

Evaluation of Diagnostic Accuracy of Alpha-Fodrin Antibody in Iranian Patients with Sjogren's Disease

Abstract

Background: Sjogren's syndrome, as a chronic autoimmune disease, involves in lymphocytic infiltration in the exocrine glands. As the result of exocrine glands disruption, the clinical hallmark of this disease including dryness of mouth and eyes along with fatigue and joint pain occur. However, heterogeneity of clinical presentations among newly diagnosed adult patients with Sjogren's syndrome leads to difficulty in its diagnosis. One of the diagnostic criteria for Sjogren's syndrome is the presence of autoantibodies in patient serum. One of the novel biomarkers suggested for diagnosis of Sjogren is alpha-fodrin antibody. In this study, we aimed to evaluate the diagnostic power of anti- α -fodrin antibody among the Iranian population for the first time. **Materials and Methods:** We recruited 82 individuals in this study. Alpha-fodrin were measured in case and control with Elisa kit as 16.71 (9.84) and 18.44 (11.54). **Results:** There was no any significant difference between two groups regarding alpha-fodrin level ($P = 0.35$). Then we applied the receiver operating characteristic (ROC) curve analysis to determine the predictive value of alpha-fodrin for diagnosing Sjogren's disease. The area under curve of the ROC curve was calculated as 0.5453. Also, there were significant association between age and alpha-fodrin antibody. **Conclusions:** Alpha-fodrin test did not have acceptable predictive power for predicting Sjogren's disease; however, it could be associated with disease progression.

Keywords: Alpha-fodrin, biomarkers, diagnosis, Sjogren's syndrome

Introduction

Sjogren's syndrome is a chronic autoimmune disease, characterized by lymphocytic infiltrates in the exocrine glands, specifically the salivary and lacrimal glands. Visceral damages and other clinical features can also occur in Sjogren's syndrome patients.^[1] Primary Sjogren's syndrome is a common systemic autoimmune disease.^[2] The hallmark of the disease is disrupted exocrine gland function, which often results in dryness of the mouth and eyes, fatigue, and joint pain.^[3] Like most autoimmune diseases, SS has a female predominance with a high female-to-male ratio (9:1) similar to that seen in systemic lupus. The peak incidence is in the 40–55 year age group.^[4] Based on formal criteria for the diagnosis,^[5] which requires the presence of immunologic abnormalities (the presence of serum anti-SSA antibodies or focal lymphocytic sialadenitis on biopsy of labial salivary glands), the estimated prevalence is

0.3–1/1000 persons.^[2] The major diagnostic challenge relates to the fact that mouth and eye dryness, limb pain, and fatigue are very common in the general population and may be associated with fibromyalgia or other pain syndromes, while primary Sjogren's syndrome is relatively rare.^[6] In line with the described diagnostic challenge, a recent patient survey by the Sjogren's Syndrome Foundation demonstrated that in the United States, the mean duration between symptom onset and time of diagnosis is up to 4 years.^[7]

In recent years, the heterogeneity of clinical presentations among newly diagnosed adult patients with Sjogren's syndrome has been better appreciated. Approximately 80% of the overall patient group presents with some form of sicca syndrome.^[8] The most common scenario is dry eyes followed by dry mouth and other sicca symptoms. Less commonly, xerostomia may be the presenting manifestation of the sicca symptoms that may develop simultaneously. Other common presenting manifestations of Sjogren's syndrome may therefore include

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Received: 18 October 2020

Revised: 11 April 2021

Accepted: 12 June 2021

Published: ***

Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_260_20

Quick Response Code:



How to cite this article: Rostamian A, Baradaran A, Mottaghi P, Salesi M, Karimifar M, Mansouri V. Evaluation of diagnostic accuracy of alpha-fodrin antibody in Iranian patients with Sjogren's disease. Adv Biomed Res 2022;11:64.

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inflammatory joint and muscle pain, chronic fatigue, swollen salivary glands, demyelinating disease, neuropathies, or abnormal lab values.^[4] The remaining 20% of Sjogren's syndrome patients present in so-called atypical fashion because, at the time these individuals enter the health care system, their sicca symptoms may be minimal or nil.

In recent years, the identification of these unusual cases has been facilitated by improvements in diagnostic and/or classification criteria that allow the diagnosis of SS to be made based solely on the findings of objective tests. Novel biomarkers such as anti- α -fodrin, has gained attention to be used in diagnose of this syndrome. However, several studies assessed the use of this biomarker for diagnose of Sjogren's syndrome in different populations, To the best of our knowledge, there is no previous study on the diagnostic value of anti- α -fodrin antibody among the Iranian population. We aimed to conduct a study to evaluate the diagnostic value of anti- α -fodrin antibody among the Iranian population for the first time.

Materials and Methods

This study is a cross-sectional study to evaluate the diagnostic value of anti- α -fodrin antibody in the Iranian population. 40 patients with primary Sjogren's Syndrome were enrolled in our study. Patients were recruited from the rheumatology clinics affiliated to Isfahan University of Medical Sciences from April 2018 to August 2019. All patients fulfilled the ACR/EULAR criteria for the classification of primary Sjogren's Syndrome.^[9] Those showing signs or symptoms of other associated diseases were excluded. Also, simultaneously, a total of 110 age and sex-matched patients with chronic rheumatic autoimmune diseases who did not fulfilled the ACR/EULAR criteria were recruited as a control group.

Patients' sera were used for measuring anti- α -fodrin antibody. Antibodies binding to α -fodrin of immunoglobulin (Ig) class G were measured using an ELISA kit (Orgentec, Mainz, Germany). Antigen coated to microplates was recombinant human α -fodrin obtained using the standard procedure. The sera were diluted 1:101. Results were expressed as arbitrary units (U) by reading off a standard curve. Furthermore, ANA and anti-SSA of each patient were recorded from their files.

After complete explanation of study goals, written informed consent was obtained from patients. This study was approved by the ethical committee of the Isfahan University of Medical Science (Project number: IR. MUI. MED. REC.1398.398).

McNemar's Chi-square test for comparison of results from different lot number kits, Fisher's exact test for comparison of anti-body prevalence, and Mann-Whitney U test for comparison of mean values of antibody levels were used. All data were analyzed by SPSS version 24. A $P < 0.05$ was considered statistically significant.

Results

We recruited 82 individuals in this study. Eight (9%) of them were male and their mean of age was 44.08 (13.22). Forty-one of them had Sjogren (case group) and 41 of them were in control group the average duration of having Sjogren in case group was 3.92 (2.62) the two group were not statistically different regarding age and gender. It should be noted that individuals in control group, in their medical history, had lupus (73% [$n = 30$]), rheumatic arthritis (9% [$n = 4$]), and autoimmune hepatitis (2% [$n = 1$]). The rest of control group consisted of health blood donors [Table 1].

Alpha-fodrin were measured in case and control as 16.71 (9.84) and 18.44 (11.54). There was no any significant difference between two groups regarding alpha-fodrin level ($P = 0.35$) [Figure 1]. Evaluating the correlations between the biomarkers and demographic variables, there was significant direct association between alpha-fodrin and duration of disease ($P = 0.011$). Also, there were significant association between age and Alpha-fodrin antibody and anti-SSA antibody (anti-Sjögren's-syndrome-related antigen A autoantibodies), while there was no significant correlation between age and alpha-fodrin in control group [Table 2].

Comparison of the alpha-fodrin by gender and study group (case/control), there were no significant difference regarding either gender independent of study groups or study groups independent of genders [Table 3].

Next, we applied the receiver operating characteristic (ROC) curve analysis to determine the predictive value of alpha-fodrin for diagnosing Sjogren's disease. The area under curve of the ROC curve was calculated as 0.5453 [Figure 2]. In next level, we determined the two cutoffs corresponding with highest sensitivity \times specificity value. Then we analyzed the data according to two cutoffs and presented kit cutoff. The odds ratio for having

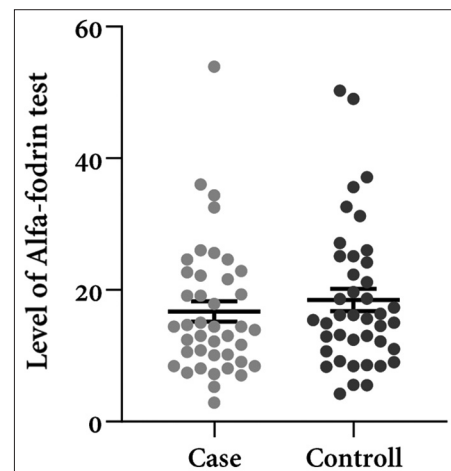


Figure 1: Alpha fodrin test level in patients with Sjogren versus control group (Mann-Whitney U-test $P = 0.35$)

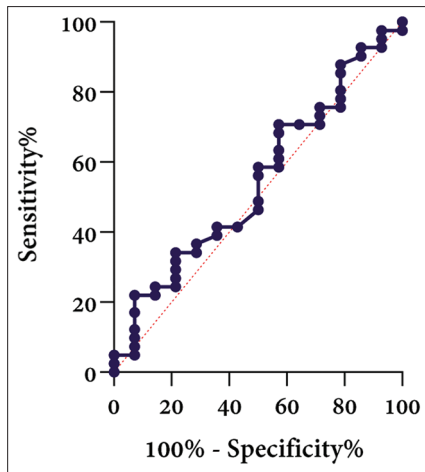


Figure 2: Receiver operating characteristic curve of alpha-fodrin level in predication of Sjogren's syndrome (area under curve = 0.5453)

Sjogren's disease were 1.085, 1.464, and 1.085, when analyzed data for 8.5, 10, and 19.5 as cutoff, respectively. The sensitivity and specificity of the test considering each cutoff are presented in Table 4.

Discussion

In this study, we found a nonsignificant difference in the alpha-fodrin levels between Sjogren patients and control group. The predictive power of alpha-fodrin test for diagnosis of the Sjogren's disease was a bit higher than 50%, which corresponds to low, unreliable discrimination for this test. However, different studies provide inconsistent results regarding the utility of this test in diagnosis of Sjogren. In a screening and diagnostic test study among 9 patients with primary Sjogren's syndrome, 15 patients with Sjogren's syndrome secondary to lupus erythematosus (LE), and 44 patients with LE alone, anti- α -fodrin antibody was more commonly detected in patients with primary (7/9; $P < .001$) and secondary (9/15; $P < .001$) Sjogren's syndrome than in those with LE alone (3/44). When patients with primary and secondary Sjogren's syndrome were combined and compared with those with LE alone, the sensitivity of anti- α -fodrin antibody was 67%, specificity was 93%, and both positive and negative predictive values were 84%. The presence of anti- α -fodrin antibody was associated with pernio, hyperglobulinemia, rheumatoid factor positivity, and the presence of anti-SS-B (La) antibody ($P < 0.01$) but not with annular erythema, photosensitivity, vasculitis, or renal disorder.

Also, we found a variation in the cutoff points of the alpha-fodrin test. Although, the factory-provided cutoff value for this test was ten IU/ml, we found other values to have more predictive accuracy. It should be noted that, as presented in Table 4, the sensitivity and reliability of the test were calculated based on different cutoff values. We consider two approaches in determination of the cutoff, and subsequently the sensitivity and specificity of the test.

Table 1: The characteristics of the participants

Variable	Patient with Sjogren	Control	P [†]
Age	46.5±11.83	41.14±14.74	0.136
Gender			
Male	2 (4.9)	6 (14.6)	0.137
Female	39 (95.1)	35 (85.4)	
Disease duration	3.92±2.62	N/A	N/A

[†]Chi-square and Mann-Whitney U-test were used. N/A: Not applicable

Table 2: Correlation between biomarkers together and with demographic characteristics in patient with Sjogren

Variables ⁺	Age	Alpha-fodrin antibody	Anti-SSA	Duration of disease
Age		0.033*	0.035*	0.052
Alpha-fodrin antibody	0.330		0.499	0.011*
Anti-SSA	-0.440	0.148		0.570
Duration of disease	0.302	0.391	0.125	

⁺Data is presented as pearson R in the lower left of the table and significance (P) at the upper right part of the table. *Significant at $P < 0.05$ SSA: Sjogren's syndrome antibody

Table 3: Comparison the alpha-fodrin level by gender and study groups

Level of alpha-fodrin	Male	Female	P
Patients with Sjogren	24.5	16.56	0.380
Control group	20.1	18.21	0.843
P	0.643	0.324	

As it already reported that alpha-fodrin could be elevated in other rheumatologic disease, the specificity of this test become of importance, however, the discrimination power of this test for separating normal individuals from Sjogren patients, contribute to importance of sensitivity. So, we suggest to define cutoff values in multilevel manner, so that lower levels could rule out the occurrence of Sjogren, while high levels could be differentiating for Sjogren from other rheumatologic diseases. Besides, other studies reported various cutoff values.

We also found a significant direct association between the number of having Sjogren and the alpha-fodrin level. It seems that although, this test could not act as a reliable test in diagnosis, it could serve as a potential candidate for evaluation of disease severity and progression. However, further studies are needed to confirm it.

The mechanism underlying the development of Sjogren's syndrome is the destruction of the epithelium of the exocrine glands, as a consequence of abnormal B cell and T cell responses to the autoantigens Ro/SSA and La/SSB, among others.^[10] Overexpression of several inflammatory cytokines in minor salivary glands has been demonstrated, including tumor necrosis factor-alpha, interleukin-6 (IL-6), IL-1, IL-18, and IL-22.^[11-16]

Table 4: Odds ratios of the variables according to cutoff of the test kit (10) and the two best-bound cutoffs

Cutoff Test result	8.5*		10		19.5*	
	Negative	Positive	Negative	Positive	Negative	Positive
Actual result						
Negative	6	35	9	32	27	14
Positive	9	33	32	10	30	12
Sensitivity	21.95		26.83		70.73	
Specificity	92.86		78.57		42.86	
OR (P)	1.464 (0.421)		1.085 (0.840)		1.085 (0.584)	

*Two best cutoffs were selected by the highest sensitivity×specificity. Cutoff 8.5 was selected with higher sensitivity and cutoff 19.5 was selected with higher specificity. OR: Odds ratio

Diagnostic criteria for Sjogren's syndrome include the detection of autoantibodies in patient serum and histological analysis of biopsied salivary gland tissue. In 1997, Haneji *et al.* found that serum samples from an NFS/sld mouse model of human Sjogren's syndrome reacted with a 120-kD protein, α -fodrin, which was specifically expressed in the lesional salivary glands. There is some evidence that this protein plays a critical role in the development of exocrinopathy. First, it induces proliferative T-cell responses and their type I cytokine production. Second, neonatal immunization with this protein prevented the development of the disease in mice. Third, serum samples from 41 (95%) of 43 patients with primary SS and 5 (63%) of 8 patients with secondary Sjogren's syndrome reacted with this antigen by immunoblotting, even though serum samples from all SLE, RA, and healthy controls were negative.^[17] Considered together, it is not surprising that α -fodrin, a substrate of calpain, is involved in the development of Sjogren's syndrome.

Although anti- α -fodrin antibody was detected in patients with Sjogren's syndrome and those with LE, it seemed to be more valuable for the diagnosis of Sjogren's syndrome than anti-SS-A (Ro) was because anti- α -fodrin was much less prevalent in patients with LE alone. Also, the association of this novel autoantibody with some extra glandular manifestations characteristically seen in these patients should be taken into account.^[18] Hu, Qin *et al.* reported that the anti- α -fodrin showed moderate accuracy for the diagnosis of SS with high specificity and relative low sensitivity in their study.^[19] Also, Bizarre *et al.* showed low sensitivity of anti- α -Fodrin antibodies in patients with primary Sjogren's Syndrome.^[20] In another study, anti-SSA and SSB antibodies, anti-M3 receptor antibodies and anti-alpha-fodrin (IgG) antibodies are specific antibodies for the diagnosis of Primary Sjogren's syndrome.^[21] Otherwise, Ruffatti *et al.* noted that the prevalence of IgA and IgG anti-alpha-fodrin antibodies in patients with primary Sjogren's Syndrome and other chronic autoimmune diseases have induced them to doubt their use as diagnostic markers of primary Sjogren's Syndrome,^[22] so, the results are still conflicting and different in each population.

Limitations and strengths

Major limitation of this study was the low prevalence of the Sjogren patients, contributing to long recruitment time. In addition, different cutoff values of different manufacturers kits, make data comparison difficult.

Major strength of this study, was that this study, to best of our knowledge, was the first study on Iranian population to evaluate the predictive power of alpha-fodrin test for Sjogren diagnosis.

Conclusions

Alpha-fodrin test did not have acceptable predictive power for predicting Sjogren's disease, however it could be associated with disease progression. Further studies with wide sample size is required for validating the results.

Acknowledgments

We thanked the patients and their families for their compliance and support.

Financial support and sponsorship

This work was supported by Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

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