. This increases nuclear transcription factor-kB (NF-kB) activity by increasing the production of reactive oxygen species (16). Increased NF-kB also mediates the expression on endothelial cell of adherence molecules such as the ICAM₁, VCAM₁, VAP₁ and E-selectin. The increase in adherence molecules favors the adherence to the endothelial cells of single cells / macrophages and contributes to atherosclerosis (17).

Indirect mechanisms are due to cytokine production at distant locations that transmit atherosclerosis through effects other than the direct participation of CMV-gene products. CMV infection increases the alpha-factor of TNF-α (tumor necrosis)(18). Increased titer of proinflammatory cytokines increases the risk of plaque breaking and sensitivity to CAD and the complications of the patient (19). Furthermore, CMV can be used as a prothrombotic agent and increase thrombin production, thereby triggering the coagulation cascade. The increased generation of thrombin worsens the risk of CAD and atherosclerosis (20). The diagnosis of CMV infection can be confirmed by: electron microscopic detection of the virus, histologic or CMV cytopathology detection, tissue and blood CMV antigen detection and amplification of CMV in tissue, DNA amplification and serology (19). Systemic CVM medicines are ganciclovir or valganciclovir, foscarnet and cidofovir, which are approved for treatment (21,22).

The aim of the study is to determine and compare any possible association of human cytomegalovirus with atherosclerosis pathogenesis.

Materials and Methods:
Blood samples obtained from 240 individuals containing 120 (60 males and 60 females) cardiovascular patients and 120 (60 males and 60 females) healthy people in a control group (60 males and 60 females) are aged between 25 and 86 years. Three (3) ml samples of blood were collected by means of a vein puncture with a disposable syringes from each patients & controls; was put in a plain test tube and then separated at room temperature by centrifuge and obtain sera. All sera were frozen at (-20°C) immediately until used. Next, by enzyme linked immunosorbent assay (ELISA), screened for cytomegalovirus antibody (IgG). Age, gender, residence, cardiovascular presentation and other diseases were criteria for selection. Diluted serum specimens (1:100) have been incubated in an antigen-coated well for 20 minutes to permit certain CMV antibodies to bind. CMV specific IgG is detected using rabbit anti-human IgG, conjugated with horseradish peroxidase, after washing off unbound antibodies and other serum constituents. After 20 minutes, the unbound conjugated substance is washed away and the substrate of TMB enzyme is added for 10 minutes. When CMV antibodies are present, it produces a blue color. Addition to the stop solution, the optical density of controls is yellow, and the standard(s) and samples are measured via a microplates reader, optical density is read at 450nm(23).

Interpretation of Result:
Negative samples: OD < 3 IU/ml standard OD
Positive samples: OD >/= 3 IU/ml standard OD

Statistical Analyses
SPSS statistical data package (version21.0 for windows, SPSS, Chicago, IL, USA) was used to perform the statistical analyzes. Data for quantitative variables, number and percentage for qualitative variables are presented as a mean ± SD. Relations with
the Chi-square test have been studied. T-test students were used to test group differences. **P-value <0.05 was considered statistically significant.**

**Results:**

240 cases were studied: 120 patients with cardiovascular diseases and 120 people with control. The patients were between 25-90 years old and 55.6±16.1 years old. Cases of control were 60 ± 16.6 (25-86) years.

Sixty patients and 60 women and 60 male and 60 female control cases had been reported. The residence of patients was predominantly urban 79 (65.9%) and the remainder were rural 41 (34.1%). Forty-two (35%) cases have had other disease, but 78 (65%) have not.

**Table 1: Shows the cardiovascular presentation in patient group**

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple</td>
<td>6</td>
<td>5.0</td>
</tr>
<tr>
<td>Angina</td>
<td>16</td>
<td>13.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>29</td>
<td>24.2</td>
</tr>
<tr>
<td>Heart failure</td>
<td>52</td>
<td>43.3</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>13</td>
<td>10.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>120</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Table(2) observed the occurrence of IgG CMV antibody in coronary artery disease patients and healthy people according to age which demonstrate that 9 (22.0 percent) in age 46-55 have cytomegalovirus IgG seropositivity, whereas 6 (13.3%), 4 (15.4%) occur in age group 56-65, <36 respectively have positive cytomegalovirus IgG antibody. The occurrence of positive antibody was found to be statistically non-significant (Chi square = 8.56, P-value = 0.074) in studied groups according to age.
Table 2: Allocation of IgG CMV presence in examined groups according to age

<table>
<thead>
<tr>
<th>Age</th>
<th>Count</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;36</td>
<td>4</td>
<td>22</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>%</td>
<td>15.4%</td>
<td>84.6%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>36-45</td>
<td>2</td>
<td>27</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>%</td>
<td>6.9%</td>
<td>93.1%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>46-55</td>
<td>9</td>
<td>32</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>%</td>
<td>22.0%</td>
<td>78.0%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>56-65</td>
<td>6</td>
<td>39</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>%</td>
<td>13.3%</td>
<td>86.7%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>66+</td>
<td>6</td>
<td>93</td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>%</td>
<td>6.1%</td>
<td>93.9%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>213</td>
<td></td>
<td>240</td>
</tr>
<tr>
<td>%</td>
<td>11.3%</td>
<td>88.8%</td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table (3) reviewed the incidence of Cytomegalovirus-IgG antibody in patients and healthy groups, among the atherosclerosis patients 26(21.7%) have CMV-IgG antibody positive, but among the control groups only 1(0.8%) have CMV-IgG antibody positive. P value = 0.005, Chi-square test = 26.1. There was a significant relation between the presence of IgG CMV in cases and control. IgG CMV concentration was 0.503 if they were negative to CMV and 0.905 in positive cases. The difference was significant (P = 0.005; t-test = 2.925).

Table 3: Incidence of IgG CMV presence in cases and control

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Count</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMVPatients</td>
<td>26</td>
<td>94</td>
<td></td>
<td>120</td>
</tr>
<tr>
<td>%</td>
<td>21.7%</td>
<td>78.3%</td>
<td></td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27</td>
<td>213</td>
<td></td>
<td>240</td>
</tr>
<tr>
<td>%</td>
<td>11.3%</td>
<td>88.8%</td>
<td></td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table (4) reveals the prevalence of cytomegalovirus-IgG antibody according to gender. 18 (26.5%) females have positive CMV-IgG antibody but only 8 (15.4%) males have positive CMV-IgG antibody. The Chi square = 2.13, P value = 0.144. Statistically no significant relation between positive CMV-IgG antibody and gender.
Discussion:

Cardiovascular diseases are a major cause of death in industrialized societies. Other etiological factors have been suggested in addition to known risk factors like high blood pressure, hypercholesterolemia and diabetes mellitus. In the last three or four decades, many research groups discussed the possibility of a link between different infections and the development of atherosclerosis and especially coronary arteries. Many pathogens, including viruses and Chlamydia pneumoniae and other bacterial agents, have been identified. Cytomegalovirus (CMV) was and still is one of the main candidates for a potential role in atherogenesis.

The presentation of the antigen in the T cells by the macrophages is a crucial step in the formation of atheromate plaque. This antigen can be a fragment of oxidized low-density protein (oxLDL), thermal shock proteins (e.g., \( \beta \)-glycoprotein) or a virus and a bacterial antigen fragment. For the reciprocal interaction of these cells, the presence of a CD40 receptor on the surface and of a CD40 ligand on the surface of T cells is important. Their attachment stimulates both the cellular response (T helper 1–Th1), as well as the humoral response (T helper 2–Th2). The immune response type Th1 was found to facilitate the progression through the mediators of atherosclerosis: interleukins (IL) – IL-1, -6, -12 and -18, tumor necrosis factor alpha (TNF-\( \alpha \)) and interferon gamma (IFN-\( \gamma \)). On the other hand, an immune response Th2 and IL-4, -5, -10 and 13 intermediators are activated to decelerate the inflammatory process. Atherosclerosis is usually found during lipid and foam cell deposition in the internal lamina of the arteries of adults. More subtle lesions in the arterial wall have recently been suggested to play a key role in atherogenesis, as the chemotaxy for inflammatory cells and the activation of cytokine cascade the Low lipoprotein (LDL) aggregation in the sub-endothelial region contributes to the atheromatous plaque. The transformation of a chronic inflammatory disease into ox-LDL is much easier to phagocytize by activated macrophages.

The first association between HCMV and atherosclerosis (AS) was reported by Adam et al, in 1987, and more studies later have found that endothelial cells infected with HCMV are play an role in the development of AS.

The current study nearly compatible with other studied showed that the CMV infection has been further studied and is known to be a possible atherosclerosis and coronary artery disease cause. Another study by Haoran Wang et al showed that a significant increase in the relative risk for CVD was associated with cytomegalovirus infections.

In developing countries, studies such as India, Iran, Saudi Arabia and Turkey showed a link between CMV and CAD development. However, in the United States, the Netherlands, Canada, Belgium and Ireland, studies show no association between CMV and CAD infection. This is consistent with higher seroprevalence of CMV in developing countries and lower seroprevalence in developed countries. Countries such as India, Iran, Saudi Arabia, and Turkey have reported >80% seropositivity of CMV, while European and North American countries have seropositivity ranging from 30% to 55%. However, in Russia, which is a developed country, Nikitskaya et al. have shown positive association between CMV and CAD. This could be due to the high CMV seropositivity in Russia of 80 to 90%. Georges and others have found that CMV and CAD are closely related. The finding is inconsistent with Hoffmeister et al's study.

In another study we found that high serum CMV-IgG antibody titers are correlated with active coronary diseases; In this study the anti-CMV titer in patients was assessed (men and women aged 40–80 years) with risk factors for atherosclerosis.
patients (men and women aged 40–80 years). The statistical difference between the patients and the control group was highly significant \((p<0.0001)\), this result was nearly agreed with current study which found that there was a significant relation between the presence of IgG CMV in cases and control, the difference was significant \((P = 0.005)\).

Namlk K. E's others have shown that the CMV seropositivity prevalence is an independent CAD predictor, in our study population the CAD had a high rate of CMV infection (30).

The study was carried out on 120 patients with coronary angiography for clinical suspicion of CAD who were diagnosed. In this study the CMV-specific IgG antibodies in the serum were detected using ELISA method. CMV colonization in the vessel walls is one of the suggested mechanisms for CMV's presence in atherogenesis. The anti-CMV IgG seropositivity according to the results of the study were 26(21.7%) in coronary artery disease and only one case 0.8% with significant difference was found between the groups with and without CAD; P value = 0.005. This study was almost like a study in South Iran, which documented a high rate of seropositive IgG anti-CMV in CAD patients (27).

We have found a link in this study between CMV and coronary atherosclerosis and this outcomes was nearly compatible with many studies that clarify the role of CMV in the development of coronary arteriosclerosis have yielded controversial results and several authors found whereas others did not. Several epidemiological studies have identified typical risk factors, such as hypercholesterolemia, arterial hypertension, diabetes mellitus, smoking and a family history of vascular diseases. In addition, several studies have found a correlation to certain persistent bacterial and viral pathogens between atherosclerosis (31). In addition, the findings of the Nikolopoulou et al.'s research indicated that the prevalence of CMV anti-IgG in patients with an angiographic CAD stable was 95.5% (32).

Positive and negative associations have been reported between cytomegalovirus (CMV) and coronary artery disease (CAD). The susceptibility to CMV-induced CAD may be linked to patterns of CMV inflammation and immune responses, and sex could influence these reactions. Cytomegalovirus antibodies can be associated with coronary-artery disease development, a virus that is dormant in many adults, and the prevalence of the coronary artery disease was linked directly to high levels of viral antibodies among women. Another Study showed of 45 women with CAD, 40 (89%) had anti-CMV IgG antibodies (33).

The different study findings can explain variations in study design, prevalence of people with chronic CMV infection and regional differences.

Finlay as conclusion, the Cytomegalovirus infection is significantly associated with increased risks of coronary artery diseases.

References:


