Cytomegalovirus Seropositivity and Serum Total Cholesterol Levels in Young Patients

M. Kent Froberg,1 Nicole Seacotte,1 and Emily Dahlberg 2

1 Department of Pathology and Medical Microbiology and Immunology, University of Minnesota-Duluth School of Medicine, Duluth, Minnesota
2 Department of Biological Sciences, Siren High School, Siren, Wisconsin

Abstract. Atherosclerosis is a chronic inflammatory disease of arteries, associated with multiple genetic and environmental factors, including hypertension, diabetes mellitus, cigarette smoking, modified and elevated LDL cholesterol, elevated plasma homocysteine, and infectious microorganisms such as Chlamydia pneumoniae and cytomegalovirus (CMV). CMV has been implicated in atherogenesis by epidemiological studies, animal research, and molecular analyses that have demonstrated CMV nucleic acids within human atherosclerotic lesions. Studies have suggested that CMV infection may alter lipid metabolism and lead to accumulation of cholesterol within atheromatous plaques. Few studies have examined the relationship between CMV infection and serum cholesterol levels in younger individuals when much of atherogenesis occurs. To test if CMV-seropositivity is associated with high levels of serum total cholesterol in relatively young patients, CMV IgG levels and total cholesterol concentrations were analyzed in sera from 172 patients, age <50 yr. Based on univariate analysis of variance, serum total cholesterol was significantly correlated to age and to CMV-seropositivity when gender was a cofactor, but not to gender or CMV-seropositivity alone. In 39 CMV-seropositive women, serum total cholesterol concentration averaged 218 ± 50 mg/dL (mean ± SD), which was significantly higher than in 53 CMV-seronegative women (194 ± 39 mg/dL, p <0.02). No significant difference was observed between the serum total cholesterol concentrations in 26 CMV-seropositive men and 51 CMV-seronegative men (198 ± 42 mg/dL versus 212 ± 48 mg/dl, respectively). Thus, this study provides evidence that CMV-seropositivity is associated with higher serum total cholesterol levels in female patients under 50 yr of age, but not in male patients of comparable age. (received 22 December 2000; accepted 15 February 2001)

Key Words: atherosclerosis, cytomegalovirus, serum cholesterol

Introduction

Atherosclerosis is the most common form of cardiovascular disease in the United States and is the leading cause of preventable death in the western world. Atherosclerosis is believed to be a chronic inflammatory response to endothelial injury, which may be multifactorial and includes infection by microorganisms (eg, CMV) [1,2]. Herpsevirus infection as a possible etiology of atherogenesis was first suggested when an avian herpesvirus named Marek’s disease virus (MDV) was shown to cause atherosclerotic lesions in the aortas of infected chickens [3,4]. MDV infection also alters lipid metabolism and leads to accumulation of cholesterol and cholesterol esters within the aortas of infected chickens [5].

In vitro studies have demonstrated increased incorporation of cholesterol in CMV-infected human saphenous vein smooth muscle cells, compared to mock infected cells [6]. Most epidemiological studies have shown an association between CMV seropositivity and...
atherosclerosis [7-10]. Studies of human atherosclerotic lesions have identified CMV antigens and nucleic acids within plaque tissues [11-14]. In at least one study, the presence of CMV in lesions was associated with more severe atherosclerosis [14]. CMV infection accelerates restenosis of coronary arteries following angioplasty [15] and enhances de novo atherosclerosis of grafted vessels following cardiac transplantation [16]. Studies have indicated that CMV infection can accelerate the formation of atherosclerotic lesions in animal models of atherogenesis [17,18] and that formation of atherosclerotic lesions in the animals is enhanced by a high-fat diet.

These studies collectively suggest that herpesvirus infection may alter cholesterol metabolism, perhaps by incorporating neutral lipids into atheromatous lesions, and might be associated with higher blood cholesterol levels. Few studies have examined the relationship between serum cholesterol levels and CMV exposure and none have examined this relationship in younger patients. This is significant because atherosclerosis is believed to start as fatty streaks that begin to appear in the arterial wall during childhood [1,2]. To determine if CMV seropositivity is associated with higher levels of serum total cholesterol in younger patients, we determined CMV seropositivity in a group of patients <50 yr old, in whom serum total cholesterol levels were known.

**Materials and Methods**

This study was approved by the Internal Review Board of the University of Minnesota-Duluth School of Medicine. Waste serum samples from 172 patients were obtained from the clinical laboratory of Saint Mary’s Duluth Clinic in Duluth, MN. The patients were all <50 yr old. Their serum samples had been analyzed for total cholesterol concentration by an enzymatic method, using a “LX20” automated analyzer (Beckman Coulter, Inc., Brea, CA). Aliquots of the serum samples (1 ml) were stored at -20°C until they were analyzed for CMV antibody. To preserve confidentiality, the only data available for each serum sample were the serum total cholesterol concentration, the patient’s sex, and (in 89 cases) the exact age.

CMV IgG antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Biotest, Denville, NJ). On two consecutive days, batches of sera were rapidly thawed and assayed in batches according to the manufacturer’s protocol. Briefly, the diluted serum samples were incubated in microwells coated with CMV antigen (20 min, 25°C), washed to remove unbound antibody and serum components, incubated with peroxidase-conjugated goat anti-human IgG (20 min, 25°C), washed to remove unreacted conjugate, and incubated with 3,3'5,5'-tetramethylbenzidine (10–12 min, 25°C). The color reaction was terminated by addition of “stop solution” and color intensity was read at 450 nm, using a microplate reader (Molecular Devices, Inc., Sunnyvale, CA). The optical density (OD) of the solution in each microwell was measured against the reagent blank.

Positive high, low, and negative serum controls were included on each microwell plate and were used to establish the mean OD of the low positive control; this value was used with the manufacturer’s correction factor to compute an OD ratio (the OD of each sample divided by the mean OD of the low positive control). Samples with OD ratios ≥1.10 were classified as seropositive for CMV; samples with OD ratios ≤0.90 were classified as seronegative for CMV; and samples with OD ratios from 0.91-1.09 were classified as equivocal for CMV.

Cholesterol concentrations in serum samples that were either seropositive or seronegative for CMV were compared using Student’s t test (Statview software, Abacus Concepts, Inc., Berkeley, CA). For a subgroup of 89 patients for whom exact age was available, univariate analysis of variance was performed (SPSS software, Version 10, SPSS, Inc., Chicago, IL) to factor for age differences in the test subjects. Data were considered significant at the \( p < 0.05 \) level.

**Results**

The CMV IgG OD ratios were determined for serum samples from a total of 172 patients (93 women, 79 men) who were <50 yr old. Sixty-five samples (37.8%) were seropositive for CMV; 104 samples (60.5%) were seronegative; 3 samples (1.7%) were equivocal.

The data for CMV serology status and serum cholesterol concentrations are listed in Table 1. In 39 CMV-seropositive women, serum total cholesterol concentration averaged 218 ± 50 mg/dL (mean ± SD),
which was significantly higher than in 53 CMV-seronegative women (194 ± 39 mg/dL, p <0.02). In 26 CMV-seropositive men, serum total cholesterol concentration averaged 198 ± 42 mg/dL (mean ± SD), which was not significantly different from 51 CMV-seronegative men (212 ± 48 mg/dL).

Exact date of birth was available for 89 patients; their ages ranged from 15 to 49 yr (mean, 38.3 yr). Data for serum cholesterol concentrations and CMV seropositivity in this subgroup (Table 2) were similar to the entire group. In this subgroup, univariate analysis of variance showed that serum total cholesterol level was associated with age (F = 4.67; p = 0.034), and with CMV-status with gender as a cofactor (F = 6.77, p = 0.011), but not with gender alone (F = 1.41, p = 0.24), or with CMV status alone (F = 0.020, p = 0.89).

Discussion

During the past two decades, epidemiological, molecular, and animal studies have implicated CMV infection in atherogenesis. CMV is an ubiquitous herpesvirus; persons are often exposed to CMV during childhood, when atherogenic lesions may first develop. CMV establishes a latent lifetime infection that may be reactivated by changed immune status. Since CMV infects endothelial cells and monocytes (ie, cells critical to atherogenesis), CMV is well situated to influence atherogenesis [19]. In vitro studies indicate that CMV infection increases the expression of cytokines, chemokines, and inflammatory cell adhesion molecules that are involved in atherogenesis [20-22]. A possible connection of CMV infection and altered lipid metabolism also suggests a role for CMV infection in atherogenesis. Murine CMV infection accelerates atherogenesis in aortas of apolipoprotein E knockout mice that have cholesterol levels approximately 5x normal [17] and in immunosuppressed BALB/c mice [18]. Interestingly, MCMV-infected BALB/c mice have significantly higher mean concentrations of serum total cholesterol than uninfected mice, when both groups are fed a high-fat diet [18].

Few epidemiological studies have tried to correlate serum lipid levels with CMV infection. Some authors have examined CMV seropositivity and serum total cholesterol as risk factors for cardiovascular disease, but they did not determine whether CMV seropositivity and serum lipid levels were statistically related [23,24].

Table 1. Relationship of CMV serology and serum total cholesterol concentration in 169 patients <50 yr old.a

<table>
<thead>
<tr>
<th>Gender &amp; CMV-status</th>
<th>No. of subjects</th>
<th>Serum total cholesterol (mg/dL; mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seronegative</td>
<td>53</td>
<td>194 ± 39</td>
</tr>
<tr>
<td>CMV-seropositive</td>
<td>39</td>
<td>218 ± 50b</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seronegative</td>
<td>51</td>
<td>212 ± 48</td>
</tr>
<tr>
<td>CMV-seropositive</td>
<td>26</td>
<td>198 ± 42c</td>
</tr>
<tr>
<td>Women and Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seronegative</td>
<td>104</td>
<td>203 ± 44</td>
</tr>
<tr>
<td>CMV-seropositive</td>
<td>65</td>
<td>210 ± 48d</td>
</tr>
</tbody>
</table>

a Excluding 3 patients with equivocal CMV-antibody titers.  
b p = 0.012 versus CMV-seronegative women by t test.  
c p = 0.21 vs CMV-seronegative men by t test.  
d p = 0.33 vs CMV-seronegative men and women by t test.

Table 2. Relationship of CMV serology and serum total cholesterol concentration in the subgroup of 88 patients for whom exact ages were available.a

<table>
<thead>
<tr>
<th>Gender &amp; CMV-status</th>
<th>No. of subjects</th>
<th>Mean age (yr)</th>
<th>Serum total cholesterol, mean ± SD (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seronegative</td>
<td>29</td>
<td>38</td>
<td>189 ± 39</td>
</tr>
<tr>
<td>CMV-seropositive</td>
<td>18</td>
<td>41</td>
<td>215 ± 43</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seronegative</td>
<td>30</td>
<td>37</td>
<td>201 ± 46</td>
</tr>
<tr>
<td>CMV-seropositive</td>
<td>11</td>
<td>38</td>
<td>176 ± 29</td>
</tr>
<tr>
<td>Women and Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seronegative</td>
<td>59</td>
<td>37.5</td>
<td>194 ± 43</td>
</tr>
<tr>
<td>CMV-seropositive</td>
<td>28</td>
<td>39.8</td>
<td>200 ± 42</td>
</tr>
</tbody>
</table>

a Univariate analysis of variance showed that serum total cholesterol level was associated with age (F = 4.67; p = 0.034), and with CMV-status with gender as a cofactor (F = 6.77, p = 0.011), but not with gender alone (F = 1.41, p = 0.24), or with CMV status alone (F = 0.020, p = 0.89).
Adam et al [10] analyzed CMV-seropositivity in relation to serum total cholesterol and triglyceride concentrations in patients requiring vascular surgery for atherosclerosis. No correlations were observed between CMV-seropositivity and serum lipid levels, but the patients were all male and their mean age was 58 yr. Whether the patients received lipid lowering drugs or dietary treatment is not mentioned. Nieto et al [9] did not find any correlation between serum cholesterol levels and CMV antibody levels in patients with atherosclerosis, but cholesterol levels were simply categorized as ≥240 or <240 mg/dL, and patients’ ages were 45-64 yr at the start of this longitudinal study.

Previous studies have not searched for a possible connection between serum total cholesterol levels and CMV-seropositivity in younger patients, during the period when much of atherogenesis is known to develop. In the present study, CMV-seropositivity and serum total cholesterol levels were determined in patients who were <50 yr old, with mean age about 20 yr less than in previous studies. Serum cholesterol levels are known to increase with age, and this was true in our study. It is unclear why CMV-seropositivity was associated with higher serum cholesterol levels in women, even when adjusted for age, but not in men. Studies with larger numbers of patients of both sexes are needed to confirm or refute our findings. The presence of atherosclerotic disease was not evaluated in this study, nor were other risk factors for atherosclerosis investigated. The finding of higher serum cholesterol levels in CMV-seropositive women may be associated with genetic and environmental factors, including other infectious agents.

The finding of higher cholesterol levels in CMV-seropositive females could be clinically significant. Since CMV infection and elevated serum cholesterol levels may both accelerate atherogenesis, CMV-seropositive individuals with higher serum cholesterol levels could develop more severe cardiovascular disease. This is consistent with the findings of Hendrix et al [14], who showed by PCR analysis that 90% of severe (grade III) human atherosclerotic lesions contained CMV nucleic acids, while only 53% of low-grade lesions (grade I) were CMV-positive in age- and sex-matched controls.

In summary, this study provides evidence that CMV-seropositivity is associated with higher serum total cholesterol levels in female patients under 50 yr of age, but not in male patients of comparable age.

Acknowledgements

The authors are grateful to Tom Nelson, M.D., and the laboratory staff of Saint Mary’s Duluth Clinic for providing the serum samples and to Ms. Kate Beattie for performing the statistical analyses. This study was supported in part by a financial contribution from Siren High School, Siren, Wisconsin.

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