Comparative Analysis of Autoantibodies Against α-Fodrin in Serum, Tear Fluid, and Saliva from Patients with Sjögren’s Syndrome

SULE YAVUZ, EBRU TOKER, MUGE BICAKCIGIL, GONCA MUMCU, and SEZEN CAKIR

ABSTRACT. Objective. To evaluate levels of IgA and IgG antibodies against α-fodrin in serum, tear fluid, and saliva and compare them with anti-Ro and anti-La antibody levels in the same samples of patients with Sjögren’s syndrome (SS).

Methods. Samples from 25 patients with SS (17 primary and 8 secondary), 8 patients with systemic lupus erythematosus (SLE), and 7 patients with rheumatoid arthritis (RA) as well as 20 healthy blood donor controls were collected. Antibodies were measured using ELISA.

Results. Although 40% of patients with primary SS had IgG anti-α-fodrin in their sera, it was also found in 36% and 32% of samples of their tear fluid and saliva, respectively. IgA α-fodrin antibodies were detected in 32% of SS sera, 20% of tear fluid samples, and 32% of saliva samples. Although the level of IgG anti-α-fodrin was significantly greater in serum, tear fluid, and saliva of SS patients compared to controls (p < 0.001), a significant difference was observed only in serum and saliva. While anti-Ro was detected in 48%, 56%, and 24% of serum, tear fluid, and saliva samples, respectively, anti-La was found in 40%, 44%, and 28%. Significant association was observed between serum IgG antibodies against α-fodrin and dry eye symptom score and rose bengal staining score. A negative association was also noted between tear IgA antibodies against α-fodrin and Schirmer I test.

Conclusion. Correlation of IgG and IgA antibodies against α-fodrin with the severity of eye involvement suggests that these autoantibodies may be considered activation markers of SS. (J Rheumatol 2006;33:1289–92)

Key Indexing Terms:
SJOGREN’S SYNDROME ANTI-α-FODRIN ANTIBODIES

Sjögren’s syndrome (SS) is one of the most common systemic autoimmune diseases in rheumatology clinics, characterized by a progressive lymphocytic and plasma cell infiltration of exocrine glands. Although the presence of several autoantibodies in blood such as antinuclear antibodies, rheumatoid factor, and anti-Ro(SSA) and anti-La(SSB) is well known, none of these is specific for SS. In the absence of a gold standard, diagnosis of SS is based on criteria comprising a number of clinical, serological, functional, and morphological variables. Recently, Haneji, et al reported a 120 kDa cleavage product of α-fodrin as a candidate autoantigen, and IgG antibodies against α-fodrin have been reported to be highly sensitive and specific markers for diagnosis of SS.

Studies have been done to search for IgA antibodies against α-fodrin in serum, tear fluid, and saliva from patients with SS, that frequency has not been reached in subsequent studies. Because IgA autoantibodies are locally produced in salivary glands of patients with SS, additional studies have been done to search for IgA antibodies against α-fodrin. In these studies, the prevalence of IgA antibodies against α-fodrin in serum varied by 43% to 64% in patients with primary SS.

We have reported that antibodies against Ro(SSA) and La(SSB) antigens were found in tear fluid despite their absence in the sera of patients with SS. Therefore, it would make sense to screen for these antibodies in the sites where they might be locally produced. We analyzed IgG and IgA antibodies against α-fodrin as well as anti-Ro(SSA) and anti-La(SSB) antibodies in serum, tear, and saliva samples from patients with SS.

MATERIALS AND METHODS

Patients. Serum, tear fluid, and saliva samples of 17 patients with primary SS and 8 patients with secondary SS diagnosed according to revised European/US criteria were studied along with the serum, tear, and saliva samples of 15 patients with other connective tissue disorders, 8 with systemic lupus erythematosus (SLE) and 7 with rheumatoid arthritis (RA), and 20 healthy controls.

All patients and controls were evaluated at the Department of Rheumatology and Ophthalmology Outpatient Clinics in the University of...
Statistical analysis. All statistical analysis was performed using SPSS software, version 11.0. Differences in autoantibody levels were determined by Fisher’s exact test. Associations between variables were analyzed using Spearman’s rank-correlation test.

RESULTS
In sera, tear fluid, and saliva samples from 20 healthy controls, the mean ± SD concentrations of IgA and IgG antibodies against α-fodrin were 20.4 ± 19.2, 8.6 ± 6.2, 3.1 ± 1.3 U/ml and 58.7 ± 13.7, 1.6 ± 1.4, and 1.6 ± 0.9 U/ml, respectively. Similarly, the mean ± SD concentrations of anti-Ro/SSA and anti-La/SSB antibodies in serum, saliva, and tears from healthy subjects for each evaluation.

Anti-La/SSB and anti-Ro/SSA autoantibodies were also measured by ELISA according to the manufacturer’s protocol (Genesis Diagnostics, Cambridgeshire, UK). Results greater than the mean ± 2 SD of anti-Ro/SSA and anti-La/SSB levels in healthy donors were defined as positive.

Statistical analysis. All statistical analysis was performed using SPSS software, version 11.0. Differences in autoantibody levels were determined by one-way ANOVA. Frequencies of autoantibodies between groups were compared using Fisher’s exact test. Associations between variables were analyzed by Spearman’s rank-correlation test.

Table 1. Frequency of IgG and IgA autoantibodies against alpha-fodrin in patients with SS and controls. Disease controls include patients with SLE and RA.

<table>
<thead>
<tr>
<th></th>
<th>SS Patients</th>
<th>All, n = 25 (%)</th>
<th>Disease Controls, n = 15 (%)</th>
<th>Healthy Controls, n = 20 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha-fodrin IgG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>7 (41)*</td>
<td>10 (40)*</td>
<td>3 (20)</td>
<td>—</td>
</tr>
<tr>
<td>Saliva</td>
<td>5 (29)*</td>
<td>8 (32)*</td>
<td>2 (13.3)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Tear</td>
<td>6 (35)*</td>
<td>9 (36)*</td>
<td>4 (26.7)</td>
<td>1 (5)</td>
</tr>
<tr>
<td><strong>Alpha-fodrin IgA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>7 (41)*</td>
<td>8 (32)*</td>
<td>1 (6)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Saliva</td>
<td>6 (35)*</td>
<td>5 (33)*†</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>Tear</td>
<td>4 (23.5)</td>
<td>5 (20)</td>
<td>1 (6)</td>
<td>2 (10)</td>
</tr>
</tbody>
</table>

* Significant differences between patients with SS and controls (p < 0.02). † Significant differences between disease controls and healthy controls (p < 0.02).
frequency of anti-Ro/SSA and anti-La/SSB antibodies compared to IgG antibodies against \( \alpha \)-fodrin in serum, tear, and saliva samples, both anti-Ro/SSA and anti-La/SSB antibodies were found in significantly greater frequency compared to IgA antibodies against \( \alpha \)-fodrin both in serum (p < 0.0001 vs p < 0.02) and in tear (p < 0.01 vs p < 0.01) samples of patients with SS.

There was a negative correlation between both unstimulated and stimulated salivary flow rate and serum IgG antibodies against \( \alpha \)-fodrin in SS patients (r = –0.6, p < 0.05 and r = –0.7, p < 0.03, unstimulated and stimulated, respectively). Significant correlations were observed between serum levels of IgG antibodies against \( \alpha \)-fodrin and patients’ eye symptoms (Figure 1) as well as rose bengal staining (r = 0.6, p < 0.01, for both). A negative correlation was noted between Schirmer I test and tear levels of IgA antibodies against \( \alpha \)-fodrin (r = –0.56, p < 0.01; Figure 2).

**DISCUSSION**

We detected IgA and IgG antibodies against \( \alpha \)-fodrin in similar frequency to anti-Ro/SSA and anti-La/SSB in the serum of patients with SS. Based on reported data, it is still controversial whether anti-fodrin antibodies should replace the classic anti-Ro and anti-La antibodies in the diagnosis of SS. Initially, Haneji, et al. suggested the presence of anti-\( \alpha \)-fodrin antibodies was a highly specific marker for SS, with a frequency of 92% in sera; however, subsequent studies showed much lower sensitivity and specificity for SS. Witte and colleagues showed that even focusing on IgA antibodies against \( \alpha \)-fodrin does not add sensitivity or specificity to diagnosis of SS. In our study, patients with SS showed a frequency of 40% and 32% for IgG and IgA antibodies against \( \alpha \)-fodrin, respectively; these are comparable to the percentages reported by Witte, et al.2 as well as Zandbelt and coworkers. Previous studies have shown that several IgA antibodies are produced in salivary glands of SS patients. Salivary and lacrimal glands are the major target tissues in SS; therefore, local \( \alpha \)-fodrin autoantibody measurement might be helpful in the diagnosis of SS. To address this issue, tear fluid and saliva IgG and IgA autoantibodies against \( \alpha \)-fodrin were analyzed and compared with the frequency of anti-Ro/SSA and anti-

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**Table 2.** Frequency of anti-Ro/SSA and anti-La/SSB antibodies in patients with SS and controls.

<table>
<thead>
<tr>
<th></th>
<th>Primary, n = 17 (%)</th>
<th>Secondary, n = 8 (%)</th>
<th>All, n = 25 (%)</th>
<th>Disease Controls, n = 15 (%)</th>
<th>Healthy Controls, n = 20 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-SSA/Ro</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>12 (48)*</td>
<td>—</td>
<td>12 (48)*†</td>
<td>1 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Saliva</td>
<td>4 (23.5)</td>
<td>2 (25)</td>
<td>6 (24)</td>
<td>1 (6)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Tear</td>
<td>10 (58.8)*</td>
<td>4 (50)*</td>
<td>14 (56)*</td>
<td>4 (26.7)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Anti-SSB/La</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>8 (47)*†</td>
<td>2 (25)*</td>
<td>10 (40)*</td>
<td>1 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Saliva</td>
<td>7 (41)*†</td>
<td>—</td>
<td>7 (28)*†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tear</td>
<td>9 (52.9)*†</td>
<td>2 (25)</td>
<td>11 (44)*†</td>
<td>2 (13)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

* Significant differences between patients with SS and healthy controls (p < 0.001). † Significant differences between patients with SS and disease controls (p < 0.04) as well as healthy controls (p < 0.005).
La/SSB antibodies both in patients with SS and in controls. While there was no significant difference in autoantibody prevalence in saliva, both anti-Ro/SSA and anti-La/SSB were found to be more prevalent in tears compared to IgA antibodies against α-fodrin in SS patients. On the other hand, serum levels of IgG antibodies against α-fodrin and salivary flow rate, rose bengal staining, and patient’s dry eye symptoms seemed to be well correlated. Additionally, a negative correlation was observed between tear level of IgA anti-α-fodrin and Schirmer I test. These results suggest it may be more relevant to consider IgG and IgA anti-α-fodrin as activation markers of SS.

All patients with SS except one were receiving hydroxychloroquine treatment at the time of sample collection. Although there is no controlled study, hydroxychloroquine is known for lowering immunoglobulin levels and erythrocyte sedimentation rate in patients with SS11. However, the frequency of autoantibodies against α-fodrin detected in this study was comparable with those of previous studies2,7, suggesting that hydroxychloroquine treatment is less likely to reduce the concentration of antibodies in serum. Recently, it has been shown that lymphocytic infiltration of salivary glands is decreased after hydroxychloroquine treatment12,13. Because the concentration of anti-α-fodrin is correlated to the degree of lymphocytic infiltration in the salivary glands, treatment with hydroxychloroquine may cause the lower prevalence of anti-α-fodrin as seen in saliva samples.

A remarkable finding in inflamed salivary glands is that there are many plasma cells that appear to be producing autoantibodies and rheumatoid factor within the gland, whereas the remaining B cells, which comprise about 10% of lymphocytes, are naive or memory B cells14,15. Ro and La antigens are found in all nucleated cells, as is α-fodrin, and they are expressed on the surface of apoptotic cells14,16. During increased apoptosis of salivary epithelium, it is possible that an immune response to these antigens may contribute to the disease pathogenesis. Anti-dsDNA antibodies disappear after immunosuppressive therapy and anti-Ro and anti-La antibodies remain in the serum of SLE patients, so different B cell subtypes involved in the immune response to Ro and La antigens and α-fodrin may explain the discrepancy among studies.

This is the first study investigating concentrations of local antibodies against α-fodrin in tear fluid and saliva. Our findings suggest that these autoantibodies may contribute to the pathogenesis of lacrimal gland involvement and can be useful for following the severity of ocular involvement in patients with SS. Because they were present in the same frequency as anti-Ro/SSA and anti-La/SSB antibodies, we conclude that testing for anti-α-fodrin antibodies has a complementary role in the diagnosis of SS. Based on their association with ophthalmologic signs and symptoms, their importance as a disease activation marker cannot be ruled out. Additional measurement of tear fluid and saliva levels of these autoantibodies may serve as a valuable diagnostic aid for SS. A prospective study evaluating the role of hydroxychloroquine treatment on autoantibody production in patients with SS is now under way.

REFERENCES