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Research Article

Anti-SSA and Anti-dsDNA Autoantibodies in Rheumatoid Arthritis Patients and their Association with Disease Severity: A Case-Control Study in Kerbala Province

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Abstract

Background: Rheumatoid arthritis (RA) is a lifelong autoimmune inflammatory disease of unknown origin. An early diagnosis of RA could improve the outcome. Several autoantibodies have been found in RA patients. ***Objective***: To evaluate the incidence of autoantibodies against Sjögren's syndrome antigen A (SSA) and double-stranded deoxyribonucleic acid (dsDNA) in RA patients and to detect the association between their presence and disease severity markers. ***Methods***: Blood samples were drawn from participants to be used in the detection of ESR and for the simultaneous detection of rheumatoid factor (RF), anti-cyclic citrullinated protein antibodies (ACCP), anti-SSA, and anti-dsDNA by the ELISA technique. ***Result***: The level of autoantibodies differs significantly between patients and healthy people. Anti-SSA was present in about 95.12% and 2.43% of patients and controls, respectively. Additionally, anti-dsDNA was present in 82.92% and 14.63% of patients and controls, respectively. A significant positive correlation between ESR and autoantibody levels was detected. A non-significant correlation was detected between disease activity score 28 (DAS28) and the existence of autoantibodies. ***Conclusion***: ROC analysis demonstrated that RF, ACCP, anti-SSA, and anti-dsDNA had high discriminatory power. The mean levels of these autoantibodies vary dramatically between sick and healthy individuals. The level of RF autoantibody varies dramatically with illness duration. According to DAS28, no autoantibody levels differed considerably.

Keywords: Rheumatoid arthritis, RF, ACCP, Anti-SSA, Anti-dsDNA, DAS28

الأجسام المضادة الذاتية المضادة ل SSA و dsDNA في مرضى التهاب المفاصل الرثوي وارتباطها بشدة المرض: دراسة حالات وشواهد في محافظة كربلاء

الخلاصة

الخلفية: التهاب المفاصل الرثوي (RA) هو مرض التهابي مناعي ذاتي مدى الحياة من أصل غير معروف. التشخيص المبكر لالتهاب المفاصل الرثوي يمكن أن يحسن النتيجة. تم العثور على العديد من الأجسام المضادة الذاتية في مرضى التهاب المفاصل الرثوي. **الهدف**: تقييم حدوث الأجسام المضادة الذاتية ضد مستضد متلازمة سجوجرن A (SSA) وحمض الديوكسي ريبونوكلييك المزدوج الشريط (dsDNA) في مرضى التهاب المفاصل الرثوي والكشف عن الارتباط بين وجودهم وعلامات شدة المرض. **الطريقة**: تم سحب عينات الدم من المشاركين لاستخدامها في الكشف عن ESR وللكشف المتزامن عن عامل الروماتويد، والأجسام المضادة للسيترولين (ACCP)، ومضاد SSA ، ومضاد dsDNA بواسطة تقنية ELISA. **النتيجة**: يختلف مستوى الأجسام المضادة الذاتية اختلافا كبيرا بين المرضى والأشخاص الأصحاء. كان مضاد SSA موجودا في حوالي 95.12٪ و 2.43٪ من المرضى والضوابط، على التوالي. بالإضافة إلى ذلك، كان مضاد dsDNA موجودا في 82.92٪ و 14.63٪ من المرضى والضوابط، على التوالي. تم الكشف عن علاقة إيجابية كبيرة بين ESR ومستويات الأجسام المضادة الذاتية. تم الكشف عن ارتباط غير معنوي بين درجة نشاط المرض (DAS28) ووجود الأجسام المضادة الذاتية. **الأستنتاج**: أظهر تحليل ROC أن RF و ACCP و anti-SSA و anti-dsDNA تتمتع بقوة تمييزية عالية. تختلف المستويات المتوسطة لهذه الأجسام المضادة الذاتية بشكل كبير بين الأفراد المرضى والأصحاء. يختلف مستوى الأجسام المضادة الذاتية RF بشكل كبير مع مدة المرض. وفقا ل DAS28، لم تختلف مستويات الأجسام المضادة الذاتية بشكل كبير.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown origin that can cause a wide range of clinical symptoms, including mild to severe joint inflammation that can cause pain, dryness, and joint destruction, as well as joint deformity and disability [1]. RA affects 0.5% to 1% of the global population [2]. RA has a female/male ratio of 2:1, and its prevalence increases with age [3]. RA can be difficult to diagnose, particularly in the early stages of the disease. The development of autoantibodies is critical for RA categorization, according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for RA [4]. An accurate early diagnosis of RA can aid in more successful treatment of the condition. In the serological evaluation of RA, several autoantibodies are employed. RF, an autoantibody to the Fc region of IgG, was found in numerous autoimmune disorders, including RA and systemic lupus erythematosus (SLE) [5]. RF can be found in up to 70-80% of RA patients [6]. ACCPs are autoantibodies with a high specificity for RA. When the enzyme peptidyl arginine deiminase (PAD) deaminates the amino acid arginine, a nonstandard amino acid termed citrulline is formed. Citrullination is a frequent biological process that occurs during cell death, inflammation, and differentiation [7]. ACCPs can be identified with high specificity (85-99%) in up to 80% of individuals with well-known RA. Furthermore, the presence of ACCPs has been linked to improved radiographic development and therapeutic response, making them valuable prognostic indicators. ACCPs have been found in the serum of RA patients, years before the disease manifests itself [8]. Anti-Ro/SSA antibodies are the most common in several autoimmune illnesses, including SLE and Sjögren's Syndrome (SS)/SLE overlap syndrome [9]. SS can occur on its own, as primary SS, or in conjunction with other rheumatic disorders, as secondary SS, such as RA. The presence of SS with RA worsens the condition and increases RA mortality and morbidity [10]. Anti-Ro/SSA antibodies, which contain anti-Ro/SSA-52kD and anti-Ro/SSA-60kD sub-specificities, are the result of an autoimmune reaction against the two Ro subunits that comprise the intracellular ribonucleoprotein (Ro52-kD and Ro60-kD) [11]. Anti-Ro/SSA antibodies were found in RA at rates ranging from 3 to 15% [12]. Anti-dsDNA antibodies can be produced by RA patients; however, they frequently coexist with therapy. They are a type of autoantibody that frequently emerges briefly after therapy and has a low avidity. Anti-dsDNA antibodies particularly target genetic material. In some situations, the presence of these antibodies may result in a lupus-like state [13]. SLE symptoms, as well as the presence of high-specificity antibodies such as anti-dsDNA, anti-Smith, and ACCP antibodies, are linked to Rhupus syndrome (RhS), a deforming and erosive symmetric polyarthritis [14]. The existence or lack of anti-SSA and anti-dsDNA antibodies in RA patients living in Kerbala governorate, Iraq, has never been examined. The goal of this study was to determine the prevalence of autoantibodies against Ro/SSA and dsDNA in RA patients, as well as the association between the presence of these autoantibodies and RA development and disease severity indices.

METHODS

Study Design and patient selection

This research was carried out at the University of Kerbala's College of Applied Medical Sciences using a case-control study design. There were 82 subjects in all (41 RA sufferers and 41 healthy ones) enrolled in the study. Rheumatologists at Al-Hindiya Teaching Hospital and Al-Imam Al-Hassan Al-Mujtaba Hospital in Kerbala, Iraq, made the diagnoses between November 2022 and February 2023. In terms of sex, age, and residency location, the patients were matched with healthy volunteers.

Inclusion and exclusion criteria

To enroll patients and control groups in the current investigation, the following inclusion criteria were used: The patients' group included patients with RA who were clinically diagnosed by physicians; both male and female patients were included; the patients' ages were above 18 years; and healthy subjects with no history of RA who matched the same age, gender, and residency as the patients' group were included. Subjects were excluded if they were under the age of 18 and had comorbidities such as osteoarthritis, psoriatic arthritis, gout, or fibromyalgia.

Outcome evaluation and measurements

Five milliliters of venous blood were drawn from each participant. About 1.5 ml of blood was collected in the ESR tube for ESR detection, and 3.5 ml of blood was drawn in a gel tube and left to clot at room temperature for 15 minutes. Serum was separated by centrifuging it for 10 minutes at about 3000 rpm. The serum sample was used for measurement of the RF, ACCP, anti-SSA, and anti-dsDNA autoantibodies using the ELISA technique.

Ethical consideration

This study was approved by the Ethical Committee at the College of Applied Medical Science, University of Kerbala, and the Ethical Committee at Al-Hindiya Teaching Hospital and Al-Imam Al-Hassan Al-Mujtaba Hospital. All subjects involved in this work were informed, and their consent was obtained verbally before the samples' collection.

Statistical analysis

Descriptive statistics were utilized to determine frequencies, the mean, standard deviation, median, range, and cross-tabulation. Bivariate correlations were analyzed to determine

significant positive and negative correlations between variables if they were present. An independent sample T-test and the Analysis of Variance (ANOVA) test were utilized to compare mean significance. The chi-square test evaluated the categorical variables. ROC curve analysis was utilized to calculate the cut-off values of autoantibodies. A $p<0.05$ was considered statistically significant. The Statistical Package for Social Sciences (SPSS) version 22 program (IBM Corp., NY, USA) was utilized to conduct all statistical analyses.

RESULTS

Out of 82 individuals involved in this study (41 RA patients and 41 controls), the female patient frequency was higher than that of males (34:7). The ages of participants ranged from 27 to 77 years, and the mean±SD of age for those with RA and healthy people were 48.29±11.22 and 48.37±11.12, respectively. Regarding the BMI of participants, the mean±SD of patients and healthy people was 30.88±5.86 and 29.82±5.45, respectively. The mean value for the disease's onset age was 40.64±14.22, as shown in Table 1.

Table 1: Descriptive data of RA patients and Controls

Parameter	RA patients *n*=41	Control *n*=41
Age (year), Median (Range)	49 (27-77)	49 (27-75)
Mean±SD	48.29±11.22	48.37±11.12
Gender *n*(%)		
Male	7 (17.07)	7 (17.07)
Female	34 (82.92)	34 (82.92)
BMI kg/m^2	30.89±5.87	29.82±5.46
Family history *n* (%)	11(26.82)	0 (0)
Disease duration	7.65±8.36	---
The disease's onset age (year)	40.63±14.22	---
Morning stiffness *n* (%)	33 (80.48)	---

Analysis of Receiver Operating Characteristics (ROC) revealed that the overall AUC, sensitivity, and specificity for RF, ACCP, SSA, and dsDNA were as follows: (0.979, 0.976, 100), (0.987, 0.976, 0.902), (0.995, 0.951, 0.976), and (0.938, 0.829, 0.854), respectively, as shown in Figure 1 and Table 2. This study showed that the levels of autoantibodies significantly differ between patients and control groups, as shown in Table 3.

Table 2: ROC values of autoantibodies

Variables	Cut-off point	AUC	Sensitivity	Specificity
RF	0.901	0.979	0.976	100
ACCP	0.328	0.987	0.976	0.902
Anti-SSA	20.95	0.995	0.951	0.976
Anti-dsDNA	0.298	0.938	0.829	0.854

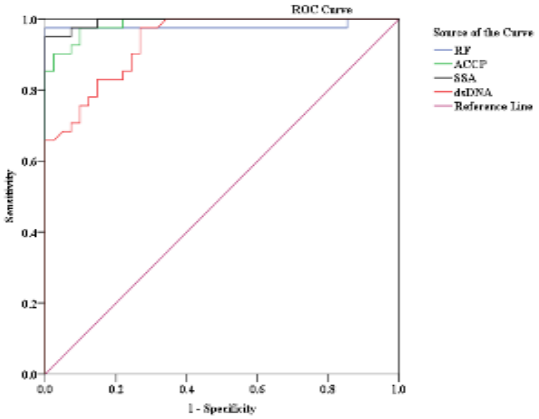

Figure 1: ROC curve illustrating the sensitivity and 1-specificity values for RF, ACCP, SSA, and dsDNA, levels.

Table 3: Comparison of Autoantibodies between RA patients and controls

Autoantibodies	Patients			Control			Chi-square *p*-value		*p*-value
	Positive *n*(%)	Negative *n*(%)	Mean ±SD	Positive *n*(%)	Negative *n*(%)	Mean±SD	Positive	Negative	Total
RF	40(97.56)	1(2.43)	2.08±1.09	0(0)	41(100)	0.74±0.08	0.00	0.00	0.00
ACCP	40(97.56)	1(2.43)	0.52±0.18	4(9.75)	37(90.24)	0.26±0.04	0.00	0.00	0.00
Anti-SSA	39 (95.12)	2(4.87)	35.5±12.1	1(2.43)	40(97.56)	14.21±3.26	0.00	0.00	0.00
Anti-dsDNA	34(82.92)	7(17.07)	0.51±0.21	6(14.63)	35(85.36)	0.26±0.04	0.00	0.00	0.00

The current study revealed the presence of significant differences in RF autoantibodies with disease duration, and non-significant differences were found regarding ACCP, anti-SSA, and anti-dsDNA autoantibodies, as revealed in Table 4.

Table 4: Distribution of autoantibodies presence according to disease duration.

Autoantibodies Mean± S.D	Disease duration			ANOVA *p*-value
	<5 year *n*=21	5-10 year *n*=10	>10 year *n*=10	
RF	2.08±1.09	1.49±0.37	2.67±1.34	0.05
ACCP	0.52±0.18	0.50±0.23	0.56±0.17	0.73
Anti-SSA	36.46±12.10	33.78±12.82	35.32±12.58	0.85
Anti-dsDNA	0.51±0.21	0.61±0.28	0.42±0.13	0.16

As shown in Table 5, all tested autoantibodies significantly differ between patients and controls in both normal and high ESR groups. The study also showed no significant differences in autoantibody levels between patients whose ESR was normal and those whose ESR was high in all studied autoantibodies.

Table 5: Distribution of autoantibodies presence according to ESR

Sample	ESR group	*n*	RF Mean± SD	ACCP Mean± SD	Anti-SSA Mean± SD	Anti-dsDNA Mean± SD
Control	Normal	36	0.75±0.08	0.27±0.04	14.08±3.23	0.26±0.04
	High	5	0.77±0.12	0.25±0.05	15.18±3.73	0.27±0.06
	Total	41	0.75±0.08	0.27±0.04	14.21±3.26	0.26±0.04
	p-value		0.61	0.21	0.49	0.72
Patient	Normal	13	1.71±0.54	0.47±0.14	33.46±11.97	0.52±0.20
	High	28	2.25±1.25	0.55±0.20	36.49±12.29	0.51±0.23
	Total	41	2.08±1.10	0.52±0.19	35.53±12.12	0.51±0.22

A similar finding was observed in the control group. There was a non-significant difference in autoantibody titers according to DAS28 for all tested autoantibodies, as shown in Table 6.

Table 6: Distribution of autoantibodies presence according to DAS28

Autoantibodies	DAS28 (Mean±SD)					ANOVA *p*-value
	Remission *n*=5	Low activity *n*=1	Moderate activity *n*=23	High activity *n*=23	Total *n*=41	
RF	1.85±0.48	0.94±0	2.20±1.21	2.06±1.08	2.08±1.10	0.680
ACCP	0.51±0.14	0.38±0	0.56±0.22	0.47±0.12	0.52±0.19	0.457
Anti-SSA	33.65±11.02	31.65±0	36.62±12.63	34.53±12.83	35.53±12.12	0.927
Anti-dsDNA	0.51±0.20	0.46±0	0.52±0.22	0.49±0.25	0.51±0.22	0. 977

The present study has shown a significant positive correlation between ESR and autoantibody titers, as shown in Table 7. The study showed a negative correlation between DAS28 and autoantibodies, as shown in Table 8.

Table 7: Correlation between autoantibodies and ESR

		RF	ACCP	Anti-SSA	Anti-dsDNA	ESR
RF	*r*	1	0.438	0.500	0.364	0.467
	p-value		0.000	0.000	0.001	0.000
ACCP	*r*		1	0.717	0.443	0.414
	p-value			0.000	0.000	0.000
Anti-SSA	*r*			1	0.507	0.396
	p-value				0.000	0.000
Anti-dsDNA	*r*				1	0.258
	p-value					0.020
ESR	*r*					1
	p-value					

Table 8: Correlation between autoantibodies and DAS28

		DAS28	ESR	RF	ACCP	Anti-SSA	Anti-dsDNA
DAS28	*r*	1	0.460**	-0.080	-0.098	-0.123	-0.073
	p-value		0.003	0.621	0.542	0.443	0.652

DISCUSSION

This research evaluates the prevalence of autoantibodies against Ro/SSA and dsDNA in RA patients resident in Kerbala governorate and detects the relationship between the existence of these autoantibodies and RA development and disease severity markers. This study revealed that female patient frequency was higher than that of males (4.8:1). This finding was in agreement with other previous studies [3,15,16]. It has been documented that the reason why the incidence of autoimmune diseases in females is higher than that in males is due to several factors, including the microbiome, behavior, hormones, and genetics, including the inactivation of genes in the X chromosome [17]. Regarding the BMI of participants, the mean values of patients and healthy people were 30.88±5.86 and 29.82±5.45, respectively. This finding was in agreement with the previous study [18]. The mean for the disease's onset age was 40.64 ±14.22. This finding was in agreement with a previous study that showed the mean for the disease's onset age was 41±2.1 [3]. The overall AUC, sensitivity, and specificity for RF, ACCP, SSA, and dsDNA were as follows: (0.979, 0.976, 100), (0.987, 0.976, 0.902), (0.995, 0.951, 0.976), (0.938, 0.829, 0.854), respectively. Comparable results were documented in previous studies that showed the sensitivity and specificity for RF and ACCP were (84.0% and 80%) and (68% and 97.5%), respectively [19]. Another previous study revealed that the sensitivity and specificity for RF and ACCP were 70%, 95%, 78.8%, and 100%, respectively [20]. Another previous study revealed the sensitivity and specificity of RF and ACCP were 86.9%, 96%, 73%, and 100%, respectively [6]. Aiman *et al*. showed in their study that the AUC, sensitivity, and specificity of both RF and ACCP were 100%, 100%, and 1.00 [5]. Additionally, a previous study revealed that the sensitivity and specificity of ACCP were 72.7% and 98.8%, respectively [21]. The current study revealed that 40 (97.56%) of patients had positive RF autoantibodies titres higher than the cut-off value, and all the subjects in the control group were negative (RF autoantibodies titres lower than the cut-off value), as shown in Table 3. This result was in agreement with a prior study in which the author reported that RF was found in all RA patients' serum samples (100% positive results) with 100% negative results in healthy controls [22]. Additionally, another study documented negative detection (100%) of antibodies for RF in the control group [23]. The study also revealed that 40 (97.6%) and 4 (9.8%) of patients and controls, respectively, had ACCP titers higher than that of the cut-off value, as shown in Table 3. Similar findings were reported in other previous studies [24-26]. A lower percentage was found in another study in which the ACCP was positive in 53.1% of RA patients and 4.7% of controls, and the frequency of RF was 61.87%. However, a higher percentage of RF was reported in the control group (17.66%) [27]. Concerning anti-SSA, 39 (95.12%) and 1 (2.43%) patients and controls, respectively, had anti-SSA titres higher than the cut-off value, as shown in Table 3. Lower percentages were reported in previous studies, ranging from 3–15.23% [28–30]. Regarding anti-dsDNA, the current study also revealed that 34 (82.92%) and 6 (14.63%) patients and controls, respectively, had dsDNA autoantibodies titers higher than the cut-off value, as shown in Table 3. Little studies focused on the level of dsDNA autoantibodies in RA patients. However, one of the previous studies showed that 66.1% of RA patients had anti-DNA autoantibodies [31]. And another study revealed that 51% of RA patients had ANA autoantibodies [29]. A lower percentage (6%) was reported in a previous study [32]. This study showed that the levels of autoantibodies significantly differ between patients and control groups, as shown in Table 3. Similarly, the results of previous studies revealed that the ACCP level differs significantly between patients and controls [5, 33, 34], in the RF and ACCP levels [26, 34], and in the anti-dsDNA level [31]. Regarding disease duration, Al-ubaidi et al. reported no significant difference in ACCP autoantibodies [35]; Badran also reported in his study that RF and ACCP didn't significantly differ [36]. Additionally, Abd-Ali et al. reported no significant difference in RF and ACCP autoantibodies [24]. The difference in RF between the present study and the studies by Al-Ubaidi et al., Badran, and Abd-Ali et al. could be due to variances in patient numbers and duration of disease categories between the studies. The current study revealed that the mean of DAS28 in patients was 4.32±1.166. This finding agreed with a previous study [16]. This study revealed that the mean of DAS28 in patients was 4.32±1.166. This result agreed with a prior study [16]. There was a non-significant difference in autoantibody levels according to DAS28 for all tested autoantibodies. A similar finding was reported in a previous study in which the author reported no significant difference in RF titer according to disease activity [37]. Theoretically, it is assumed that the disease activity score is increased by increasing the mean of autoantibodies, but the current study showed inconsistent results. Perhaps the reason is due to the inaccurate measurement of the DAS28 as a result of the bias that results from the wrong global assessment of patients' health conditions. The present study showed a significant positive correlation between ESR and

autoantibody titers. Similar findings were observed in other prior studies that found a significant correlation between RF and ACCP [5,24,38], between ESR and both RF and ACCP [39], and between anti-Ro/SSA and both RF and ANA [26]. And between ACCP and ESR [33]. Inversely, this result was inconsistent with other previous studies that showed no significant correlation between anti-dsDNA with RF and ACCP [13], between anti-Ro with both RF and ACCP [40], between ACCP and RF [41], between ACCP and ESR [38], and between anti-Ro/SSA with both ACCP and ESR [26]. The study discovered a negative correlation between DAS28 and autoantibodies. Similar findings were reported in a prior study that showed no correlation between ACCP titers and the activity of disease [42].

Conclusion

ROC analysis demonstrated that RF, ACCP, SSA, and dsDNA have high discriminatory power. There was no significant impact of age on the existence of autoantibodies. The levels of RF autoantibodies vary significantly related to disease duration but not according to DAS28 for all tested autoantibodies. ESR and autoantibody titers have a favorable relationship.

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Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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