The prevalence of infectious agents in patients with systemic sclerosis

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1. Introduction
The relationship between infection and autoimmunity has been increasingly defined in recent years. Bacterial, viral, and parasitic infections are known to induce and exacerbate autoimmune diseases (1,2). Systemic sclerosis (SSc) is an autoimmune disease characterized by microvascular injury, excessive extracellular matrix deposition, and fibrosis of the skin and internal organs. Bacterial and viral infectious agents have been suspected to be contributing factors in the development and progression of the pathologic features of SSc.

2. Materials and methods
2.1. Groups of patients
Thirty SSc patients who were admitted to the rheumatology unit of the Konya Training and Research Hospital between April 2009 and March 2012 and 30 sex- and age-matched healthy controls were included. The presence of 9 different antibodies (IgM and IgG) against Helicobacter pylori, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and parvovirus B19 were investigated in sera samples obtained from the 60 participants using an enzyme-linked immunosorbent assay method. The characteristics of current and past infections with H. pylori, CMV, EBV, and parvovirus B19 were evaluated by determining the seropositivity of the tested bacterial and viral agents.

Results: The prevalences of H. pylori, CMV, EBV, and parvovirus B19 were determined to be higher in patients with SSc than in the control group.

Conclusion: SSc is associated with a higher rate of certain infections, which deserves further investigation in order to assess the role of infections in disease etiology/pathogenesis.

Key words: Systemic sclerosis, infection, etiology

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Background/aim: Systemic sclerosis (SSc) is an autoimmune disease characterized by microvascular injury, excessive extracellular matrix deposition, and fibrosis in the skin and internal organs. Bacterial and viral infectious agents have been suspected to be contributing factors in the development and progression of the pathologic features of SSc.

Materials and methods: In this study, 30 SSc patients who were admitted to the rheumatology unit of the Konya Training and Research Hospital and 30 healthy controls were included. The presence of 9 different antibodies (IgM and IgG) against Helicobacter pylori, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and parvovirus B19 were investigated in sera samples obtained from the 60 participants using an enzyme-linked immunosorbent assay method. The characteristics of current and past infections with H. pylori, CMV, EBV, and parvovirus B19 were evaluated by determining the seropositivity of the tested bacterial and viral agents.
The following agents were used for the detection of antibodies: anti-CMV (IgM and IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), EBV EBNA-1 (IgM) (IBL International GmbH, Hamburg Germany), EBV EBNA-1 (IgG) (EUROIMMUN Medizinische Labordiagnostika AG), EBV-CA (IgM) (EUROIMMUN Medizinische Labordiagnostika AG), anti-\(H.\) pylori (IgM and IgG) (EUROIMMUN Medizinische Labordiagnostika AG), and antiparvovirus B19 (IgM and IgG) (EUROIMMUN Medizinische Labordiagnostika AG). All tests were evaluated by using a microplate reader (Biotek ELx 800, BioTek Instruments, Inc., Winooski, VT, USA). Interference from rheumatoid factor was avoided by the addition of sheep IgG to the sample dilution buffer. The participants underwent comprehensive examinations and laboratory evaluations, including full blood count, erythrocyte sedimentation rate, and renal and liver functions.

A power analysis was conducted after recruitment. Using an \(\alpha\) level of 0.05, \(\beta\) level of 0.20, and sample size of 30, power was found as 70.7% for the study. Therefore, the sample size of \(n = 30\) may be sufficient. SSc is a rare disease with an incidence rate of 2.3–10 per million (5). Hence, the maximum amount of patients had been detected to be included in the study.

2.2.1. ELISA measurements
Patient samples were diluted 1:101 with a sample buffer (10 \(\mu\)L of serum in 1.0 mL of sample buffer), and photometric measurements were made at wavelengths of 450 nm and 630 nm for all the tests that were included in the study.

2.2.2. Anti-CMV IgM assay
The measurement unit was a ratio (extinction of the control or patient samples/extinction of calibrator 2). Results were evaluated as follows: <0.80 ratio was negative, \(\geq 0.80\) to <1.1 ratio was borderline, and \(\geq 1.1\) ratio was positive. The detection limit of the test was 0.01 ratio, intraassay CVs were 3.3%–5.8%, and interassay CVs were 5.5%–6.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.3. Anti-CMV IgG assay
The measurement unit was the COI. Results were evaluated as follows: <0.8 COI was negative, 0.8–1.2 COI was equivocal, and >1.2 COI was positive. Intraassay CV was 7.8%, and interassay CV was 12.1%. Specificity and sensitivity rates of the test were >95% for each.

2.2.4. EBV EBNA-1 IgM assay
The measurement unit was the cut-off index (COI). Results were evaluated as follows: <0.8 COI was negative, 0.8–1.2 COI was equivocal, and >1.2 COI was positive. Intraassay CV was 4.2%–5.4%, and interassay CV was 5.7%–6.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.5. EBV EBNA-1 IgG assay
The measurement unit was RU/mL. Results were evaluated as follows: <16 RU/mL was negative, \(\geq 16\) to <22 RU/mL was borderline, and \(\geq 22\) RU/mL was positive. The linearity of the test was 12–126 RU/mL, the detection limit of the test was 0.9 RU/mL, intraassay CVs were 4.8%–8.9%, and interassay CVs were 8.4%–10.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.6. EBV CA IgM assay
The measurement unit was a ratio (extinction of the control or patient samples/extinction of calibrator 2). Results were evaluated as follows: <0.80 ratio was negative, \(\geq 0.80\) to <1.1 ratio was borderline, and \(\geq 1.1\) ratio was positive. The detection limit of the test was a 0.01 ratio, intraassay CVs were 3.3%–5.8%, and interassay CVs were 5.5%–6.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.7. EBV CA IgG assay
The measurement unit was RU/mL. Results were evaluated as follows: <16 RU/mL was negative, \(\geq 16\) to <22 RU/mL was borderline, and \(\geq 22\) RU/mL was positive. The linearity of the test was 2–200 RU/mL, the detection limit of the test was 1 RU/mL, intraassay CVs were 4.2%–7.4%, and interassay CVs were 3.2%–8.2%. Specificity and sensitivity rates of the test were 100% for each.

2.2.8. Anti-\(H.\) pylori IgM assay
The measurement unit was the COI. Results were evaluated as follows: <0.8 COI was negative, 0.8–1.2 COI was equivocal, and >1.2 COI was positive. Intraassay CV was 8.1%, and interassay CV was 11.3%. Specificity and sensitivity rates of the test were >95% for each.

2.2.9. Anti-\(H.\) pylori IgG assay
The measurement unit was RU/mL. Results were evaluated as follows: <16 RU/mL was negative, \(\geq 16\) to <22 RU/mL was borderline, and \(\geq 22\) RU/mL was positive. Detection limit of the test was 0.7 RU/mL, intraassay CVs were 3.1%–3.2%, and interassay CVs were 3.4%–4.6%. Specificity and sensitivity rates of the test were 100% for each.

2.2.10. Antiparvovirus B19 IgM assay
The measurement unit was a ratio (extinction of the control or patient samples/extinction of calibrator 2). Results were evaluated as follows: <0.80 ratio was negative, \(\geq 0.80\) to <1.1 ratio was borderline, and \(\geq 1.1\) ratio was positive. The detection limit of the test was a 0.01 ratio, intraassay CVs were 3.3%–5.8%, and interassay CVs were 5.5%–6.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.11. Antiparvovirus B19 IgG assay
The measurement unit was international units (IU/mL). Results were evaluated as follows: <4 IU/mL was negative, \(\geq 4\) to <8 IU/mL was borderline, and \(\geq 8\) IU/mL was positive. The linearity of the test was 2–200 IU/mL, the detection limit of the test was 0.05 ratio, intraassay CVs were 3.3%–5.8%, and interassay CVs were 5.5%–6.9%. Specificity and sensitivity rates of the test were 100% for each.

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≥4 to <5.5 IU/mL was borderline, and ≥5.5 IU/mL was positive. The linearity of the test was 1–100 IU/mL. The detection limit of the test was 0.1 IU/mL, intraassay CVs were 2.5%–7.7%, and interassay CVs were 1.6%–6.0%. Specificity and sensitivity rates of the test were 100% for each.

2.3. Ethical approval
Ethical approval was provided by the Ethics Committee of Meram Medical School, Selçuk University (Konya, Turkey) (ref: 2011/259). The patients provided both verbal and written consent prior to their participation.

2.4. Statistical analysis
The results of tests were expressed as the number of observations (n), mean ± standard deviation, median, and min–max values. The results of the homogeneity (Levene test) and normality tests (Shapiro–Wilk) were used to decide which statistical methods to apply in the comparison of the study groups. Normally distributed groups and groups with homogeneous variances were compared using the Student t-test. According to those tests results, parametric test assumptions were not available for some variables, so the comparisons of 2 independent groups were performed by Mann–Whitney U test. Categorical data was analyzed with the Fischer exact test and chi-square test. P < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results
The mean age of the subjects was 35 ± 15.25 years (19 females and 11 males, age range 7–60) for the SSc patients and 37 ± 16.35 years (18 females and 12 males, age range 8–59) for the control group. Table 1 summarizes the demographic characteristics of the study population.

In our population, 73.3% of SSc patients had H. pylori antibodies (IgM and/or IgG), compared with 46.6% of the control group (P < 0.05). Anti-CMV antibodies (IgM and/or IgG) were detected in 93.3% of SSc patients, compared with 73.3% of the control group (P < 0.05). EBV antibodies (IgM and/or IgG) were detected in 93.3% of SSc patients, compared with 66.6% of the control group (P < 0.05). Parvovirus B19 antibodies (IgM and/or IgG) were detected in 86.6% of SSc patients, compared with 63.3% of the control group (P < 0.05). Table 2 shows the prevalence of antibodies against these infections in patients with SSc and patients in the control group.

There was no statistically significant difference between the limited SSc and diffuse SSc patients for H. pylori, CMV, EBV and parvovirus B19 infections. The prevalence of recent infections (IgM-positive but IgG-negative) was similar between the SSc patients and controls.

4. Discussion
Infections can trigger autoimmune diseases. This fact is well established in the literature. SSc is among the less investigated autoimmune diseases, and not much information exists on the disease pathogenesis and triggers (6–8). In the current study, we investigated the relationship between past and recent infections and the etiology of SSc.

Many agents have been postulated as being involved in the cause of the disease. Bacterial and viral infectious agents have been suspected to be contributing factors in the development and progression of SSc. The rationale for this infection hypothesis is that many SSc-like symptoms are transiently elicited by infectious agents in otherwise healthy individuals. Some researchers have suggested that the production of specific autoantibodies in SSc patients is the result of an antigen-driven response caused by molecular mimicry. Molecular mimicry is a mechanism of autoimmune disease that may explain the pathogenicity of antibodies against viral proteins in SSc patients (9). Self-reactive antibodies against a virus may induce endothelial cell apoptosis through the interaction with the integrin α3β1 and α6β1 NAG-proteins complex (9). Endothelial injury represents one of the first steps in the pathogenesis of SSc. Endothelial cells may be infected by bacteria or viruses that may be instrumental in inducing vasculitis (10).

Attempts to establish a link between the H. pylori infection status and SSc have yielded conflicting results. One study identified higher incidence rates of H. pylori

### Table 1. Demographic characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SSc</th>
<th>Controls</th>
<th>P-value Limited+diffuse SSc vs. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>18</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Female/male</td>
<td>1.57</td>
<td>2</td>
<td>1.72</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>34 ± 16.25</td>
<td>36 ± 13.45</td>
<td>35 ± 15.25</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>2.7</td>
<td>3.1</td>
<td>2.9</td>
</tr>
</tbody>
</table>

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infection in patients with rheumatic diseases, including SSc, as detected by serologic analysis (11). In contrast, 2 larger studies discovered there was no difference in the *H. pylori* infection rates between patients with SSc with Raynaud phenomenon and healthy controls (12,13). A recent study (14) indicated that, although there is no difference in the *H. pylori* infection rate between control subjects and patients with SSc, 90% of patients with SSc were infected with the virulent CagA strain compared with 37% of infected control subjects. In a study of patients with primary Raynaud phenomenon, the eradication of *H. pylori* infections was associated with the complete disappearance of the episodes of Raynaud phenomenon in 17% of treated patients and a reduction in symptoms in an additional 72% (15). In the present study the prevalence of *H. pylori* antibodies (both IgM and IgG) was significantly higher among SSc patients than in controls. CMV may play a role in SSc pathogenesis due to its ability to infect both endothelial and monocyte/macrophage cells, and through the upregulation of fibrogenic cytokines and the induction of immune dysregulation (16,17). CMV, as a possible trigger for SSc, has been suggested by the reported appearance of SSc shortly after acute episodes of viral infections (18). The most direct evidence of a link between CMV and SSc is the presence of high-titer IgG antibodies against the polyglycine motifs of CMV (19). In addition, antitopoisomerase I (Scl-70) autoantibodies, which are characteristic of diffuse SSc, can cross-react with a peptide sequence of the UL70 protein of CMV (20). One difficulty in establishing a clear association between CMV and SSc is the fact that 60% to 90% of adults display serologic evidence of past CMV infection, yet SSc affects at most 3 in 10,000 people. We discovered that the prevalence of anti-CMV antibodies (both IgM and IgG) is higher among SSc patients than in controls.

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**Table 2.** Prevalence of antibodies against infections in patients with systemic sclerosis and control groups.

<table>
<thead>
<tr>
<th>Prevalence of <em>H. pylori</em> antibodies, % of patients</th>
<th>SSc (30)</th>
<th>Controls (30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM+, IgG-</td>
<td>5.5</td>
<td>3.3</td>
<td>P = 1.0</td>
</tr>
<tr>
<td>IgM+, IgG+</td>
<td>16.6</td>
<td>10</td>
<td>P = 0.706</td>
</tr>
<tr>
<td>IgM-, IgG+</td>
<td>50</td>
<td>33.3</td>
<td>P = 0.190</td>
</tr>
<tr>
<td>IgM+ and/or IgG+</td>
<td>72.2</td>
<td>46.6</td>
<td>P = 0.035</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prevalence of CMV antibodies, % of patients</th>
<th>SSc (30)</th>
<th>Controls (30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM+, IgG-</td>
<td>0</td>
<td>3.3</td>
<td>P = 1.0</td>
</tr>
<tr>
<td>IgM+, IgG+</td>
<td>22.2</td>
<td>13.3</td>
<td>P = 0.731</td>
</tr>
<tr>
<td>IgM-, IgG+</td>
<td>72.2</td>
<td>56.6</td>
<td>P = 0.100</td>
</tr>
<tr>
<td>IgM+ and/or IgG+</td>
<td>94.4</td>
<td>73.3</td>
<td>P = 0.038</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prevalence of EBV antibodies, % of patients</th>
<th>SSc (30)</th>
<th>Controls (30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM+, IgG-</td>
<td>5.5</td>
<td>6.6</td>
<td>P = 1.0</td>
</tr>
<tr>
<td>IgM+, IgG+</td>
<td>11.1</td>
<td>6.6</td>
<td>P = 0.671</td>
</tr>
<tr>
<td>IgM-, IgG+</td>
<td>72.2</td>
<td>56.6</td>
<td>P = 0.100</td>
</tr>
<tr>
<td>IgM+ and/or IgG+</td>
<td>94.4</td>
<td>66.6</td>
<td>P = 0.010</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prevalence of parvovirus B19 antibodies, % of patients</th>
<th>SSc (30)</th>
<th>Controls (30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM+, IgG-</td>
<td>5.5</td>
<td>0</td>
<td>P = 1.0</td>
</tr>
<tr>
<td>IgM+, IgG+</td>
<td>5.5</td>
<td>3.3</td>
<td>P = 1.0</td>
</tr>
<tr>
<td>IgM-, IgG+</td>
<td>72.2</td>
<td>60</td>
<td>P = 0.273</td>
</tr>
<tr>
<td>IgM+ and/or IgG+</td>
<td>88.8</td>
<td>63.3</td>
<td>P = 0.037</td>
</tr>
</tbody>
</table>
EBV DNA has been detected in the lungs of SSc patients (21). It has been observed that an antibody against hnRNP autoantigen called p542 may develop in mononucleosis, and IgG anti-p542 has been discovered in SSc (22). In these patients, autoantibodies against a 60–62-kDa cellular protein, which cross-reacts with the glycine/alanine repeat in EBNA-1, may have been detected (19). Farina et al. (23) showed that SSc fibroblasts have greatly diminished IFN-inducible gene response upon TLR7/9 agonist stimulation. This is possibly one of the reasons why EBV is able to infect and persist in SSc fibroblasts. They suggested that EBV infection could contribute to fibrosis in SSc skin. In the current study, the prevalence of EBV antibodies (both IgM and IgG) was significantly higher among SSc patients than in controls.

Parvovirus B19 has been proposed as a causative agent in rheumatoid disease and other vascular injury syndromes, including Wegener granulomatosis (24), microscopic polyarteritis nodosa (25), Henoch–Schönlein purpura (26), and dermatomyositis (27) in SSc. A direct correlation between the extent of degenerative endothelial cell alterations and the degree of B19RNA expression suggested a causal role of B19 in the propagation of endothelial cell dysfunction (28). A possible role for parvovirus B19 infection in the evolution of SSc is suggested by the presence of B19 at a high percentage (57%) in bone marrow biopsies from unselected SSc patients in the absence of B19 viremia (29). It has been suggested that bone marrow may represent a reservoir from which the B19 virus could spread to SSc target tissues (30). We detected a significantly higher prevalence of parvovirus B19 antibodies (both IgM and IgG) in SSc patients than in controls.

We discovered a higher prevalence of infectious agents in SSc patients than in healthy controls. SSc likely occurs as a result of interactions between the infectious agent and a cascade of host-specific factors and events. This is not surprising, because the immune response to infection is highly individual. It is controlled by multiple genes, age, and the route of infection. In a disease as varied, complex, and rare as SSc, infection prevalence alone should not be expected to provide sufficient evidence for or against a pathological role in the disease. Therefore, additional factors such as coinfections, differences in the strains of infectious agents, and immunological and genetic host factors will have to be further identified and controlled to understand the role of infectious agents in SSc.

Our study had several limitations. First, the relationship between exposure time to the viral infections and clinical onset of the disease was not determined. Second, the study group was small owing to the rarity of the disorder. This condition is estimated to occur in 2.3–10 people per 1 million (5). Third, the impact of environmental factors in the onset of SSc was not analyzed.

References


