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

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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[1]. Indeed, α-fodrin is specifically expressed in the salivary glands and it could play a role in the development of exocrinopathy. Although initial studies showed 92% positivity of IgG antibodies against α-fodrin in SS, that frequency has not been reached in subsequent studies[1,2]. 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[2,3].

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[4]. 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[5] 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

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Marmara School of Medicine. For clinical assessments of dry eye, dry eye symptom score, Schirmer I test, Schirmer test with topical anesthesia, fluorescein and rose bengal staining scores, and tear breakup time were used as reported[4]. Of those with secondary SS, 4 patients had SLE, 3 patients had RA, and another patient had systemic sclerosis. All patients and controls were women and the mean age was 54.4 ± 15.9, 53.5 ± 11.9, 42.9 ± 10.4, and 52.5 ± 13.2 years for patients with primary SS and secondary SS, SLE, and RA and controls, respectively. Patients with systemic manifestations of SS were not included in the study. All but one patient with SS were receiving hydroxychloroquine treatment when the samples were collected.

Methods. IgA and IgG anti-α-fodrin antibodies in 100-fold dilution were assessed by ELISA (Aesku Diagnostics, Wendelsheim, Germany). Results were considered positive when the optical density at 450 nm after correction for background exceeded the mean + 2 standard deviations (SD) of a pool of 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.

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, tear fluid, and saliva samples of controls were 7.1 ± 2.5, 6.2 ± 3.3, 4.0 ± 2.3, and 6.3 ± 4.3, 5.0 ± 3.5, and 2.4 ± 1.0 U/ml, respectively. Using a cutoff defined as mean concentration in the sera, tear, and saliva of healthy individuals plus 2 SD, the percentages of samples containing antibodies against α-fodrin and Ro/SSA and La/SSB antigens are given in Tables 1 and 2.

IgG antibodies against α-fodrin were detected in 10 of the 25 sera (40%) from patients with SS. In addition, IgG antibodies against α-fodrin were detected in 1 of 8 (12.5%) sera from patients with SLE and 2 of 7 (28.5%) sera from patients with RA without SS. We also observed that 9 of 25 (36%) tear and 8 of 25 (32%) saliva samples of patients with SS had IgG antibodies against α-fodrin, while it was detectable in 2 of 8 (25%) SLE and in 2 of 7 (28.5%) RA patients without SS in tear fluid and in 2 of 8 (25%) SLE patients without SS in saliva samples, respectively. Specificity for IgG antibodies against α-fodrin was 93% for both serum and tear fluid and 67% for saliva.

Eight serum samples from 25 SS patients and one serum sample from 20 controls had IgA antibodies against α-fodrin (Table 1). One SLE patient without SS had IgA antibodies against α-fodrin in both serum and tear samples. It was also noted that 2 of 7 RA and 3 of 8 SLE patients without SS had IgA antibodies against α-fodrin in their saliva samples. Specificity for IgA antibodies against α-fodrin was 80%, 73%, and 87% for serum, tear, and saliva, respectively. No control or SLE or RA patients had either IgG or IgA antibodies against α-fodrin in all 3 samples; 3 SS patients had IgA anti-α-fodrin and one patient had IgG anti-α-fodrin antibodies in all 3 samples.

Either anti-Ro/SSA or anti-La/SSB or both were detected in sera of 12 of 17 primary and 2 of 8 secondary SS patients, respectively. Anti-Ro/SSA or anti-La/SSB antibody was found in the tear fluid and/or saliva samples of 4 of 5 primary and 5 of 6 secondary SS patients, even though neither appeared in their serum. Overall, 2 patients with anti-Ro/SSA and 3 patients with anti-La/SSB were found to be positive in all 3 samples. Additionally, 2 patients who had neither anti-Ro/SSA nor anti-La/SSB antibodies in any of their samples had IgA antibodies against α-fodrin in both serum and tear samples. IgG antibodies against α-fodrin were also observed in all serum, tear fluid, and saliva samples of one of these patients. All patients had at least one of these autoantibodies (anti-Ro and/or anti-La and/or IgG and/or IgA anti-α-fodrin) in their tear fluid and/or saliva irrespective of their presence in serum.

Although no significant difference was found in the fre-

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.

	SS Patients				
	Primary, n = 17 (%)	Secondary, n = 8 (%)	All, n = 25 (%)	Disease Controls, n = 15 (%)	Healthy Controls, n = 20 (%)
Alpha-fodrin IgG					
Serum	7 (41)*	3 (37.5)*	10 (40)*	3 (20)	—
Saliva	5 (29)*	3 (37.5)*	8 (32)*	2 (13.3)	1 (5)
Tear	6 (35)*	3 (37.5)*	9 (36)*	4 (26.7)	1 (5)
Alpha-fodrin IgA					
Serum	7 (41)*	1 (12.5)	8 (32)*	1 (6)	1 (5)
Saliva	6 (35)*	2 (25)	8 (32)*	5 (33)†	2 (10)
Tear	4 (23.5)	1 (12.5)	5 (20)	1 (6)	2 (10)

* Significant differences between patients with SS and controls ($p < 0.02$). † Significant differences between disease controls and healthy controls ($p < 0.02$).

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

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

* 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 ($p < 0.005$).

quency of anti-Ro/SSA and anti-La/SSB antibodies compared to IgG antibodies against α-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 α-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 α-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 α-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 α-fodrin ($r = –0.56$, $p < 0.01$; Figure 2).

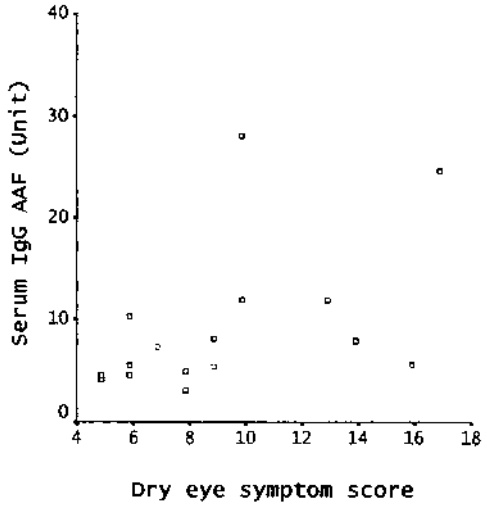

Figure 1. Relationship between levels of IgG α-fodrin antibodies (AAF) in serum and dry eye symptom score.

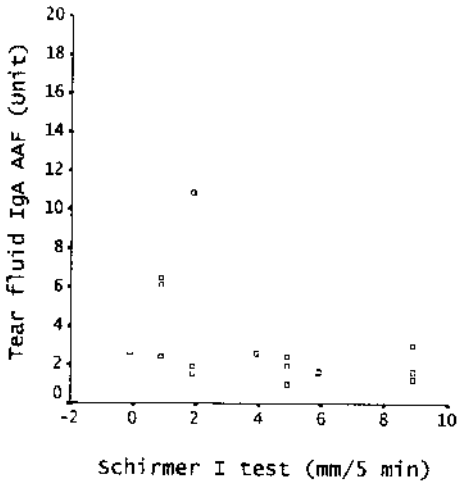

Figure 2. Relationship between levels of IgA α-fodrin antibodies in tear fluid and Schirmer I test.

DISCUSSION

We detected IgA and IgG antibodies against α-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[2,3,6-8]. Initially, Haneji, *et al*[1] suggested the presence of anti-α-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[2,3]. Witte and colleagues showed that even focusing on IgA antibodies against α-fodrin does not add sensitivity or specificity to diagnosis of SS[2]. In our study, patients with SS showed a frequency of 40% and 32% for IgG and IgA antibodies against α-fodrin, respectively; these are comparable to the percentages reported by Witte, *et al*[2] as well as Zandbelt and coworkers[7]. Previous studies have shown that several IgA antibodies are produced in salivary glands of SS patients[9,10]. Salivary and lacrimal glands are the major target tissues in SS; therefore, local α-fodrin autoantibody measurement might be helpful in the diagnosis of SS. To address this issue, tear fluid and saliva IgG and IgA autoantibodies against α-fodrin were analyzed and compared with the frequency of anti-Ro/SSA and anti-

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 SS[12]. However, the frequency of autoantibodies against α-fodrin detected in this study was comparable with those of previous studies[2,7], 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 treatment[12,13]. 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 cells[14,15]. Ro and La antigens are found in all nucleated cells, as is α-fodrin, and they are expressed on the surface of apoptotic cells[14,16]. 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.

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