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Research Article **Open Access**

Autoantibody profile, disease activity and organ involvement in Iranian systemic lupus erythematosus patients

Mahmoud Mahmoudi[1], Maryam Rastin[1]*, Maryam Sahebari[2], Shahrzad Zamani[1] and Nafiseh Tabasi[1]

[1]Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [2]Rheumatic Disease Research Center, Internal Medicine Department, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Autoantibodies have been implicated with increased risk of organ involvement in systemic lupus erythematosus (SLE). In the present study, we looked for autoantibody profiles and their association with clinical symptoms in a group of Iranian patients with SLE. In this study, 98 lupus patients (78 females and 20 males) were evaluated for the presence of autoantibodies against nRNP/Sm, Sm, SSA, Ro52, SSB, Scl-70, Jo-1, CENP B, nucleosomes, histones and Rib-P protein using immunoblotting technique. Anti-dsDNA was the most prevalent autoantibody (69.1%). The increased amount of autoantibodies, of the affected organs, and presence of anti-histone and anti-dsDNA correlated with disease activity. In the SLE patients with renal involvement, presence of anti-nucleosome (54.8% vs. 39.4%, P= 0.04) and decreased levels of anti-SSB (14.3% vs. 26.3%, P= 0.007) were significantly different compared with patients without renal involvement. Our results showed that elevated levels of autoantibodies including anti-dsDNA and anti-histone, and increasing number of involved organs, could be used as predictors for assessment of disease activity in patients with lupus. In addition, the increased levels of anti-nucleosome and the lower occurrence of anti-SSB could be used in the verification of renal damage.

Keywords: anti-dsDNA, autoantibody, organ involvement, systemic lupus erythematosus.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with unknown etiology and intermittent courses of relapse and remission, in which multiple organs are affected [1,2]. Patients with SLE present diverse clinical symptoms such as skin, kidney, central nervous system (CNS) or other organs involvement [3,4]. The pathogenesis of lupus is not well understood, but development of autoantibodies, which may exist many years before the onset of clinical symptoms and diagnosis, is of great importance [1, 5]. In addition to their diagnostic application, autoantibodies seem to be involved in the increasing risk of organs involvement [6, 7]. Why autoantibodies are developed is not well known. However, breakdown of self-tolerance in B and T lymphocytes [3, 8, 9], increased presentation of modified self-antigens as a result of increased apoptosis [10-13] or decreased clearance of apoptotic materials [14,15] are possible factors contributing to the development of autoantibodies [3, 16].

In patients with lupus, clinical manifestations are variable and the reasons why some organs are involved in a number of patients but not in others is not well known. Autoantibodies play a significant role in disease manifestations [3, 6, 17, 18] and are involved in the active phase of the disease [6, 19, 20].

Clinical manifestations and severity of lupus vary in different regions of the world [6, 21]. Moreover, ethnic, racial and environmental factors are involved in the development of SLE and its symptoms.

The aim of this study was to determine, for the first time, autoantibodies profile and their relationship with organ involvement in Iranian patients with SLE.

Methods and Materials

Pateints

In the current cross sectional study, blood samples from 98 SLE patients (78 females and 20 males) were collected for profiling of autoantibodies. Patients with SLE were consecutively referred to the immunology research center to be examined by a rheumatologist. All SLE patients fulfilled at least four of the revised SLE criteria of the

* Corresponding Author: Maryam Rastin, E-mail: rastinm@mums.ac.ir, Tel: +98 9155044497, Fax: +98 511 7112596
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American college of rheumatology (1997 ACR revised criteria) for the classification of SLE (Hochberg, 1997). The study was approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS. REC.1389.5). Written informed consents were obtained from all patients participating in the study. Serum samples, after collection from whole blood of patients, were deeply frozen at -70°C until analysis.

Inclusion criteria of the patients were new diagnosis of Lupus (before starting treatment).

Being in remission and taking a maximum dose of 10 mg/day of prednisolone and/or 200 mg/day of hydroxychloroquine.

In those with major organ involvement sampling was performed before starting cytotoxic or a high dose of corticosteroid therapy.

On the other side, exclusion criteria were:

- having a diagnosis of drug induced lupus.
- being under treatment with cytotoxic and other medications that could induce autoantibodies.
- having overlapping syndromes.

Clinical variables from patients were obtained using medical history records and physical examinations; disease activity for each patient was determined using SLEDAI (systemic lupus erythematosus disease activity index). Organ involvement was also defined according to SLEDAI criteria. In addition, patients were inquired about a history of deep vein thrombosis.

Antibody profiling

Autoantibody profile was determined by a commercial kit (EUROIMMUN, Germany) using an immunoblotting technique, according to the manufacturer's instructions. Briefly, 1.5 mL of each diluted serum was incubated with a strip of pre-coated antigens (nRNP, Sm, SSA, SSB, Ro-52, CENP-B, Jo-1, Scl-70, nucleosomes, dsDNA, histones and Rib-p protein) for 30 minutes at room temperature. Strips were then washed three times using washing buffer, and then incubated with 1.5 mL of enzyme conjugated anti-human IgG for 30 minutes. After washing, strips were incubated with 1.5 mL of substrate solution for 10 minutes, washed, air dried and evaluated. Interpretation of the results was done using manufacturer's data sheets and software.

Anti-dsDNA autoantibody was screened using an enzyme-linked immunosorbent assay kit (ELISA; EUROIMMUN, Germany).

Statistical analysis

Statistical analysis was performed using SPSS for windows version 16.0. The normality of the variables was first examined using descriptive statistics. Comparison between nominal variables was made using Chi-Square or if necessary Fisher's exact-tests. Comparison between continuous variables was made by student's t-test or if necessary Mann-Whitney test. All the results were presented as mean±standard deviation (SD). P values of less than 0.05 were considered statistically significant.

Results

Specification of patients

In the current study, the mean age of the patients was 27.1±7.9 years (range, 14 to 45 years; Fig. 1). At the sampling time, most of the patients had injuries in different organs and in 56.2% of them three to four organs were involved (Fig. 2). In the patient group, joint involvement was the most common clinical disorder (60.2%), followed by skin involvement (54.1%). About 42.9% of SLE patients had renal involvement and 13.3% had involvement of the visceral organs such as heart and lungs. The prevalence of ocular involvement was 2%; the minimum prevalence of a manifestation in SLE patients of this study (Table 1). Alternately, six SLE patients had a family history of lupus, in their mothers, brothers or sisters.

Frequency of autoantibodies

In this study, most of the SLE patients had different types of autoantibodies, and the mean amount of autoantibodies at the sampling time was 3.7±1.99. Anti-dsDNA was the most common autoantibody (69.1%) among the patients, followed by anti-SSA (42.9%), and anti Ro-52 (37.8%). In patients with a familial history of lupus, the most common autoantibody was anti-histones. Anti Scl-70 and anti Jo-1 autoantibodies were detected in none of the patients. Data of the prevalence of autoantibodies are presented in Table 2.

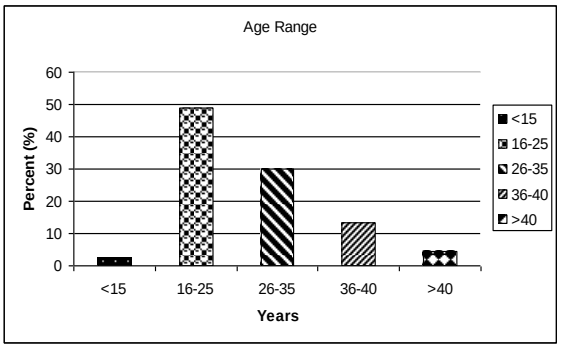

Fig. 1. Age distribution in patients with systemic lupus erythematosus

Table 1. Clinical and demographic variables in patients with systemic lupus erythematosus

Characteristic	Frequency (%)
Arthritis/arthralgia	60.2
Cutaneous involvement	54.1
Renal involvement	42.9
Haematological disorder	42.9
Neurological manifestations	19.4
Visceral involvement	13.3
History of abortion	7.1
Thrombotic disorders	6.1
Positive Family History of SLE	6.1
SLADAI (Systemic Lupus Erythematosus Disease Activity Index)	10.07 ± 3.77
Age (years)	27.1 ± 7.9
Duration of disease (years)	3.93 ± 0.37

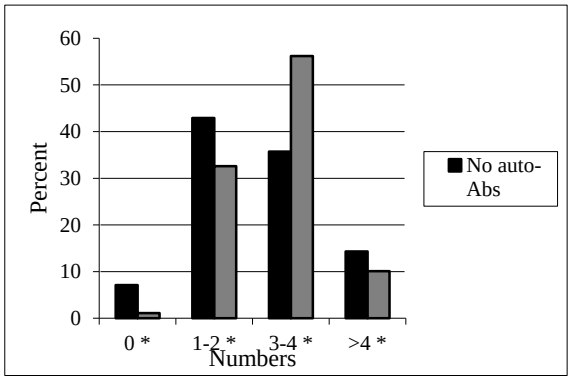

Fig. 2. Distribution of the number of autoantibodies and involved organs in patients with systemic lupus erythematosus

Table 2. Frequency of autoantibodies in patients with systemic lupus erythematosus

Autoantibody	Frequency (%)
Anti-dsDNA	69.1
Anti-SSA	42.9
Anti-Ro-52	37.8
Anti-nRNP	19.3
Anti-Nucleosomes	27.6
Anti-Histone	24.5
Anti-Rib.p-Protein	17.3
Anti-Sm	16.3
Anti-SSB	10.2
Anti-CENP B	2.0

Correlation between autoantibodies and clinical variables

It was found that in each affected organ, particular autoantibodies were most frequent (Table 3). However, only increased amounts of anti-nucleosomes (54.8% vs. 39.4%, P= 0.04) and decreased levels of anti-SSB (14.3% vs. 26.3%, P= 0.007) in patients with renal involvement were significantly different compared with patients without renal involvement.

Concurrent presence of autoantibodies

We determined the simultaneous presence of different autoantibodies, and realized that autoantibodies were mainly observed in two different clusters. The first one is consisted of anti-Sm, anti-nRNP, anti-SSA and anti-Ro52 (group 1), and the second one is consisted of anti-dsDNA, antinucleosomes and anti-histones autoantibodies (group 2).

Association of group 1 and group 2 autoantibodies with demographic data

Autoantibodies in group 1 were more common in newly diagnosed SLE patients compared with patients at the remission phase (35.0% vs. 8.0%; P= 0.04). Moreover, these antibodies were more frequent in female patients in comparison to males (56.0% vs. 8.0%; P= 0.008). However, there was no association between disease activity and the presence of group 1 or 2 autoantibodies.

Correlation between autoantibodies and disease activity

The mean SLEDAI score in lupus patients of this study was 10.07±3.77. In assessment of the relationship between SLEDAI and presence of each autoantibody, a significant positive correlation was detected between SLEDAI and anti-histone antibody levels (SLEDAI score of 12.87±3.1 in anti-histone positive patients vs. 8.20±2.76 in anti-histones negative patients, P<0.05). Nevertheless, the positive correlation that we found between anti-dsDNA and SLEDAI was predictable because anti- dsDNA is included in SLEDAI score calculation (11.25±3.5 in anti-dsDNA positive patients vs. 7.33±3.58 in anti-dsDNA negative patients, P= 0.001). In patients with renal involvement, SLEDAI had a positive correlation with proteinuria (10.5±3.65 in patients with proteinuria vs. 7.8±3.59 for patients without proteinuria, P= 0.009).

Table 3. Most frequent autoantibodies in systemic lupus erythematosus patients with different clinical and demographic variables

Variables	Most frequent autoantibody	%
Cutaneous involvement	Anti-dsDNA	56.3
Arthritis/arthralgia	Anti-dsDNA	68.2
Renal involvement	Anti-nucleosomes	54.8
Neurological manifestations	Anti-SSA/ Anti-Ro52	55.6
Ocular involvement	Anti-nRNP/ Anti-Rib-p protein	100
Oral ulcer	Anti-nRNP	75.0
Visceral involvement	Anti-SSA / Anti-dsDNA	54.5
History of abortion	Anti-SSA / Anti-Ro52	71.4
Haematological disorder	Anti-dsDNA	53.7
Thrombotic disorders	Anti-nucleosomes	100
Positive Family History of SLE	Anti-dsDNA	56.3

The increased number of autoantibodies ($P< 0.05$) and involved organs ($P<0.05$) were significantly correlated with SLEDAI.

Discussion

We studied the profile of autoantibodies and their relationships with organs involvement in a population of Iranian patients with lupus. Our findings showed that in most of the SLE patients at the sampling time, there existed different types of autoantibodies, while anti-dsDNA was the most common. Increased levels of autoantibodies, increased number of involved organs and presence of anti-histone and anti-dsDNA were positively correlated with disease activity. In patients with renal involvement, the increased rate of proteinuria had a positive correlation with disease activity. In agreement with our results, some studies reported an association between the increasing number of involved organs and disease activity [22]; some showed that autoantibodies were useful predictors for disease activity [4-6] and others demonstrated that the increased titer of anti-dsDNA could alert the physician about a possible flare on the way ahead [21, 23-25].

In previous surveys, presence of autoantibodies was suspected with increased risk of tissue involvement [1, 5, 7]. In our study, considering the correlation between autoantibodies and tissue involvement, a significant increase in anti-nucleosomes and a remarkable decline in anti-SSB in patients with renal involvement were observed. In agreement with our results, association of anti-SSB with decreased involvement and severity of renal diseases was observed in some other studies [23, 24, 26]. In most studies, anti-dsDNA antibody was demonstrated to be correlated with lupus nephritis [13, 14, 23, 27]; however, our study failed to confirm these correlations. Nonetheless, anti-nucleosome antibodies were significantly increased in our patients with nephritis, which was in accordance with some previous studies [13, 14, 20]. Some authors suggest that nucleosomes are the target of autoantibodies and, as a bridge, mediate the binding of autoantibodies and immune-complexes to the anionic glomerular basement [13, 14]. Sherer et al. showed that anti-nucleosome antibodies could be detected even prior to the development of anti-dsDNA and anti-histone antibodies in lupus patients [23].

Autoantibody production, disease severity, clinical symptoms and also the progression stage of SLE are highly influenced by ethnic background and genetic differences [6, 21, 25, 28, 29]. Our results demonstrated that the presence of anti-dsDNA antibody was not a sufficient and sole factor leading to the occurrence of renal damage in all ethnicities. However, in agreement with some previous studies [17, 18], anti-nucleosomes rather than anti-dsDNA could be a better factor for evaluation of renal involvement in Iranian patients with SLE.

Early evaluation of disease activity and diagnosis of major organ involvement in patients with SLE are crucial to the physician, because appropriate treatment will reduce subsequent damage to the organs.

Our study had some limitations; it was a cross sectional study, all of the patients held a unique Iranian background, while most of them were newly diagnosed patients in the active phase of the disease. Further longitudinal studies in different geographical regions and ethnic populations could help to identify the role that autoantibodies play in SLE.

Conclusion

In summary, our results showed that an increase in the amount of autoantibodies and involved organs with increased presence of anti-dsDNA and anti-histone, could be used as predictors for assessment of disease activity in patients with SLE. In addition, the increased levels of anti-nucleosome antibody and the lower occurrence of anti-SSB could be used in the verification of renal damage.

Conflict of interest
The authors declare no conflict of interest.

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