Association between Antinuclear Antibodies Patterns and Immunological Markers with Systemic Lupus Erythematosus in Iraqi Female Patients

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KEYWORDS

Systemic lupus erythematosus, Autoantibodies, ANA, Indirect immunofluorescence

ABSTRACT

Background: Systemic lupus erythematosus is a condition where the immune system attacks various tissues and organs, affecting commonly women between the ages of 15 to 44. It is caused by genetics or environmental factors. A critical factor in the diagnosis by antinuclear antibodies at a level of ≥1:80. Objective: To assess the disease activity and identification of antinuclear antibody patterns and titer on Hep-2 cells with immunological markers. Methods: This was a case-control study conducted at Baghdad Teaching Hospital/Medical City between January 25, 2024, to April 25, 2024. 60 SLE patients were contrasted with 60 healthy controls. The American College of Rheumatology SLE criteria were used to make the diagnosis. Results: SLE patients had a mean age of 32.12±10.54 years, while controls had a mean age of 31.37±10.65 years (P=0.699). positive family history represents 15.0 % while negative family history of SLE patients is 85.0%. The mean disease duration was 5.25±4.77 years. The disease activity index (SLEDI) was (27.80 ±10.40). The most common patterns of Antinuclear Antibodies (ANA) are speckled (56.7%), homogeneous (26.7%), peripheral (1.7%), mixed (3.3%), and negative (11.7%) patterns. Conclusion: Antinuclear antibodies are considered the gold standard diagnostic test. A few healthy individuals display speckled patterns at a ratio of 1:40. The disease activity no significant with pattern.

1. Introduction

Systemic lupus erythematosus is a chronic autoimmune disease that mainly affects any part of the body and is indicated by tissue inflammation that leads to the production of autoantibodies (Fujita K. et al., 2023). It is related to important mortality and morbidity, which mainly affects females than males with a prominent 9:1 ratio, those who occur between the ages of 16 and 55 years (Bello N. et al., 2022). The worldwide prevalence of SLE and newly diagnosed individuals was expected to be 5.14 per 100,000 person-years. With larger percentages among females 8.82 per 100,000 person-years compared to males 1.53 per 100,000 person-years (Tian J. et al., 2023). Antinuclear antibodies (ANAs) are important laboratory indications for SLE to assist with screening and monitoring (Abozaid H.S.M. et al., 2023). The indirect fluorescence on Hep-2 is the golden standard and the classical method for the identification of ANA for testing in clinical practice because of its high sensitivity (Mahler M. et al., 2014). Immunological markers for SLE are Anti-double-stranded DNA antibodies that are very specific to the diagnosis (Lee E.E. et al., 2020).

2. Methodology

This case-control study involved 60 female patients diagnosed with SLE aged between 16 to 55 years from the (Rheumatology clinic at Baghdad Teaching Hospital/Medical City) who met the 2019 ACR criteria for SLE diagnosis. The control group consisted of 60 healthy female individuals selected from both patients and healthy controls based on a questionnaire. The research took place from January 25, 2024 to April 25, 2024.

Immunological tests: Quantitative measurement anti-nuclear antibodies by indirect immunofluorescence tests Mosaic: Hep-2/Liver (Monkey) (IIFT) Kit from (EUROIMMUN, Germmany), quantitative measurement by Enzyme-linked immunosorbent assay (ELISA) of human Kit from (Sunlong biotech, Chain). Inclusion criteria, adult female patients with SLE. Exclusion criteria Patients who have experienced other autoimmune diseases. Pediatric patients with systemic lupus erythematosus. Male patients with systemic lupus erythematosus.

A data collection sheet with questionnaires contained the following information general demographic data: Name, age, residency, education level, occupation, marital status, number of children, Abortions, body measurement, height, weight, body mass index, family history, menstrual history, drugs intakes and duration of disease.

Ethical issue, approval, and official permission: Before data collection, a verbal agreement was acquired from each participant after describing the study's goal, ensuring data protection, and approval from appropriate health authorities (2639-22/1/2024).
Statistical analysis. Statistical analysis was carried out using the Social Sciences (SPSS) version 26.0. Mean, SD were used to express variables. The statistical significance of such associations was assessed by Chi-square (X^2) test.

3. Result and Discussion

Demographical Characteristics of the Study Groups

The socio-demographic characteristics of the studied samples are shown in Table 1. The range of females age from (16 to 55) years. Patients had an age of 32.12±10.54, while healthy individuals had a mean age of 31.37±10.65. Approximately 85% of patients in the group had no family history**.** Over the observation period, the mean SLEDAI value was 27.80 ± 10.40. Characteristics of medication use there were hydroxychloroquine, prednisolone, azathioprine, non-steroidal anti-inflammatory drugs, mycophenolate mofetil, rituximab, methotrexate and cyclophosphamide (93.3%), (91.7%), (51.7%), (36.7%), (13.3%), (8.3%), (3.3%), and (3.3%), respectively.

Table 1: Demographical Characteristics of the Study Groups

Parameters		**Patient (n=60)** No. (%)	**Control (n=60)** No. (%)
Age	Mean ± SD	32.12±10.54	31.37±10.65
Family history	No	51 (85.0)	60 (100)
	Yes	9 (15.0)	0 (0)
Duration of disease (years)	Mean ± SD	5.25±4.77	-
Physicians Global Assessment	0 None	1 (1.7)	-
	1 Mild	32 (53.3)	

	2 Moderate	18 (30.0)	-
	3 Severe	9 (15.0)	-
SLEDAI score	Mean ± SD	27.80±10.40	-
Medication	Hydroxychloroquine	56 (93.3)	-
	Prednisolone	55 (91.7)	-
	Azathioprine	31 (51.7)	-
	NSAIDs	22 (36.7)	-
	Mycophenolate Mofetil	8 (13.3)	-
	Rituximab	5 (8.3)	-
	Methotrexate	2 (3.3)	-
	Cyclophosphamide	2 (3.3)	-

NSAIDs: Non-steroidal anti-inflammatory drugs

Patterns of ANA Pattern and the Correlated Titers among Different Study Groups

The speckled (56.7%) and homogeneous (26.7%) ANA patterns were most commonly seen, with peripheral (1.7%) and mixed (3.3%) patterns following. 11.7% of patients had negative patterns. 40.0% of patients had higher ANA IIF titers at 1:160. The ANA IIF titer 1:160 demonstrates titers in mixed patterns. The highest titers were seen in patients with Peripheral patterns, with 100.0% having ANA titer 1:320, followed by homogeneous and speckled patterns at 37.5% and 35.3% respectively. Table 2 and fig. (1), (2), (3), (4), (5).

Table 2: Titers and patterns of antinuclear antibodies in study groups.

Titer		Patient Pattern					Total Patient	Total Control	Total
		Speckled	Homogenous	Peripheral	Mixed	Negative			
1:40	No.	2	0	0	0	0	2	1*	3
	%	5.9	0.0	0.0	0.0	0.0	3.3	1.7	2.5
1:80	No.	6	2	0	0	0	8	0	8
	%	17.6	12.5	0.0	0.0	0.0	13.3	0.0	6.7
1:160	No.	14	8	0	2	0	24	0	24
	%	41.2	50.0	0.0	100	0.0	40.0	0.0	20.0
1:320	No.	12	6	1	0	0	19	0	19
	%	35.3	37.5	100	0.0	0.0	31.7	0.0	15.8
Negative	No.	0	0	0	0	7	7	59	66
	%	0.0	0.0	0.0	0.0	100	11.7	98.3	55.0
Total	No.	34	16	1	2	7	60	60	120
	%	56.7	26.7	1.7	3.3	11.7	50	50	100

Chi-square: statistics 133.976, df =16, p=0.0001; Kruskal=Wallis's test: statistics =110.019, df=4, p=0.0001; P< 0.01 that mean (HS); * One of the healthy individuals that appears to titer 1:40 speckled pattern of ANA

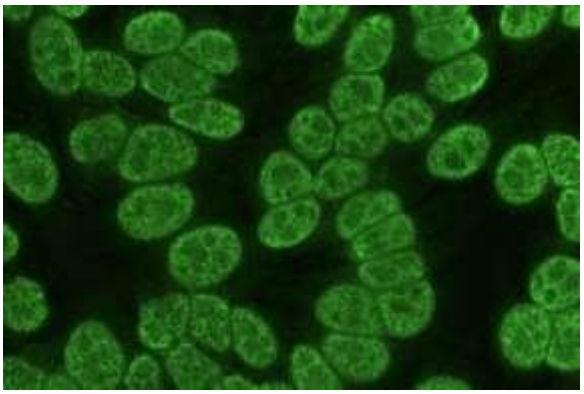

Figure 1 (Speckled Pattern)

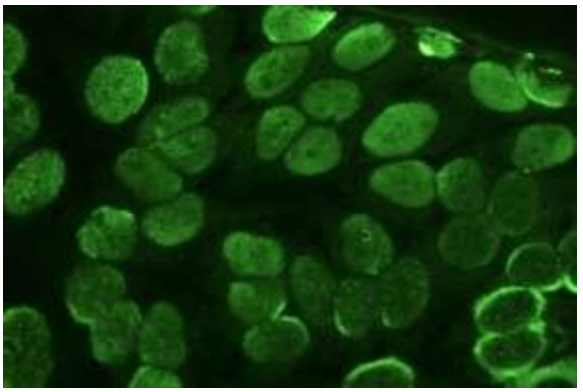

Figure 2 (Homogenous Pattern)

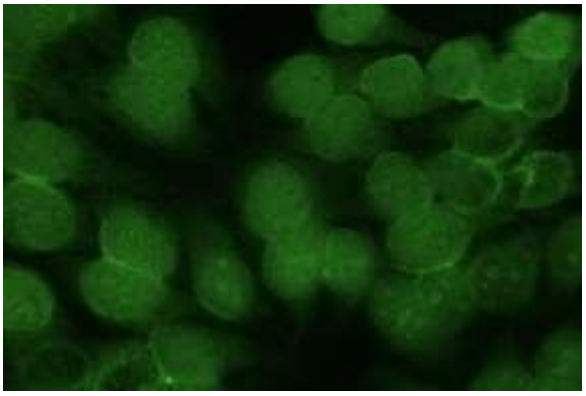

Figure 3 (Mixed Pattern)

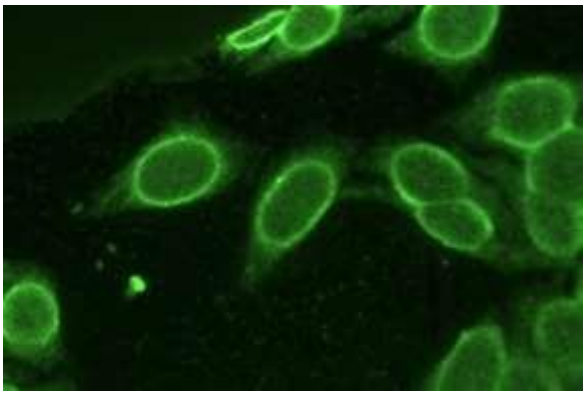

Figure 4 (Peripheral Pattern)

Figure 5 (Negative Pattern)

Patterns of ANA and the Correlated Physician's Global Assessment among SLE Group.

Classification of the PGA of disease activity into four stages (None) for activity disease showed 1.7% from the patient group, 100% with speckled, and other patterns 0.0%. (Mild) 53.3% of the patient group showed 59.4% within speckled, 25.0% with homogenous, 0.0% peripheral, and 6.3% with mixed, While 9.4% with negative pattern. (Moderate) 30.0% from the SLE Patient group showed 55.6% with speckled, 22.2% with homogenous, 5.6% with peripheral, 0.0% with mixed, and 16.7% with negative pattern. (Severe) 16.0% of the patient group showed 44.4% with speckled, 44.4% with homogenous, 0.0% with peripheral and mixed, and 11.1% with negative pattern. The Findings of this study no correlation between patterns of ANA and PGA this detail is shown in Table 3.

Table 3: Association physician's global assessment and patterns of ANA in the SLE group.

ANA patterns	Patient n=60				P-value
	None	Mild	Moderate	Severe	

		N=1	N=32	N=18	N=9	
Speckled	**No.**	1	19	10	4	0.702
	%	100	59.4	55.6	44.4	
Homogenous	**No.**	0	8	4	4	0.563
	%	0.0	25.0	22.2	44.4	
Peripheral	**No.**	0	0	1	0	0.613
	%	0.0	0.0	5.6	0.0	
Mixed	**No.**	0	2	0	0	0.499
	%	0.0	6.3	0.0	0.0	
Negative	**No.**	0	3	3	1	0.865
	%	0.0	9.4	16.7	11.1	

Association between Biomarkers and Anti-nuclear Antibody IIF

Within the ANA-IIF pattern, a speckled pattern was observed in 88.2% in anti-dsDNA, while anti-SS-A was present in 52.9%, anti-SSB in 17.6%, anti-Sm in 47.1%, and anti-RNP in 17.6%. Additionally, C3 was present in 73.5%, and C4 in 47.1%. The homogenous pattern showed anti-dsDNA in 87.5%, anti-SS-A in 56.3%, anti-SSB in 12.5%, anti-Sm in 43.8%, and anti-RNP in 25.0%.C3 was found in 75.0%, and C4 in 50.0%. All details can be seen in Table 4.

Table 4 : Association between Biomarkers and Anti-nuclear Antibody IIF in the Study Groups

parameters	No. %	Patient					Total patient n=60	Control n=60	Total n=120	P-value
		Speckled N=34	Homogenous N=16	Peripheral N=1	Mixed N=2	Negative N=7				
Anti-dsDNA	**No.**	30	14	1	2	5	52	0	52	0.001**
	%	88.2	87.5	100	100	71.4	86.7	0	43.3	
Anti-SSA	**No.**	18	9	0	0	4	31	0	31	0.001**
	%	52.9	56.3	0.0	0.0	57.1	51.7	0	25.8	
Anti-SSB	**No.**	6	2	0	0	1	9	0	9	0.063
	%	17.6	12.5	0.0	0.0	14.3	15.0	0	7.5	
Anti-Smith	**No.**	16	7	0	0	4	27	0	27	0.001**
	%	47.1	43.8	0.0	0.0	57.1	45.0	0	22.5	
Anti-RNP	**No.**	6	4	0	0	0	10	0	10	0.004*
	%	17.6	25.0	0.0	0.0	0.0	16.7	0	8.3	
C3	**No.**	25	12	1	2	4	44	0	44	0.001**
	%	73.5	75.0	100	100	57.1	73.3	0	36.7	
C4	**No.**	16	8	1	2	2	29	0	29	0.001**
	%	47.1	50.0	100	100	28.6	48.3	0	24.2	

Discussion

In this result the no family history of the auto immune disease that high (85%) that agree to Kandane's research, a significant majority (92.6%) of individuals with SLE do not have a family history of the disease (Kandane-Rathnayake R. *et al.*, 2022).Proposing that mutations in the individual's germline are

more prone to causing SLE in individuals without a familial background of the disease (Harley I.T.W. and Sawalha A.H., 2022). Support Fatima's research findings on the severity of SLE disease in females from Kerbala, with percentages indicating mild (58.3%), moderate (30.6%), and severe (11.1%).(Fatimah Abdul Hussein K. *et al.*, 2024). HCQ, primarily utilized for treating SLE, has demonstrated effectiveness in reducing disease activity, mainly in mild to moderate cases, preventing disease flare-ups, and reducing the need for long-term glucocorticoids. The multiple advantages of HCQ also apply to pregnancy and the period of breastfeeding (Dima A. *et al.*, 2022).

This study and several others have shown that the percentages of speckled (56.7%) and homogeneous (26.7%) are comparable. Aishwarya Ramachandran's study also found that the percentages of speckled (52.9%) and homogenous (27.5%) are similar to those of the current study. In an Egyptian female, the speckled pattern was the most common (41.3%) (ELAMIR A.M. *et al.*, 2019). Anis's study, on the other hand, was speckled (56%) and disagreed with his study in homogenous (42%) (Anis S. *et al.*, 2023, Ramachandran A. *et al.*, 2023). Similar patterns were found in another Swedish research with 219 individuals, with homogeneous and speckled patterns being the most common; however, homogeneous patterns (54.3%) were more common than speckled patterns (22.4%). A mixed pattern came in third in prevalence, with an accounting prevalence of 11.0% (Frodlund M. *et al.*, 2013). The research investigation reveals a negative (11.7%), which is greater than the 6.2% negative result obtained by Choi and less than the 17.6% negative result obtained by Hanan Sayed M. Abozaid (Choi M.Y. *et al.*, 2019, Abozaid H.S.M. *et al.*, 2023).

The physician global assessment, a visual analog score, is a measure of the overall activity of SLE disease as assessed by the clinician (Chessa E. *et al.*, 2020). The current study supports the findings of another researcher that there is no relationship between ANA patterning and SLEDAI-determined disease activity (Zhang T. *et al.*, 2022).
In this outcome, the highest percentage was 56.7% for speckled and 26.7% for homogenous findings. The strong correlation between Anti-nuclear antibody IIF with anti-dsDNA, and C4 is highly significant with a P-value of 0.001. This aligns with previous studies that also found significant differences at 0.007 and 0.003, respectively. However, the C3 P-value of 0.066 shows no significant differences (Al-Mughales J.A., 2022). There is no agreement with another researcher that the P-value is greater than 0.01 (Ramachandran A. *et al.*, 2023).

4. Conclusion and future scope

Anti-dsDNA is a communal antibody responsible for the activity of diseases. ANA patterns commonly in patients are speckled and also the speckled pattern was positive for low titer (1:40) in healthy control. The disease activity no significant with pattern.

Limitations

A few possible limitations should be taken into account when interpreting the results of this study. The important note was the small number of patients, which was partly because rheumatic disorders are uncommon. Additionally, all subjects were from a single institution. It is essential to repeat this study with a bigger sample size as our patients may not be typical of all Iraqi patients.

Conflict of interest

There is no conflict of interest.

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