Enterovirus Infections as a Possible Risk Factor for Myocardial Infarction

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Background—An increasing body of evidence suggests that, in addition to the well-known classic risk factors, some microbial infections may be associated with the development of atherosclerosis and myocardial infarction (MI). The aim of our study was to evaluate the possible role of enteroviral infections in the pathogenesis of MI.

Methods and Results—Stored sera, collected in Eastern Finland in 1977, from a set of 12 155 randomly selected men and women aged 25 to 64 years were used in prospective, nested case-control study. The study sample comprised 183 men and 81 women with MI and matched controls. The sera were tested for IgG antibodies to a newly identified enterovirus-common (EVC) antigen, to heat-denatured coxsackievirus B5 (CBV-5), and to adenovirus hexon protein. Raw data from enzyme immunoassays were converted to relative units before analysis. In univariate analysis, EVC antibodies were significantly associated with the risk of MI in men (P=0.009) but not in women. Men with MI had a significantly higher mean level of EVC antibodies than matched controls (P=0.014). High antibody levels to EVC were associated with an increased risk of MI in men aged 25 to 49 years (relative risk [RR] 4.34, P<0.001) but not in older men (>50 years of age). Women with MI also showed a trend toward higher antibody levels than control women, but the difference was not statistically significant. Antibody levels to whole CBV-5 or adenovirus hexon protein appeared to be no different among case patients versus control subjects.

Conclusions—If we assume that a high level of EVC antibodies reflects a history of relatively frequent enterovirus infections, the present observation might suggest that enterovirus infections increase the risk of MI at least in middle-aged men. Further studies are needed to understand possible clinical significance of this observation. (Circulation. 1998;98:2534-2537.)

Key Words: viruses ■ myocardial infarction ■ follow-up studies

The pathogenesis of atherosclerosis is a complex multifactorial process. In addition to the well known risk factors, such as high serum cholesterol, smoking, and hypertension, reactions of the immune system may also be involved in the process of atherosclerosis. Atherosclerotic lesions bear many features characteristic of inflammatory conditions, the most striking of which is the accumulation of macrophages and activated T-lymphocytes. According to the current view, these lymphocytes are more than bystanders during atherogenesis, although the antigens responsible for their activation have not been identified. A rising body of epidemiological evidence suggests that at least some microbial infections are associated with atherosclerosis and myocardial infarction (MI). Furthermore, acute respiratory symptoms with fever often precede myocardial infarction. The agent related to respiratory infections most often associated with coronary heart disease (CHD) is Chlamydia pneumoniae. There is also evidence suggesting that some viruses (eg, herpes simplex virus and cytomegalovirus) are involved in the pathogenesis of atherosclerosis and MI. The association of coxackie B viruses (CBV), members of the enterovirus genus, with acute and chronic myocarditis in humans is well-known. In some studies, concomitant increases in antibodies to CBVs were seen at diagnosis of MI, suggesting the association of infections due to this virus group with MI as well. However, the association was not seen in some other studies. We have used a newly identified enterovirus group antigen and carried out a prospective seroepidemiological analysis on the possible association of enterovirus infections with a risk of MI.

Methods

Subjects
Using a nested case-control study design, the subjects were identified from a population-based random sample of 12 155 men and women between 25 and 64 years of age; they were from 2 provinces in Eastern Finland and had been examined in 1977 in connection with the North Karelia Project. The survey procedures have been described in detail elsewhere. Serum total cholesterol, systolic blood pressure values, data on smoking habits, diabetes, and family history of CHD were recorded in 1977. Serum cholesterol was determined...
TABLE 1. Characteristics and Classic Risk Factors of Male and Female Cases and Controls Taken From our Analysis

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Cases</th>
<th>P (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>228</td>
<td>183</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>52.6±7.2</td>
<td>52.5±7.1</td>
<td>0.962</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>6.83±1.18</td>
<td>7.20±1.36</td>
<td>0.004</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>148.9±20.9</td>
<td>152.9±19.3</td>
<td>0.044</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.5±3.7</td>
<td>27.1±3.9</td>
<td>0.164</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>47.1±50</td>
<td>62.5±49</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>4.8±21.5</td>
<td>6.0±23.8</td>
<td>0.596</td>
</tr>
<tr>
<td>Positive family history of CHD, %</td>
<td>13.2±33.9</td>
<td>29.0±45.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>151</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>56.6±7.1</td>
<td>57.0±6.8</td>
<td>0.694</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>7.12±1.34</td>
<td>7.62±1.41</td>
<td>0.008</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>157.9±25.7</td>
<td>164.0±24.3</td>
<td>0.076</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3±4.8</td>
<td>28.3±5.3</td>
<td>0.983</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>4.0±19.7</td>
<td>13.8±24.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>2.6±16.1</td>
<td>6.2±24.2</td>
<td>0.187</td>
</tr>
<tr>
<td>Positive family history of CHD, %</td>
<td>17.2±37.9</td>
<td>25.9±44.1</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Values are mean±SD. BMI indicates body mass index.

using the Lieberman-Burchard reaction, and blood pressure was measured in sitting position after 5 minutes’ rest. Data on smoking, diabetes, and family history of CHD were obtained by questionnaire. Positive family history of CHD was defined as MI of angina pectoris before the age of 60 in either parent. People with previous history of CHD were excluded from the present study. New cases of MI were identified during a 9-year follow-up and 183 male and 81 female case patients with MI were included in our analysis (Table 1). Control subjects, at least one for each case patient, were randomly chosen from the same cohort and matched according to sex for 5-year age strata. They remained free of MI at the end of the follow-up. The number of control subjects in this study was 379 (228 men and 151 women).

**Solid-Phase Assay for Virus Specific Antibodies**

High-binding EIA plates (Nunc, Maxisorp) were coated by overnight incubation with denatured CBV-5 (0.06 μg/mL), purified hexon of adenovirus type 5 (0.2 μg/mL), or synthetic peptide derived from an immunodominant region of capsid protein VP1 (amino acid sequence with single-letter codes: KEVPALTAVETGAT-C; 5 μg/mL), known to be a common antigenic determinant for most enteroviruses. The wells were blocked with BSA, and sera diluted 1:1000 in EIA buffer (PBS with 1% BSA, 0.1% Tween 20, 1% FCS) were added to duplicate wells for each antigen to be tested. After 2 hours’ incubation at 37°C, the plates were washed and the reaction was visualized by horseradish peroxidase (HRP)–conjugated antihuman IgG (Medix Biochemica, Finland). The antibody-positive control serum was analyzed in all plates in each assay; the results obtained with the test sera were expressed as relative units in relation to the standard sample.

**Statistical Analysis**

Standard t test and χ² test were used to analyze the differences of classic risk factor levels and means (and log-means) of the measured viral antibodies between case patients and control subjects. The association of antibodies with the risk of MI was analyzed using conditional logistic regression models. Antibodies were used as a continuous and a dichotomized variable with the sex-specific median as a cut point because the interpretation of the actual antibody level in this context is difficult. All analyses were adjusted for smoking, serum cholesterol, blood pressure, body mass index, diabetes, and family history of CHD.

**Results**

In men, levels of enterovirus-specific antibodies (P=0.014) were 23% higher in MI case patients than in control subjects, whereas antibody levels to adenovirus hexon and heat-treated CBV-5 were almost similar in both groups (Table 2). The results remained practically similar when log transformants were tested (data not shown). In female MI case patients, enterovirus-specific antibodies were also 21% higher than in the control subjects, but the difference did not reach the level of statistic significance. Interestingly, in the female MI case patients, antibody levels to heat-treated CBV-5 were significantly lower than in the control subjects.

Life-table analysis in men revealed that MI case patients with high (above the median) enterovirus antibody levels manifested with fatal or nonfatal MI significantly faster than those having low (below the median) antibody levels (P=0.03, χ² test; data not shown). The difference was progressively divergent during the 9-year follow-up.

In univariate analysis, using enterovirus-specific antibodies as a continuous variable, there was a positive association with the risk of MI that was statistically significant in men (RR 1.03 per antibody unit, P=0.009) but not in women (RR 1.01, P=0.189). In all men, with adjustment for other risk factors in the multivariate model, the risk ratio for enterovirus antibodies was no longer significant (RR 1.08, P=0.153).

A significant interaction was seen between levels of enterovirus-specific antibodies and age (P<0.001). Therefore, the male subjects were divided into 2 different age groups (Table 3). The risk ratio for developing MI with high levels of enterovirus-specific antibodies (over the median) was statistically significant only in the younger men (aged 25 to 49 years) at baseline (RR 4.34, P<0.001). In older men such an association was not seen. The risk ratios actually decreased with age and became <1.0 after 55 years of age (data not shown). Statistically significant interactions were
also seen between EVC antibodies and systolic blood pressure ($P=0.003$).

In the multivariate analysis adjusted for total serum cholesterol, systolic blood pressure, smoking, body mass index, diabetes, and positive family history of CHD, the association of a high level of enterovirus-specific antibodies to MI remained significant (RR 3.45, $P=0.008$) in younger men. This indicates that the effect was independent of the classic coronary risk factors (Table 3).

**Discussion**

In this Finnish prospective seroepidemiological study, high levels of enterovirus antibodies were found to be associated with a risk of MI in men. Among women, MI case patients showed a trend toward higher EVC antibody levels, but the difference was not statistically significant. This might be due to the small number of case patients, especially in the age group under 50 years (only 14 case patients). In general, antibody levels to antigens tested were lower in women than in men. Antibody levels to different viruses cannot be, however, directly compared with each other because they were expressed as relative units in relation to virus-specific control sera used in each assay. Our present findings are also in accord with the results showing that the frequency and severity of symptoms of respiratory infections are independently related to the risk of MI.$^4$

Relative risk of MI by high levels of enterovirus-specific antibodies depended on age; the risk was the highest in middle-aged men. Multivariate analysis adjusted for the classic risk factors showed that a high enterovirus antibody level was an independent risk factor for MI. In addition to age, statistically significant interactions were seen between EVC antibody levels and systolic blood pressure and smoking. In earlier studies, an association has been reported between smoking and *C pneumoniae* antibodies.$^{18,23}$

Enteroviruses are a large group of pathogenic viruses (coxsackieviruses, echoviruses, polioviruses, new enteroviruses) associated with a wide range of clinical syndromes but also frequently cause short-term infections with little or no clinical symptoms. The enterovirus antibody assay used was a recently developed enzyme immunoassay$^{24,25}$ using as antigen a synthetic peptide derived from a highly antigenic region of capsid protein VP1.$^{26}$ It has been successfully used as a group antigen in serological diagnosis of enterovirus infections.$^{24,25}$ EVC antibody levels accumulate by age, showing a peak between 40 and 50 years; this is followed by a rapid decrease (Figure 1). We speculate that, at the population level, high EVC antibody levels might reflect a history of relatively frequent enterovirus infections. If this is true, frequent enterovirus infections might be considered to increase the risk for MI. Alternatively, unknown genetic factors that increase the risk for MI might also enhance extent of antibody response to EVC. A further possibility is that higher antibody levels are based on cross-reactions between EVC and host-cell proteins.$^{26}$ Autoantibodies to various antigens are known to occur in patients prone to MI.$^{27}$

The question of why the significant difference in antibody levels between MI case patients and control subjects was seen with EVC but not with heat-treated CBV-5 cannot be answered by our study. In human sera there have been a mixture of complex serological responses of polyclonal character owing to previous infections with several enterovirus serotypes. Perhaps the peptide representing the common epitope (EVC) was a more suitable antigen than the heated CBV-5 virions for recording cumulative enterovirus group–specific antibody levels. The entire CBV-5 virion, even in its heated form, is known to contain several epitopes, some of them serotype-specific. Different kinetics of antibodies targeted to different epitopes may dilute out the cumulative response to the shared epitopes.

![Levels of EVC antibodies by age in normal population. One hundred randomly selected sera in each age group of healthy people were tested for EVC antibodies. Raw data from enzyme immunoassays were converted to relative units before analysis.](image-url)
In this study, the temporal sequence between the infection and MI was clearly demonstrated. It was remarkable that curves showing the accumulation of MI cases in the high- and low–EVC antibody groups diverged progressively from the beginning of the 9-year follow-up. This suggests that the risk factor presenting with high EVC antibody levels is of a permanent nature rather than resulting from a short-term event such as a specific outbreak of enterovirus infections. In earlier studies on chlamydia infections, those that have shown positive results have been either case-control studies or studies in which the high-antibody titers have been found only a few months before the onset of acute MI. Thus the sequence of the events has remained unclear.

Although our results demonstrate that high levels of enterovirus-specific antibodies are associated with MI, further studies are needed to evaluate whether and with which mechanism enterovirus infections are involved in the pathogenesis of atherosclerosis and the development of MI.

Acknowledgments

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References

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