



## Evaluation of the Pattern of Antinuclear Antibodies (ANA) Profile among Patients Suffering from Three Major Rheumatic Diseases

Soroush Khojasteh-Kaffash<sup>2</sup>, Elham Atabati<sup>1</sup>, Ali Fanoodi<sup>2</sup>, Afsane Bahrami<sup>1</sup>, Mohammadreza Mogharrabi<sup>2</sup>, Mahmoud Zardast<sup>3</sup>, Mohammad Fereidouni<sup>1\*</sup>

1 Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

2 Student Research Committee, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

3 Department of Pathology, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

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### ABSTRACT

**Background and Objective:** Autoimmune diseases (AIDs) are characterized by tissue destruction or organ dysfunction by the body's own immune system. The anti-nuclear antibodies (ANA) test is a helpful test for diagnosing and classifying ANA-associated rheumatic diseases (AARDs). This study aimed to evaluate the frequency and profile of autoantibodies among patients suffering from systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjogren's syndrome (SS).

**Materials:** This cross-sectional study was performed on patients who referred to the Rheumatology Clinic of Birjand University of Medical Sciences. Demographic data and information about risk factors were collected by a questionnaire. In confirmed cases, the presence of autoantibodies was detected by Fluorescent method (FANA). In all positive patients, ANA profiles were identified.

**Findings:** Totally, 138 patients were included in this study. Prevalence of the three main diseases including SLE, RA, and SS was 45.5%, 39.0%, and 15.4%, respectively and being overweight was the most common risk factor. The rate of positive FANA test was 71.4%, 43.8%, and 78.9% in SLE, RA, and SS, respectively. In the case of autoantibodies, Ro-52 autoantibody had the highest prevalence. The most common co-occurrence of autoantibodies was for DFS70 with PM-Scl100.

**Conclusion:** The results of this study showed that the mean titers of ANAs were higher in SS patients. The Ro-52 autoantibody was the most commonly detected autoantibody. There was no specific profile of autoantibodies for none of the studied diseases. Further studies are needed to evaluate the correlation between the presence of particular autoantibodies with the prognosis of the patients.

**Keywords:** Antinuclear Autoantibodies (ANA); Autoimmune Diseases (AIDs); Rheumatoid Arthritis (RA); Sjogren's Syndrome (SS); Systemic Lupus Erythematosus (SLE)

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\*Corresponding Author: Corresponding author: Mohammad Fereidouni E-mail: [dr.m.fereidouni@gmail.com](mailto:dr.m.fereidouni@gmail.com)

## Introduction

Autoimmune diseases (AIDs) are characterized by tissue destruction or organ dysfunction that occur due to different immune mechanisms against the body's own antigens [1]. Clinicians typically classify AIDs as systemic or specific for tissues or organs; it is believed that infections, genetic and environmental factors contribute to the pathogenesis of autoimmunity [2–10]. Prevalence of AIDs has been increased during recent Decades in westernized societies and AIDs are the third most common types of diseases in the United State [11, 12]. In this regard, AIDs affect 5-10% of the world's population and some AIDs are among the top 10 causes of death in women over 65 years old [8, 11].

In spite of fairly low prevalence, the economic burden of AIDs is high as they are chronic diseases which may cause disability and complications in the majority of patients in long term [2, 11]. AIDs are a group of heterogeneous diseases with a broad range of non-specific symptoms including arthralgia, arthritis, myalgia, fatigue and morning stiffness which might be seen in other conditions such as infections [11]. As the symptoms of autoimmunity are not specific and there are some fluctuations in the severity of symptoms, the accurate diagnosis of these diseases is sometimes difficult [2]. To facilitate the diagnosis, laboratory tests such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF) and anti-nuclear antibodies (ANAs) routinely are requested for the patients [13].

Most of laboratory tests are not specific and may not have significant correlation with disease progression and severity. On the other hand, other diseases and medications may affect the results of these tests. Among the laboratory tests, ANA test is one of the most helpful tests for diagnosing and classifying ANA-associated rheumatic diseases (AARDs) such as systemic lupus erythematosus (SLE), Sjogren's syndrome (SS) and systemic sclerosis [14, 15]. It also has been shown that these autoantibodies can be detected in AARDs about 2 decades earlier than the onset of symptoms; therefore, performing this test in suspicious people with special medical histories can identify the disease in the early phase [14]. The effectiveness of ANA test is not confirmed for following up of patients with SLE, because this test is positive in these patients anyway [7]. However, some subtypes, such as anti-dsDNA, can be effective in determining the active stage of the disease. Due to the similarity in the criteria of diagnosing and overlapping symptoms in some AIDs, the clinicians need these tests for differential diagnosis of AIDs [7].

ANA test detects autoantibodies against various components of cell nucleus and is positive in 20% to 100% of AIDs. New methods of measuring ANA have higher sensitivity and specificity; one of these methods is called fluorescent antinuclear antibodies (FANA), which has been used in this study. The gold standard method is fluorescent microscopy, known as FANA test, which rather than detection of autoantibodies, the fluorescence patterns can be helpful for identifying the disorders [16]. In addition to FANA patterns, as some autoantibodies such as anti-dsDNA, anti-Jo1 and anti-centromere are characteristic for specific diseases, several methods such as immunoblotting were developed for semi-quantitative identification of the profile of different autoantibodies in patients [1, 14].

Considering the variation in frequency and profiles of autoantibodies in different societies, and regarding the importance and usefulness of ANA test in screening, diagnosis, classification and monitoring the clinical activity of AIDs [6, 7, 14], the aim of this study was to evaluate the frequency and profiles of autoantibodies among a group of patients suffering from three major rheumatic diseases including SLE, rheumatoid arthritis (RA) and SS in the east of Iran.

## Methods

### *Study design*

This cross-sectional study was performed on patients whom referred to the Rheumatology Clinic of Birjand University of Medical Sciences due to suffering from different AIDs. Patients who diseases were confirmed by an expert rheumatologist according to the American College of Rheumatology (ACR) classification criteria and have had a FANA test result were enrolled in this study. The study was confirmed by ethics committee of Birjand University of Medical Sciences (ethics code: IR.BUMS.REC.1399.068) and informed consent form was received from all participants. Demographic information as well as information about past medical conditions, family history of AIDs and risk factors including infection, smoking, osteoarthritis, being overweight (body mass index (BMI) > 24), presence of metabolic disorders including renal dysfunction, diabetes or hyperlipidemia were collected by a questionnaire.

### *ANA profile and FANA tests*

Information about the results of FANA tests and ANA profiles was extracted from Shafa laboratory's database. All FANA tests were performed using commercial FANA kit (IIFT Mosaic: HEp-2/Liver, Euroimmune, Germany). In patients with primary positive FANA test, different serum dilutions from 1/400 to 1/10000 were also checked to determine the FANA titers. All subjects with a FANA titer of 1/100 or higher were considered positive.

In case of FANA positive samples, ANA profile was determined using commercial immunoblotting kit (EUROLINE ANA Profile 3 plus DFS70, Euroimmune, Germany). The kit could detect autoantibodies against 16 different antigens and the results were quantified by the commercial image analysis software (EUROLineScan, Euroimmune, Germany).

### *Statistical analysis*

Data were analyzed using SPSS software version 16 (IBM, Chicago, USA). Quantitative and descriptive data were expressed as mean and standard deviation and qualitative data were expressed as number (percentage). P value less than 0.05 was considered significant.

## Results

In total, 138 patients (mean age  $45.5 \pm 12.9$  years, Female/Male ratio: 8.2) were included in this study. The prevalence of the three main diseases including SLE, RA and SS was 45.5%, 39.0% and 15.4%, respectively. Being overweight was the most common risk factor, which was seen in 53.6%, 60.4% and 78.9% of SLE, RA and SS patients, respectively. Table 1 shows the prevalence of different risk factors as well as family history of AIDs.

Diseases	SS	SLE	RA	All
	Number (%)	Number (%)	Number (%)	Number (%)
<b>Female proportion</b>	19(100)	53(94.6)	42(87.5)	114(92.6)
<b>Mean age</b>	$45.4 \pm 14.5$	$41.7 \pm 12.6$	$48.4 \pm 12.6$	$46.5 \pm 12.7$

<b>Family history of AIDs</b>	6(36.1)	30(53.6)	29(60.4)	65(52.8)
<b>Mild form of diseases</b>	9(47.5)	31(55.4)	30(62.5)	70(56.9)
<b>Controlled form of diseases</b>	10(52.6)	25(44.6)	18(37.5)	53(43.0)
<b>BMI (mean <math>\pm</math> SD)</b>	24.1 $\pm$ 2.2	23.7 $\pm$ 2.4	24.2 $\pm$ 3.1	25 $\pm$ 2.0
<b>Smoking</b>	5(26.3)	9(16.1)	17(35.4)	31(25.2)
<b>Being overweight</b>	15(78.9)	30(53.6)	29(60.4)	74(60.1)
<b>Infection</b>	1(5.3)	5(8.9)	5(10.4)	11(8.9)
<b>Osteoarthritis</b>	11(57.9)	28(50.0)	29(60.4)	68(55.2)
<b>Metabolic diseases</b>	10(52.6)	28(50.0)	28(58.3)	66(53.6)

**Table 1.** Demographic information and the prevalence of risk factors among patients with SLE, RA and SS

According to Table 2, the highest rate of co-incidence was for RA and SLE (5.8%). The rate of positive FANA test was 71.4%, 43.8%, and 78.9% in SLE, RA, and SS, respectively. The median of FANA titers were highest in SS (1/1600), followed by RA and SLE (1/1200 and 1/750, respectively).

<b>Diseases</b>	<b>Percentage</b>
<b>SS + SLE</b>	3.6%
<b>SS + RA</b>	1.4%
<b>SLE + RA</b>	5.8%
<b>SS + SLE + RA</b>	0.0%

**Table 2.** Frequency of co-incidence of the diseases among study population

In case of ANA profile, Ro-52 autoantibody had the highest prevalence, while Sm, Scl-70 and Rib.P-protein autoantibodies had the lowest prevalence. Among all patients Ro-52 autoantibody was the most common autoantibody in SS and SLE, but in RA, SS-A autoantibody had the highest frequency. Table 3 shows the frequency of different autoantibodies according to the disease.

<b>Diseases</b>	<b>SS</b>	<b>SLE</b>	<b>RA</b>	<b>All</b>
	<b>Number (%)</b>	<b>Number (%)</b>	<b>Number (%)</b>	<b>Number (%)</b>
<b>FANA</b>	15(78.9)	40(71.4)	21(43.8)	76(61.7)
<b>RNP/Sm</b>	0(0)	5(12.5)	1(4.8)	6(4.8)
<b>Sm</b>	0(0)	0(0)	0(0)	0(0)
<b>SS-A</b>	10(66.6)	12(30)	5(23.8)	27(21.9)
<b>Ro-52</b>	12(80.0)	15(37.5)	4(19.0)	31(25.2)
<b>SS-B (SSB)</b>	4(26.7)	2(5)	0(0)	6(4.8)
<b>Scl-70</b>	0(0)	0(0)	0(0)	0(0)
<b>PM-Scl100</b>	1(6.7)	3(7.5)	4(19.0)	8(6.5)
<b>PM-Scl75</b>	0(0)	1(2.5)	0(0)	1(0.8)
<b>Jo-1</b>	0(0)	1(2.5)	0(0)	1(0.8)
<b>Centromere B (CB)</b>	1(6.7)	2(5.0)	1(4.8)	4(3.2)
<b>PCNA</b>	1(6.7)	2(5.0)	0(0)	3(2.4)
<b>dsDNA</b>	1(6.7)	9(16.1)	1(4.8)	11(8.9)
<b>Nucleosomes</b>	0(0)	5(12.5)	0(0)	5(4.0)
<b>Histones (H1)</b>	0(0)	3(7.5)	2(9.6)	5(4.0)
<b>Rib.P-protein</b>	0(0)	0(0)	0(0)	0(0)
<b>AMA-M2</b>	0(0)	4(10.0)	0(0)	4(3.2)
<b>DFS70</b>	3(20.0)	9(22.5)	4(4.8)	16(13.0)

**Table 3.** Frequency of different autoantibodies in three AIDs: SLE, RA and SS

The most common co-incidence of autoantibodies in SLE, RA and SS was for AMA-M2 with SS-A (78.0%), Histones (H1) with PM-Scl100 (66.5%), and DFS70 with PM-Scl100 (84.5%), respectively. Table 4 shows the co-incidence of different autoantibodies in details.

ANA profile	Diseases	RNP /Sm	SS-A native	Ro-52	SS-B (SSB)	PM-Scl100	Centromere B (CB)	dsDNA	Nucleosomes	Histones (H1)	AMA-M2	DFS70
<b>SS-A</b>	<b>SLE</b>	2.9	100									
	<b>RA</b>	0	100									
	<b>SS</b>	—	100									
<b>Ro-52</b>	<b>SLE</b>	12.3	28.2	100								
	<b>RA</b>	20.5	9.4	100								
	<b>SS</b>	—	<b>69.2</b>	100								
<b>SS-B (SSB)</b>	<b>SLE</b>	33.3	5.6	19.6	100							
	<b>RA</b>	—	—	—	100							
	<b>SS</b>	—	30.8	36.3	100							
<b>PM-Scl100</b>	<b>SLE</b>	7.2	26.5	9.3	7.5	100						
	<b>RA</b>	0	40.2	33.4	—	100						
	<b>SS</b>	—	7.6	25.0	0	100						
<b>Centromere B (CB)</b>	<b>SLE</b>	15.8	4.5	3.0	20.0	0	100					
	<b>RA</b>	5.2	20.0	0	—	0	100					
	<b>SS</b>	—	3.2	0	25.0	0	100					
<b>dsDNA</b>	<b>SLE</b>	2.9	0	25.9	0	13.3	0	100				
	<b>RA</b>	4.5	0	27.2		0	31.8	100				
	<b>SS</b>	—	2.3	1.9	0	19.7	0	100				
<b>Nucleosomes</b>	<b>SLE</b>	13.9	32.0	0	2.2	14.3	0	13.4	100			
	<b>RA</b>	—	—	—	—	—	—	—	100			
	<b>SS</b>	—	—	—	—	—	—	—	100			

<b>Histon es (H1)</b>	<b>SLE</b>	12.3	0	19.6	2.0	<b>57.0</b>	3.0	<b>52.0</b>	23.1	100		
	<b>RA</b>	0	0	0	—	<b>66.5</b>	0	0	—	100		
	<b>SS</b>	—	—	—	—	—	—	—	—	100		
<b>AMA- M2</b>	<b>SLE</b>	7.8	<b>78.0</b>	<b>52.0</b>	17.4	<b>54.6</b>	7.8	0	24.1	0	100	
	<b>RA</b>	—	—	—	—	—	—	—	—	—	100	
	<b>SS</b>	—	—	—	—	—	—	—	—	—	100	
<b>DFS70</b>	<b>SLE</b>	42.6	9.4	3.0	4.9	37.2	0	8.9	4.3	9.0	—	100
	<b>RA</b>	<b>50.0</b>	0	<b>53.0</b>		31.8	11.1	<b>50.0</b>	—	8.9	—	100
	<b>SS</b>	—	11.1	15.6	34.3	<b>84.5</b>	46.5	36.8	—	—	0	100

Table 4. The co-incidence percentage of different autoantibodies in three AIDs: SLE, RA and SS Co-incidence  $\geq 50\%$  is bolded.

### Discussion

The increasing prevalence of AIDs and their significant socio-economic impacts on public health as well as the overlap of clinical symptoms of these diseases has increased the importance of laboratory tests for helping in diagnosis and prognosis of AIDs. At present, the main goal of treatment of AIDs is treatment in the early phase of the disease, or in other words, treatment before development tissue damage [17]. Using ANA test is beneficial due to its differential diagnostic value, low cost, and ease of performing; however, in general, its sensitivity and specificity are low and because of its subjectivity, it needs experienced personnel.

Findings of the study on the prevalence ratio in women and men and the mean age of the patients suffering from RA, SLE and SS were consistent with previous studies which confirmed the higher prevalence in women and in middle aged individuals [18]. Compared to a study conducted in the United States, the prevalence of RA in women was 13.3% higher and the mean age was 4.8 years lower than men, and this finding indicates an earlier onset of RA in our study population, which could be due to a variety of reasons including environmental factors, physical stress, microbiomes and infections [19].

According to the results, being overweight in patients suffering from SLE was seen in a larger number of people compared to a global study (53.6% versus 41.0%) [20]. Moreover, a large percentage of RA patients have had a family history of the disease, which might be one of the reasons for earlier onset of the disease, but had no effect on the prognosis of the disease and its prevalence dependence on a specific sex [21].

The results suggest that patients with osteoarthritis have had a higher chance of accompanying AIDs than patients with RA, which contradicts a study performed in the United States that found the role of RA in



AIDs more important than osteoarthritis. One possible reason for this discrepancy might be the difference in the way these studies were performed and the inclusion criteria considered in these studies [19].

Co-incidence of RA and SLE is called rhus syndrome, which the prevalence of its clinical manifestations is 0.09% [22]. However, it seems that accompany of positive ANA test without the incidence of clinical manifestations of SLE in patients suffering from RA might be higher than co-incidence of RA and SLE (rhus). One of the reasons for positive ANA test in RA patients who do not have the clinical manifestations of SLE could be the use of biologic drugs or sulfasalazine, which also causes a condition called drug-dependent lupus erythematosus [18].

According to the results, the most common autoantibody was Ro-52, which is consistent with a study in patients referred to the Referral and Triage Center in Calgary, Canada [17]. Comparing the results of our study to a study performed in Germany, it was shown that the prevalence of Ro-52 autoantibody was similar in patients with SS but was lower in patients with SLE [23]. Moreover, the prevalence of Ro-52 autoantibody in patients with SLE is also consistent with the study of Didier et al. [7]. Ro-52 autoantibodies are usually produced due to late stimulation, use of anti-TNF- $\alpha$  biologic drugs, any type of apoptosis and UV exposure [24]. The high prevalence of Ro-52 autoantibodies in this study could be due to the geographical conditions of the region and the high exposure of patients to UV rays. Also, Ro-52 and Ro-60 autoantibodies are very common in the healthy population of this region without accompanying clinical manifestations [24].

The results showed that the prevalence of SS-A autoantibody in patients with SLE and SS was lower compared to a study performed in France and almost similar compared to a study performed in Germany [7, 23]. Studies show that SS-A autoantibody is detectable in patients with SLE and SS during skin involvement and congenital heart block more than other times, respectively [7]. Comparing to other studies, the prevalence of SS-B autoantibody in patients suffering from SLE and SS was lower, which may be due to the increase of this autoantibody in skin involvement and congenital heart block [7, 23].

Comparing to the study of Ganapathy and Casiano, in our study the prevalence of DFS-70 autoantibody was higher in patients with SLE and SS and lower in patients with RA, which could be due to the increase of this autoantibody in all chronic inflammatory conditions such as cancers, chronic infections, etc. [25]. The prevalence of ds-DNA autoantibody in patients with SLE was lower in our study comparing to similar studies in Norway and France [7, 26]. Lower concentrations of ds-DNA autoantibody in patients with SLE is probably due to the fact that this autoantibody is more elevated in patients with severe forms of the diseases, while the people in this study were monitored and have received medication, and often their diseases were mild or in some cases in moderate form.

According to the results, the prevalence of Jo-1 autoantibody in patients with SLE, RA and SS was very low, which may be due to the specificity of this autoantibody for inflammatory myopathies such as dermatomyositis [18]. The prevalence of anti-histone antibody in patients with SLE was lower than other studies, which may be due to the high specificity of this autoantibody for drug-induced lupus erythematosus and non-lupus autoimmune diseases [23]. In the present study, compared to the study of Didier et al., in patients with SLE, the prevalence of RNP, Sm and nucleosome autoantibodies was lower and the prevalence of Pm/Scl autoantibody was almost similar [7]. The lower prevalence of Sm autoantibody in patients with SLE may be due to the increase of this autoantibody in lupus nephritis and the control of patients in the present study [27]. Also, due to the control of SLE patients in this study and the increase in the amount of nucleosome and ds-DNA autoantibodies in patients with acute conditions,



the low prevalence of these autoantibodies was predictable [7]. Due to the low specificity of RNP autoantibody for SLE, its low prevalence in patients with SLE was expected [7].

Although the present study suffers from a few limitation such as fairly small sample size, lack of information about the severity and trend of the diseases and subjectivity of FANA test but the strict inclusion criteria and identifying autoantibodies with method using a well-known commercial kits strengthen our study [17].

## **Conclusion**

In conclusion, according to the best of our knowledge, this study is one of the first studies performed on ANA profile patterns and AIDs in Iran. The results of this study showed that the mean titers of ANAs were higher in SS patients. Ro-52 autoantibody was the most common detected autoantibody. There was no specific profile of autoantibodies for none of studied diseases. Further studies are needed to evaluate the correlation between presence of particular autoantibodies with prognosis and outcome of the patients.

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## **Authors' Contributions**

M.F.; Designed and performed the research, analyzed results, co-wrote the paper, supervised the research. E.A.; Examined and diagnoses the cases, analyzed results, co-wrote the paper. S.K.; Performed the research, designed and collected the questionnaires, collected data, co-wrote the paper. A.F.; Performed the research, designed and collected the questionnaires, collected data, co-wrote the paper. A.B.; Performed statistical analysis. M.M.; Performed the research, collected data, co-wrote the paper. M.Z.; Performed laboratory tests.

## **Compliance with Ethics Guidelines**

The study was confirmed by ethics committee of Birjand University of Medical Sciences (ethics code: IR.BUMS.REC.1399.068) and informed consent form was received from all participants.

## **Consent for Publication**

Not applicable.

## **Data Availability**

Not applicable.

## **Competing Interests**

The authors in this study declare that they have no competing interests. Authors also indicate that they did not have a financial relationship with the organization that sponsored the research and had full control of all primary data and agree to allow the journal to review their data if requested.

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