The Association Between Infection Burden in Iranian Patients with Acute Myocardial Infarction and Unstable Angina


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ABSTRACT

Aim: to evaluate the association of ischemic heart disease (IHD) with the number of pathogens (infection burden) among individuals with infection.

Methods: a total of 120 patients with IHD as the acute myocardial infarction (AMI; n=60) or unstable angina (UA; n=60) group and 60 healthy subjects with sex- and age-matched as control group were enrolled in this study. Serum samples of all participants were tested for the presence of antibodies to Helicobacter pylori (H. pylori), cytomegalovirus (CMV), type-1 herpes simplex virus (HSV-1) and type-2 HSV (HSV-2) by using ELISA.

Results: Regarding the association of the infection burden with IHD, the prevalence ratios and 95% confidence intervals (CI) were 3.18 (CI: 1.50-6.72; P<0.001) for 3 seropositivities and 3.83 (CI: 0.84-17.43; P<0.05) for 4 seropositivities. The rate of subjects with high infection burden (≥3 seropositivities) was significantly higher in IHD group as compared to control group (53.4% vs 21.6%; P<0.01). Moreover, the mean number of seropositivities was also significantly higher in patients with IHD in comparison to control group (2.47 vs 1.68; P<0.01). The seroprevalence of anti-H. pylori antibodies in AMI and UA groups was significantly higher compared to control group (P<0.0001). The seroprevalence of anti-CMV antibodies in AMI and UA group was also significantly higher than those observed in control group (P<0.01). Moreover, the seroprevalence of anti-HSV-1 antibodies was significantly higher in AMI and UA groups in comparison to control group (P<0.001). The seroprevalence of anti-HSV-2 antibodies was similarly expressed in patients and healthy control group.

Conclusion: the infection burden was significantly higher in patients with IHD, which represent that the parameter should also be considered as an independent risk factor for development of IHD. The seroprevalence of H. pylori, CMV and HSV-1 were also higher in patients with IHD.

Key words: ischemic heart disease, pathogen burden, H. pylori, CMV, HSV-1, HSV-2.

INTRODUCTION

It has been suggested that inflammation and infection have important roles in the pathogenesis of IHD. Accumulation of leukocytes including monocytes/macrophages, T cells, B cells and PMN cells has been demonstrated in atherosclerotic lesions. Moreover, an association between IHD and some infectious agents including Chlamydia pneumoniae, H. pylori, HSV, CMV, hepatitis A, respiratory tract and dental infections has been reported in some epidemiological studies. However, the results of some prospective studies failed to demonstrate strong relationship between the presence of antibodies to C. pneumoniae, HSV-1, H pylori and CMV and the occurrence of coronary arterial disease.

Some investigators have estimated antibody response to more than one infectious agent in patients with cardiovascular disease. However, if a single infectious agent has an etiologic role for the development of cardiovascular disease, it seems that the risk of cardiovascular disease is more related to the number of pathogens, rather than a single pathogen, to which a person has been exposed. This idea was specified as pathogen burden and primarily introduced by Epstein. Presence of one infection might increase the risk of other infections or increase deleterious effects of other pathogens or both. For example, it has been suggested that combined infection with HSV and periodontal bacteria may increase the risk of cardiovascular disease through decreasing levels of high-density lipoprotein cholesterol more than each pathogen alone. Moreover, it has been demonstrated that the number of infectious agents in infected individuals was associated to the occurrence of coronary artery disease and the risk of future cardiac events. The present study was conducted for the first...
time to evaluate the number of pathogens (pathogen burden) including H. pylori, CMV, HSV-1, HSV-2 in Iranian patients with IHD to clarify any association.

**METHODS**

This descriptive cross-sectional study has been conducted between March 2007 and January 2008. A total of 120 patients (aged 40-60 years) with IHD who were admitted to Ali-ebne-Abitaleb Hospital of Rafsanjan (a city that located in Kerman province in south-east of Iran) were enrolled to study. Patients were classified into 2 groups according to well-established criteria, as having AMI (n=60) or UA (n=60). AMI was diagnosed according to the presence of two of following criteria: 1) prolonged chest pain compatible with AMI; 2) typical ECG changes; 3) increased level of cardiac enzymes. UA was defined according to the Braunwald’s classification and all patients had chest pain at rest with definite ischemic electrocardiographic changes such as ST-segment changes and/or T-wave inversion. UA patients were in class III B according to Braunwald’s classification. For randomization purposes, patients were randomly included in the study according to their admission registration number in the Cardiac Care Unit of hospital. Exclusion criteria were valvular heart disease, surgery, trauma within the prior months, cardiomyopathy, liver disease, renal failure, malignant diseases, other inflammatory disease (such as septicemia and pneumonia) and oral anticoagulant therapy. The blood samples were collected from patients during the first 3 days after admission. A third sex- and age-matched group (n=60) with similar geographic and socioeconomic background without any IHD were registered as a control group. The healthy subjects were selected among blood donors and interviewed with respect to cardiovascular symptoms. None of the healthy subjects had any history of cardiovascular or any other related diseases. Moreover, individuals with medical history of pulmonary disease, gastrointestinal diseases, diabetes mellitus, hypertension, renal failure, anemia, asthma and neoplasia were all excluded from the study. Peripheral blood (2-4 milliliter) was collected from all participants and the serum of samples were separated and stored at –20°C.

This study had been evaluated and approved by the Ethical Committee of Rafsanjan University of Medical Sciences. Moreover, patients were recruited if they agreed for blood sampling.

**Determination of immunoglobulin G (IgG) to H. pylori, CMV, HSV-1 and HSV-2**

The serum levels of anti-H. pylori antibodies were measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits (Trinity Biotec, Ireland). Antibodies against CMV, HSV-1 and HSV-2 were also measured by commercial ELISA kits (Euroimmun, Lübeck, Germany).

**Statistical Analysis**

Differences in variables were analyzed using t-test, ANOVA and Chi-square tests as appropriate and the P < 0.05 were considered significant. All the available data were analyzed by a computer program (SPSS version 18, Chicago, IL, USA).

**RESULTS**

Baseline characteristics of subjects: Baseline characteristics of AMI, UA and healthy control groups are shown on **Table 1**. The patients and control groups were similar in age and men/women ratio, but the prevalence of classical risk factors of IHD (hypertension, dyslipidemia, diabetes and smoking) was significantly higher in patients compared with control group.

**Table 1. Baseline characteristics of patients and control groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>AMI (n=60)</th>
<th>UA (n=60)</th>
<th>Control (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SEM)</td>
<td>54.6 ± 9.7</td>
<td>55.8 ± 9.6</td>
<td>52.98 ± 8.73</td>
</tr>
<tr>
<td>Sex (Men/Women)</td>
<td>39/21</td>
<td>35/25</td>
<td>33/27</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Dyslipidemia*</td>
<td>10</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes mellitus*</td>
<td>7</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Current smoking*</td>
<td>14</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

* Represent the differences between total patients with IHD and healthy control group.

Infection burden: We defined pathogen burden by considering seropositivity to all 4 pathogens, including H. pylori, CMV, HSV-1, and HSV-2. The prevalence ratios for the association of particular infection and the total infection burden (number of seropositivities) with
The prevalence ratios with 95% confidence intervals (CI) were 0.31 (CI: 0.15-0.64; P<0.001) for 1 seropositivity; 0.92 (CI: 0.46-1.82; P<0.47) for 2 seropositivities; 3.18 (CI: 1.50-6.72; P<0.001) for 3 seropositivities; and 3.83 (CI: 0.84-17.43; P<0.05) for 4 seropositivities. Accordingly, there is a clear shift of infection burden toward higher scores in patients with IHD. The rates of subjects with a high pathogen burden (≥3 seropositivities) was significantly higher in IHD group as compared with healthy group (2.47 vs 1.68; P<0.01). Similar results were obtained when AMI or UA groups compared to control group (Table 3 and Figure 1). Moreover, the mean number of seropositivities was also significantly higher in IHD group as compared with healthy group (2.47 vs 1.68; P<0.01). Similar results were obtained when AMI or UA groups compared to control group (Table 3). Infection burden and the mean number of seropositivities were similarly expressed in AMI and UA groups (Table 3 and Figure 1).

Anti-H. pylori IgG seropositivity: The overall seroprevalence of anti-H. pylori IgG was 89.2% among total patients with IHD and 58.3% among healthy control subjects (Table 2). The seropositivity rate of anti-H. pylori antibody was 86.7% and 91.7% in AMI and UA groups, respectively. The seroprevalences of anti-H. pylori antibodies in AMI group, UA group and total patients with IHD (AMI plus UA) were significantly higher compared with control group (P<0.0001). The seroprevalence of anti-H. pylori was similar in patients with AMI and UA (Figure 2).

Anti-CMV, anti-HSV-1 and anti-HSV-2 seropositivity: The overall seroprevalences of anti-CMV and anti-HSV-1 antibodies were 69.2% and 60.8% in patients with IHD and 48.3% and 33.3% in control group, respectively (Table 2). Moreover, the seroprevalence of anti-CMV and anti-HSV-1 antibodies were 68.3% and 61.7% in AMI group and 70% and 60% in UA group, respectively (Figure 2). Statistical analysis showed that the seroprevalences of anti-CMV and anti-HSV-1 antibodies in patients group were significantly higher than those observed in healthy control group (P<0.01 and P<0.001, respectively). However, the seroprevalence of anti-HSV-2 was similarly expressed in patients and healthy groups (Figure 2). No significant difference was also observed between AMI and UA groups regarding the seroprevalence of anti-CMV, anti-HSV-1 and anti-HSV-2 antibodies.

### Table 2. The prevalence ratios for the association of the particular infection and total infection burden (number of seropositivities) with IHD

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Patients (n=120)</th>
<th>Control (n=60)</th>
<th>Prevalence ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori</td>
<td>89.2%</td>
<td>58.3%</td>
<td>5.87 (2.71-12.71)</td>
<td>0.0001</td>
</tr>
<tr>
<td>CMV</td>
<td>69.2%</td>
<td>48.3%</td>
<td>2.39 (1.26-4.53)</td>
<td>0.01</td>
</tr>
<tr>
<td>HSV-1</td>
<td>60.8%</td>
<td>33.3%</td>
<td>3.10 (1.62-5.95)</td>
<td>0.001</td>
</tr>
<tr>
<td>HSV-2</td>
<td>28.3%</td>
<td>28.3%</td>
<td>1.00 (0.50-1.98)</td>
<td>1.00</td>
</tr>
<tr>
<td>1 seropositivity</td>
<td>17.5%</td>
<td>40%</td>
<td>0.31 (0.15-0.64)</td>
<td>0.001</td>
</tr>
<tr>
<td>2 seroposities</td>
<td>28.3%</td>
<td>30%</td>
<td>0.92 (0.46-1.82)</td>
<td>0.47</td>
</tr>
<tr>
<td>3 seroposities</td>
<td>41.7%</td>
<td>18.3%</td>
<td>3.18 (1.50-6.72)</td>
<td>0.001</td>
</tr>
<tr>
<td>4 seroposities</td>
<td>11.7%</td>
<td>3.3%</td>
<td>3.83 (0.84-17.43)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

### Table 3. Comparison of infection burden in patients with ischemic heart disease and healthy control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Infection burden</th>
<th>Mean number of seropositivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>AMI</td>
<td>60</td>
<td>12 (20%)</td>
<td>16 (26.7%)</td>
</tr>
<tr>
<td>UA</td>
<td>60</td>
<td>9 (15%)</td>
<td>18 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>21 (17.5%)</td>
<td>34 (28.3%)</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>5</td>
<td>24 (40%)</td>
</tr>
</tbody>
</table>
The results of the present study showed that the 53.4% of overall patients with IHD (51.7% of AMI group and 55% of UA group) have been infected by 3 pathogens, in comparison to 21.6% of control group. Moreover, the mean number of positive IgG responses to multiple pathogens was significantly higher in patients as compared with healthy control. In one study from Germany the number of antibodies against several organisms (Chlamydia pneumoniae, Helicobacter pylori, Mycoplasma pneumoniae, Haemophilus influenzae, CMV, HSV-1 and 2, and Epstein Barr Virus) has been associated with advanced atherosclerosis and carotid plaque progression over time. Another study demonstrated that the prevalence of coronary artery disease was also associated with the number of positive serology tests against common infections (Chlamydia pneumoniae, CMV, hepatitis A virus, and HSV-1 & 2). The prevalence of coronary artery disease was 48% in subjects with antibodies to ≤ 2 pathogens; 69% in those with antibodies to 3 or 4 pathogens; and 85% in those with antibodies to 5 pathogens. Antibody titers to five common infectious agents (including Chlamydia pneumoniae, H. pylori, CMV, HSV-1 & 2) were also associated with carotid plaque thickness. The association of pathogen burden with cardiovascular diseases has been also reported in other studies. These observations represent that the pathogen burden may act as an independent risk factor for the development of IHD.

Several potential mechanisms have been reported to explain how infections may participate in cardiovascular disease. However, multiple simultaneous infectious agents may have synergistic effects on the promotion of cardiovascular disease. Inflammatory processes are central components for the development of acute coronary syndromes. The infectious agents can induce several of the inflammatory mechanisms which contribute to cardiovascular events. It has been recently demonstrated a significant association between the high antibody response to multiple pathogens (including CMV, HSV-1, Hepatitis A virus, H. pylori and Chlamydia pneumoniae) with several inflammatory parameters such as interleukin (IL)-6, C-reactive protein (CRP) and fibrinogen. There is increasing evidence that CRP, a marker of inflammation, is an independent risk factor for cardiovascular disease and is a valuable tool for assessing at-risk populations. In our previous study, higher serum levels of high sensitivity (hs)-CRP were observed in patients with IHD as compared with healthy subjects. Furthermore, it has been reported that certain organisms can directly invade cells in the cardiovascular system, causing local and systemic inflammatory reactions. Induction of inflammatory cytokines is another possible mechanism by which the pathogen burden may contribute to the pathogenesis of IHD. Indeed, higher levels of some cytokines such as TNF-α, IL-6, IL-8, IL-12, IL-17 and IL-18 have been demonstrated in patients with IHD. Pathogen burden may also lead to the expression of heat shock protein (HSP)-60 on the arterial cell surface and it seems that an immune cross-reaction may occur between human and bacterial HSP-60 which in turn leads to an autoimmune reaction and local inflammation of the artery. Higher levels of antibodies
to C. pneumonia HSP-60 have been reported in patients with IHD, which is consistent with this hypothesis.24

The results of the present study showed that seroprevalence of anti-H. pylori IgG was significantly higher in patients with IHD as compared with the control group. The possible mechanisms by which H. pylori infection could increase the risk of IHD have been proposed as induction of dyslipidemia, increasing levels of fibrinogen, induction of CRP production, increasing the blood leukocyte count and homocysteine, induction of hypercoagulability, induction of immune cross-reactivity and causation of impaired endothelial function.27-30

Regarding CMV infection, the results of the present study showed that the seroprevalence of anti-CMV IgG was significantly higher in patients with AMI or UA as compared with the healthy subjects. Some sero-epidemiological studies have also demonstrated a positive correlation between anti-CMV antibodies and cardiovascular disease.5,7,31 Infecion of arterial tissue by CMV, CMV-associated oxLDL uptake, CMV-related up-regulation of PDGF receptors on smooth muscle cell (and thereby the enhancement of smooth muscle cell migration and proliferation in atherosclerotic lesions), CMV-induced up-regulation of adhesion molecules on infected endothelial cells and CMV-induced cytokine production such as IL-12, IL-18, TNF-α and IFN-γ by smooth muscle cells, macrophages and endothelial cells may be account for the involvement of CMV in cardiovascular disease.31 It has been also demonstrated that the interaction of envelope proteins of CMV with toll-like receptors in atherosclerotic lesions followed by the production of pro-inflammatory cytokines and plaque development.31,32 Furthermore, the presence of antiphospholipid antibodies in mice (following immunization with a CMV-derived peptide) and increased levels of antiphospholipid antibodies in patients with accelerated atherosclerosis have been demonstrated, which provide further evidence for autoimmune processes in the aggravation of atherosclerotic process by CMV.33,34 Moreover, antibodies against some CMV proteins have been shown to cross-react with HSP-60 and bind to non-stressed endothelial cells; thereby inducing apoptosis.33,34

The results of this study also showed an association between anti-HSV-1 seropositivity and IHD. Although the association of anti-HSV-1 antibodies with coronary heart disease had been reported in some investigations7,9, this association was not observed in several other studies.35 However, one hypothesis for the association of HSV-1 and coronary heart events may include the potential reactivation of HSV-1 in autonomic nerves that innervate the coronary arteries, subsequent endothelial injury and the initiation of an acute thrombotic event. However, our findings showed the absence of association between anti-HSV-2 antibodies and IHD.

It should be noted that the results of some studies are controversial regarding the association of infection burden with cardiovascular disease. In a study among patients with coronary disease, the number of antibodies against 8 common organisms was not associated with the degree of atherosclerosis, CRP levels or the risk of major adverse coronary events.36 Moreover, another study indicated that the total number of antibodies against C. pneumoniae, H. pylori, CMV, HSV, and hepatitis A virus was not associated with subclinical estimation of atherosclerosis, including intimal-medial thickness and coronary calcification.37 The differences in the study design or in the population background, such as the prevalence of other risk factors may be account for the differences. The race and ethnicity of participants should be also considered to explain some discrepancies of results from different studies. It seems that individuals with a more strong inflammatory response to infectious agents (perhaps due to gene polymorphisms of inflammatory factors and immune response genes) are also more likely to show vascular alterations related to infection. The gene polymorphisms of inflammatory factors such as IL-6 and TNF-α have been associated with coronary artery disease.38 Genetic background may also influence the infectious burden-host interaction and susceptibility to certain infection. Some human leukocytes antigens (HLA) have been presented as risk factors to certain infection. HLA-B35, for example, has been associated with Chlamydia pneumoniae-mediated coronary artery disease.39 Furthermore, toll-like receptor-4 (TLR-4) is an important component of the innate immune response. It has been reported that a particular gene polymorphism in TLR4, which decreases the inflammatory response to gram-negative pathogens, was associated with decreased carotid atherosclerosis and intima-media thickness.40

It should be also noted that infection burden may be an indicator of the burden of other risk factors that are the direct contributors to cardiovascular diseases. The patients with multiple medical problems, with certain genetic background or those with behavioral risk factors such as smoking may be the same subjects with a high load of common infections. Furthermore, it seems that the interplay interaction between host factors (such as behavioral and genetic factors) and infections may influence cardiovascular diseases.
CONCLUSION

The present study shows that the number of infectious pathogens, to which an individual has been exposed, was associated with IHD. These observations represent the pathogen burden as an independent risk factor for IHD. It is noticeable that infectious burden may be a target of intervention in controlling the cardiovascular diseases. These results also show that infection by H pylori, CMV and HSV-1 were significantly associated with IHD.

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