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Clinical implications of autoantibodies to extractable nuclear antigens in rheumatoid arthritis patients in tertiary care hospital in Riyadh, Saudi Arabia

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Abstract

The purpose of this study was to assess the prevalence of autoantibodies against extractable nuclear antigens (ENAs), anti-Ro/SSA, anti La/SSB, anti Jo-1, ribo-nucleoproteins (anti UI-RNP) and antibodies to Smith (anti-Sm) in rheumatoid arthritis (RA) patients and to detect the relationship between the existence of these autoantibodies and clinical presentation, radiological damage, and disease activity markers. The study, included 57 RA patients and 35 normal controls, was conducted in Al-Quwayiya General Hospital. Sera were obtained from patients and controls to carry out Hs-CRP, ESR, RF factors, ANA and ACPA IgG. Anti-Sm, anti-U1-RNP, anti-Ro/SSA, anti-La/SSB, and anti-Jo-1 were measured using the Alegria® assay. ESR was 23.8±6.5, while CRP was 16.19±7.15 in RA patients. RF was detected in 23 (40.35%) patients and ACPA in 54 (94.73%), while ANA was positive in 14 (24.56%) cases. Anti-Ro/SSA was detected in 8 (14.03%) patients, anti-La/SSB in 3 (5.26%), anti-Jo-1 was found in 2 cases (3.50%), and anti-RNP in 1 (1.75%). Anti-Ro/SSA had a significant negative relationship with sex (r=−0.220, *P*=0.001), but a positive correlation was observed with sicca symptoms (r=0.149, *P*=0.02). Anti-La/SSB antibodies correlated positively with swollen joint count (r=0. 0.225, *P*=0.001), tender joint count (r=0.219, *P*=0.001) and ANA (r=0.367, *P*<0.001). Anti-Jo-1 antibodies showed a significant positive correlation with interstitial lung disease (r=−0.429, *P*≤0.001). Anti-Ro/SSA had a significant positive correlation with RF titer (r=0.259, *P*<0.001) and ANA (r=0.498, *P*<0.001). Anti-La/SSB had significant positive correlation with ANA (r=0.372, *P*<0.001). Anti-Jo-1 showed a significant positive correlation with RF titer (r=0.141, *P*<0.040) and ANA (r=0.249, *P*<0.001). Anti-RNP showed a significant positive correlation with ACPA titre (r=0.288, *P*<0.001) and ANA (r=0.159, *P*<0.018). We conclude that ENA autoantibodies are crucial and need to be correlated with clinical diagnosis and other serological testing for early diagnosis and intervention of the RA.

Keywords: Rheumatoid arthritis, Extractable nuclear antigens, ANA and ACPA.

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Introduction

Rheumatoid arthritis (RA) disease is a chronic autoimmune illness marked by pain, swelling, synovial joint degeneration, and disability.[1] RA has numerous appearances, some of which are associated with the existence of specific auto-

antibodies, such as anti Ro/SSA (anti–Sjögren's-syndrome-related antigen A autoantibodies), which is linked to Sjögren's syndrome associated with RA.[2] Presence of anti-Jo-1 in RA patients may represent an association between anti-synthetase syndrome (ASS) and RA particularly in situations where anti-citrullinated protein antibodies (ACPA) are positive.[3]

Antinuclear antibody (ANA)-associated rheumatic diseases, such as primary Sjögren syndrome (pSS), systemic sclerosis (SSc), systemic lupus erythematosus (SLE), autoimmune myositis and mixed connective tissue disease (MCTD), all have autoantibodies directed to intracellular components (AIM). Some of these autoantibodies are extremely specific for each unique rheumatic diseases and are used to classify and/or diagnose these autoimmune illnesses.[4]

Antibodies against extractable nuclear antigens (ENA) are auto-antibodies that interact with cellular nuclear proteins, which are referred to as "extractable" because they may be extracted from nucleus by saline. Systemic autoimmune diseases are particularly associated with one or more ENA. Consequently, if ANA results are detected in patients with suspected autoimmune disorders, anti-ENA tests are carried out to screen the presence of certain diseases. However, since it was detected that anti-ENAs could be detected in ANA negative samples, there is an argument that the anti-ENA detection should be more expanded.[5]

Autoantibodies against ENAs have been investigated most extensively in SLE patients. These autoantibodies to ENAs are linked to lupus nephritis pathogenesis and play a significant role in determining disease activity and severity.[6] Antinuclear antibodies in RA were investigated extensively since 1961, when Weir et al.,[7] discovered the presence of ANA in 14 % of RA patients. The existence of an extractable nuclear antigen had been investigated in the 1970s. It was also believed that ANA positive RA patients had a lupus-like condition that needed to be managed differently, particularly with corticosteroids.[8] Many investigations on RA nuclear antigens and ANA in RA patients have been conducted since the 1980s, looking for associations with variables such as Rh factor, the Epstein Barr virus and HLA factors.[9]

The purpose of this study was to assess the prevalence of ENAs (Ro/SSA, La/SSB, UI-RNP, Jo-1, and anti-Sm) autoantibodies in RA patients and to detect if there was a relationship between the presence of these autoantibodies and clinical presentation, disease activity markers, and radiological damage.

Materials and Methods

Study design

This study was done from June 2021 to December 2021 in the rheumatology clinic and laboratory of Al-Quwayiyah General Hospital. All studied patients were selected according to the guidelines of American College of Rheumatology's, 2010.[10] and aged 18 years or more. The studied patients had a detailed rheumatological history and clinical examination, which included the clinical criteria of tender joint count (TJC), swollen joint count (SJC) and the duration of morning stiffness in minutes to determine all patients' Disease Activity (DAS 28). Sjögren's syndrome symptoms and related pulmonary symptoms were also investigated, due to the known relationship between these clinical presentations and the tested anti-ENA autoantibodies, carful clinical assessment was done to rule out any symptoms or signs associated with certain disorders, such as Raynaud's illness, sclerodactyly, telangiectasia, swollen puffy fingers, Gottron's sign, and subcutaneous calcifications. Fifty-seven patients with rheumatoid arthritis were studied. A control group included 35 participants with no prior history of joint swelling, no symptoms of autoimmune disease, and no family history of any autoimmune diseases. Al-Quwayiyah General Hospital Ethical Committee reviewed and approved the study protocol (May 2021). Each patient and control participant agreed to be enrolled in the study with provision of written informed consent.

Samples collection

A whole blood sample from each patient was collected under aseptic conditions. After allowing the blood to coagulate, the serum was

separated by centrifugation. There was no haemolysis or lipemia in the serum, as this could interfere with the processes. Serum samples were kept at -20°C for five days or kept in the refrigerator for up to five days at 2-8°C before being processed.

- Serum reactive protein (CRP), was performed by using a latex test, (Crescent diagnostics, UK), when latex particles coated with goat IgG antihuman CRP were mixed with CRP-containing samples, they agglutinated.[11]

- The erythrocyte sedimentation rate (ESR), was done using the standard Westergren method.[12]

- Rheumatoid factor (RF) was detected using, RF latex test, (Crescent Diagnostics, UK), when human gamma globulin-coated latex particles are mixed with RF-containing specimens, the antigen / antibody reaction was easily identifiable as latex agglutination.[13]

- Anti-citrullinated peptide antibody (ACPA IgG) was detected using an indirect solid phase enzyme immunoassay (Abnova, United Kingdom) for quantitative assessment of IgG autoantibodies against citrullinated proteins in human serum. An automated ELISA analyzer (evolis, Biorad, France) was used for detection of ACPA IgG. The yellow colour intensity related to antibody-antigen-complex quantity was photometrically measured at 450 nm. The calculation range was 0-1000 U/mL and a cut-off of 20 U/mL was used in this ELISA test.[14]

- Anti-extractable nuclear antigens (ENA) autoantibodies and ANA were examined in the RA patients and the control participants for proper validation of the immunological laboratory findings. Anti-Sm, anti-U1-RNP, anti-Ro/SSA, anti-La/SSB), and anti-Jo-1 antibodies were measured using an ELISA kit (Alegria® test strip assay, Orgentec Diagnostika, Germany), which used bar-coded 8-well micro strips. Each Alegria® test strip kit contained an enzyme conjugate, enzyme substrate, sample buffer, and a test-specific control. In positive samples, antibodies attached to the antigen coated on the reaction wells surfaces, generating an antibody antigen complex, which was used to make the determination. After incubation, unattached and unspecific bound molecules were removed in a first washing phase. Following that, an enzyme conjugate was added, which bound to the immobilized antibody–antigen complex. A second washing step followed the incubation to eliminate any unbound enzyme conjugate. Then enzyme substrate solution was added and incubated which caused hydrolysis and development of the colour. The blue colour intensity was proportional to the concentration of the antibody–antigen combination and evaluated photometrically at 650 nm. The test has a calculating range of 0–200 U/ml [normal 15 U/ml, borderline 15–25 U/ml, increased >25 U/ml].[15]

Radiology

The hands and wrists X-rays, as well as the forefeet, were taken. The simplified radiological Sharp/Vander Heijde score was used to assess joint injury in all patients enrolled in the study.[16]

Statistical analysis

The statistical package for the social sciences (SPSS) version 15 was used to analyse the data. ENAs were correlated with various clinical factors and disease activity indicators using univariate Pearson's correlations. *P* values less than 0.05 were considered statistically significant.

Results

Table 1 shows data of 57 patients with rheumatoid arthritis, of these 44 (77.19%) were females and 13 (22.81%) males. Their mean age was 49.12±12.63 years and the disease duration 62.18±49.61 months. There was no statistical difference regarding sex and age as compared with the control group. Age at onset of RA in the patients was 43.12±14.89 years and disease duration 62.18±49.61 years. The swollen joint count was 6.12±3.02 and 2.02±0.43 among RA cases and control respectively. Tender joint count was 6.01±2.79 and 2.13±0.50 among RA cases and control respectively. There were significant statistical differences regarding presence swollen and tender joint count in RA patients as compared with the control group (*P*=0.006 and 0.012, respectively). Subcutaneous nodules and interstitial lung

disease were found only in rheumatoid patients, represented as presented by 1 (1.75%) and 2 (3.51%), respectively.

Table 1. Demographic data, clinical presentation among the studied groups of RA patients and control.

Parameters	RA patients = 57	Control = 35	*P* value
Age	49.12±12.63	46.14±11.73	NS
Sex			
Female	44(77.19%)	10(28.6%)	NS
Male	13(22.81%)	25(71.4%)	
Age at onset of RA (years)	43.12±14.89	0	-
RA disease duration	62.18±49.61	0	-
Swollen joint count	6.12±3.02	2.02±0.43	0.006
Tender joint count	6.01±2.79	2.13±0.50	0.012
Subcutaneous nodules	1 (1.75%)	0	-
Interstitial lung disease	2 (3.51%)	0	-

P value >0.05 is. Not significant (NS).

Table 2. shows that ESR was 23.8±6.5 in RA patients, while it was 13±3.8 in control group with no statistical difference, CRP was 16.19±7.15 in RA patients, while it was 10.19±3.71 in control group with no statistical difference. RF was detected in 23 (40.35%) and ACPA was detected in 54 (94.73%), while ANA were positive in 14 (24.56%). RF, ACPA and ANA was detected in 4 (11.4%), 2 (5.7%) and 3 (8.57%) respectively in the control group. Rheumatoid factor titre was 367.1±51.2 and ACPA titre was 24.42±6.02. There were significant differences regarding rheumatoid factor titre and ACP titer when compared with control group. Anti-Ro/SSA was detected in 8 (14.03%) RA patients, anti-La/SSB in 3 (5.26%) patients, Anti-Jo-1 was positive in 2 (3.50%) patients, anti-RNP in 1 (1.75%) and no patients had anti-Sm antibodies. Anti-Ro/SSA was detected in 1(2.86%) subject of control group, while anti-Sm, anti-La/SSB, anti-Jo-1, anti-RNP auto-antibodies were not detected.

Table 2. Laboratory findings among the two studied groups (RA patients and controls).

Test	RA patients = 57	Controls = 35	*P* value
ESR 1st hour (mm/h)	23.8±6.5	13±3.8	NS
CRP (mg/dl)	16.19±7.15	10.19±3.71	NS
RF positive	24 (42.10%)	4(11.4%)	0.003
RF titer	367.1±51.2	13.1±6.2	0.001
ACPA positive	54(94.73%)	2(5.7%)	0.005
ACPA titer	24.42±6.02	11.07±3.12	0.019
ANA	14 (24.56%)	3 (8.57%)	0.040
anti-Ro/SSA	8 (14.03%)	1(2.86%)	-
Ant- anti-La/SSB	3 (5.26%)	-	-
Anti-Jo-1	2 (3.50%)	-	-
Anti-RNP	1 (1.75%)	-	-

P value >0.05 is. Not significant (NS).

Anti-Ro/SSA showed a significant negative correlation with sex (r=−0.220, *P*=0.001) being more frequent in female patients (Table 3). There was a positive correlation with sicca symptoms (r= 0.149, *P*= 0.02), Anti-La/SSB antibodies correlated positively with swollen

joint count (r= 0.225, *P*= 0.001), tender joint count (r=0.219, *P*=0.001) and ANA (r= 0.367, *P*<0.001. Anti-Jo-1 antibodies showed a significant positive correlation with interstitial lung disease (r=−0.429, *P*≤0.001), RF titre (r= 0.137, *P*= 0.037) and ANA (r= 0.251, *P*≤0.001). Such data are shown in Table 3.

Table 3. Relationship between extractable nuclear antigens and clinical features in RA patients.

Parameters	Extractable nuclear antigens							
	Pearson correlation							
	Anti-Ro		Anti-La		Anti-Jo-1		Anti-RNP	
	r	*P	r	*P	r	*P	r	*P
Age	−0.071	NS	−0.089	NS	0.127	NS	0.040	NS
Sex	−0.220	0.001	−0.127	NS	0.059	NS	−0.058	NS
Age at onset (years)	−0.080	NS	−0.079	NS	0.064	NS	−0.056	NS
Disease duration (months)	−0.016	NS	−0.015	NS	0.044	NS	0.241	<.001
Subcutaneous nodules	0.072	NS	−0.030	NS	−0.018	NS	−0.013	NS
Swollen joint count	0.121	NS	0.225	0.001	0.081	NS	0.071	NS
Tender joint count	0.117	NS	0.219	0.001	0.072	NS	0.074	NS
Interstitial lung disease	0.050	NS	−0.042	NS	0.429	<.001	−0.018	NS
Sicca symptoms	0.149	0.021	0.089	NS	0.058	NS	0.116	NS

**P* value >0.05 is. Not significant (NS).

Table 4. shows the relation between extractable nuclear antigens and laboratory and radiological findings. Anti-Ro/SSA showed a significant positive correlation with RF titre (r=0.259, *P*<0.001) and ANA (r=0.498, *P*<0.001). Anti-La had significant positive correlation with ANA (r=0.372, *P*<0.001). Anti-Jo-1 had a significant positive correlation with RF titre (r=0.141, *P*<0.040) and ANA (r=0.249, *P*<0.001). Anti-RNP was positively correlated with ACPA titre (r=0.288, *P*<0.001) and ANA (r=0.159, *P*<0.018). There was no correlation between Sharp/van der Heijde scoring and any of ENAs in RA patients

Table 4. Relationship between extractable nuclear antigens, laboratory, and radiological findings in RA patients.

	ENAs in RA patients							
	Pearson correlation							
	Anti-Ro		Anti-La		Anti-Jo-1		Anti-RNP	
	r	*P	r	*P	r	*P	r	*P
ESR 1st hour (mm/h)	0.069	NS	0.088	NS	0.024	NS	0.019	NS
CRP (mg/dl)	0.089	NS	0.131	NS	0.071	NS	0.039	NS
WBCs count ($\times 10^3/mm^3$)	−0.041	NS	−0.020	NS	−0.032	NS	−0.014	NS
RF titer	0.259	<0.001	0.029	NS	0.141	0.040	−0.061	NS
ACPA titer	0.007	NS	0.052	NS	0.019	NS	0.288	<0.001
ANA	0.498	<0.001	0.372	<0.001	0.249	<0.001	0.159	0.018
Sharp/van der Heijde scoring	0.121	NS	0.051	NS	−0.012	NS	0.069	NS

P value >0.05 is. Not significant (NS).

Discussion

Certain autoimmune diseases are characteristically related to the existence of one or more anti-ENA antibodies. Autoantibody association can help to distinguish between different autoimmune diseases and aid in the diagnosis of autoimmune disorders.[17]

In this study, 57 patients with RA were studied, 44 (77.19%) were females and 13 (22.81%) males. Their mean age was 49.12±12.63 years. There was no statistical difference regarding sex and age as compared with the control group. Moeez et al, 2013.[18] also reported that women were three times more susceptible to RA than males and the disease is highly noted in patients at 30-60 years old. Al-Moallim et al., 2020.[19] also observed in a study in a tertiary care centre in Saudi Arabia that the cases mean age was 42.9 years

In this study the swollen joint count was 6.12±3.02 and 2.02±0.43 among RA cases and controls respectively. Tender joint count was 6.01±2.79 and 2.13±0.50 among RA cases and controls respectively. Such data match with the criteria of the American College of Rheumatology (ACR)'s 2010 for RA.[10] According to ACR patients who had with swelling of one or more joints on clinical examination beside presence of laboratory markers, a score of ≥ 6 out of 10 is required to classify a patient as having definite RA.

Subcutaneous nodules and Interstitial lung disease were represented as 1.75% and 3.51%, respectively in RA cases. Subcutaneous nodules were found in 8.8% of RA patients in the series by Sayah and English, 2005.[20] However only in 1.5% in a more recent study by Nyhäll-Wåhlin et al., 2006.[21] who reported that subcutaneous nodules were the least common RF manifestation. Calatayud et al., 2007.[22] stated that interstitial lung disease was a rare manifestation of RA, its prevalence ranged from 0.4% in radiological studies to 3% in lung biopsies from RA patients.

ESR was 23.8±6.5 in RA patients and 13±3.8 in the control group with no statistical difference, while CRP was 16.19±7.15 in RA patients, and 10.19±3.71 in control group with no statistical difference., Al-Moallim and Al-harbi., 2014.[23] found that laboratory tests, such as ESR, CRP may not be specific for RA. RF was detected in 40.35% and ACPA was detected in 94.73%, while ANA was positive in 24.56%. There were significant differences regarding RF and ACP titres when compared with the control group. This was similar to study done in Jeddah, Saudi Arabia by Emad et al., 2021.[15] who reported that RF was detected in 43.9% cases, ACPA was detected in 95.7% and ANA was detected in 25.2%. Also, another study revealed that ACPA had better sensitivity than RF and could be detected earlier in the disease course.[24] Regarding ANA despite the fact that this study found a higher prevalence of ANA, none of the studied patients had any clinical manifestations indicative of ANA-associated rheumatic diseases (AARD). Similar to our findings Romero-Álvarez et al., 2019 in a recent work found higher prevalence of ANA in Mexican patients with early RA reaching 25.8%.[25]

Autoantibodies testing is a time-honoured assessment of AARD or other broad range of systemic autoimmune rheumatic disorders. Autoantibodies are used as elements of validated disease categorization in the majority of these disorders.[26]

Anti-Ro/SSA was detected in 14.03%, anti-La/SSB in 5.26%, Anti-Jo-1 in 3.50%, anti-RNP in 1.75%. Also, Emad et al., 2021[15] had quite similar results in Saudi Arabian subjects. They detected anti-Ro/SSA in 13.5%, anti-La/SSB in 4.3%, anti-Jo-1 in 2.2%, anti-RNP in 0.9% in RA patients. Cavazzana et al., 2006.[27] reported that Anti-Ro/SSA was detected in about 6% of patients with extra-articular manifestation affected by RA and 30% of SLE patients. In a previous study Izmirly et al., 2012.[28] detected anti-Ro/SSA in 90% with subgroups of SLE as old-onset more than 50 years, and in patients with hereditary complements deficiency as C2 or C4, pSS (50–60%), and 8.3% in RA patients. Semelee et al., 2021.[29] reported SSB antibodies in 3.9% of RA female patients. Zanlorenzi et al., 2012.30 detected RNAP antibodies in only 4.5% of all cases and were associated with diffuse cutaneous systemic sclerosis. Faucher et al., 2010.[31] reported that 2 cases out of 66 RA cases

had RNAP antibodies. Bunn et al., 1998.[32] found that RNAP antibodies were good predictors of scleroderma renal crisis. However Anti-Sm antibodies in the present study were not detected in any of the studied patients, this was consistent with findings of a study by Flechsig et al., 2017.[33] who reported that Anti-Sm antibodies were mainly essential for SLE diagnosis.

In this study Anti-Ro/SSA showed a significant negative correlation with sex (r=−0.220, *P*=0.001) being more frequent in female patients. There was a positive correlation with sicca symptoms (dryness of eye, mouth, nose, vagina, dry cough, and other symptoms) (r=0.149, *P*=0.02), Skopouli et al.,1988[34] noted that detection of anti-Ro/SSA antibodies were mainly in RA female patients with high prevalence of sicca symptoms in comparison with RA patients with negative anti-Ro/SSA. Zanlorenzi et al. 2012.[2] examined 385 individuals with RA and observed no correlation between positive anti-Ro/SSA and sex.

Anti-La/SSB antibodies correlated positively with swollen joint count (r= 0.225, *P*= 0.001), tender joint count (r= 0.219, *P*= 0.001) and ANA (r= 0.367, *P*< 0.001. Also, Emad et al, 2021.[14] reported association between Anti-La/SSB antibodies and tender joint count, swollen joint count and ANA. Jardel et al., 2017.[35] reported that anti-La/SSB positivity, without anti-Ro/SSA autoantibody was not related to sicca symptoms.

In the current study anti-Jo-1 antibodies were detected in two cases who could be classified as RA-Anti-synthetase overlapping syndrome. Furthermore, a significant positive correlation was found with interstitial lung disease (r=−0.429, *P*≤0.001), RF titre (r=0.137, *P*=0.037) and ANA (r=0.251, *P*≤.001). Salehi et al, 2017.[36] also reported that positive anti-Jo-1 auto-antibodies has very important role in diagnosis of the Interstitial lung disease (ILD) in patients having ASS.

In conclusion, Anti-ENAs autoantibodies were not always negative in RA patients as more than one connective tissue diseases can exist in RA patient. ENA autoantibodies were crucial and need to be correlated with clinical diagnosis and other serological testing for early diagnosis and intervention of the AR disease. Such autoantibodies should be tested at least once in RA patients when the disease first appears. Anti-Jo1 should be detected in RA patients associated with ASS which can worsen the course of RA patients with sudden start of respiratory distress and fast progressing ILD.

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Author Contributions

ESK planned and designed the study, wrote the protocol, collected the samples, performed the practical laboratory activities, participated in the interpretation of the results and analysis, drafted and critically revised the manuscript. MFAA participated in planning and designing the study, clinical evaluation of cases, sample collection, participated in the interpretation of the results. Both authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

Al-Quwayiyah General Hospital Ethical Committee reviewed and approved the study protocol (May 2021).

Informed consent

Each patient and control participant agreed to be enrolled in the study with provision of written informed consent.

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