Evaluation of serum anti-nuclear antibodies and anti-dsDNA in patients with systemic lupus erythematosus

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Abstract

Background: Systemic lupus erythematosus (SLE) is characterized by the production of autoantibodies and dysregulation of cytokines. This study aimed to describe the results of anti-nuclear antibodies (ANA) and antibodies against double-stranded DNA (anti-dsDNA) tests in patients with SLE and to investigate the association between anti-dsDNA and ANA. **Materials and methods:** A retrospective data and cross-sectional descriptive study were conducted on 147 patients diagnosed with SLE who attended Hue University of Medicine and Pharmacy Hospital from January 2018 to April 2023. **Results:** The mean age of the study population was 32.2 ± 13.6 years, with the most common age range being 20 to below 40 years (59.2%). The prevalence of SLE in females was observed in 127 out of 147 (86.4%) patients, which was 6.35 times higher than in males. The rates of ANA and anti-dsDNA positivity in SLE patients were 57.1% and 29.9%, respectively. The prevalence of positive ANA result tests in females was higher than in males, with a statistically significant difference ($p<0.05$). Specifically, the rate of positive anti-dsDNA in ANA-positive patients (52.4%) was significantly higher compared to ANA-negative patients (0.0%) ($p<0.05$). **Conclusions:** The prevalences of positive ANA and anti-dsDNA test results in patients with SLE were 57.1% and 29.9%, respectively. There is a statistically significant association between anti-dsDNA and ANA test results in patients with SLE ($p<0.05$).

Keywords: *Systemic lupus erythematosus, ANA, anti-dsDNA antibodies, ELISA.*

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that affects many organs including joints, skin, brain, lungs, kidneys, and blood vessels. The production of autoantibodies and dysregulation of cytokines are the outstanding features of the disease [1]. The disease mainly occurs in women aged 15-44 years, with a female-to-male ratio ranging from 8:1 to 15:1 [2], [3]. SLE disease has many different clinical manifestations from mild to severe disease symptoms and even life-threatening damage due to the severe consequences of the disease. The cause and the pathogenesis of the disease are still not clear. However, many studies revealed that environmental and genetic factors interact to trigger immune responses causing the overproduction of pathogenic autoantibodies and dysregulation of cytokines, thereby leading to tissue damage. The clinical heterogeneity and complex pathogenesis of SLE pose challenges in diagnosis, treatment, and prognosis [4].

SLE is characterized by the presence of antibodies against nuclear and cytoplasmic antigens [5]. ANA is a serological marker that commonly occurs in SLE patients and can be used for screening, diagnosis, and prognosis. ANA has high sensitivity ranging from 95% to 97% but low specificity of only 20% in SLE [6]. The positive ANA test can be detected in other autoimmune diseases such as rheumatoid arthritis, scleroderma, Sjögren's disease, Hashimoto thyroiditis, and Grave's disease. In addition, ANA can also occur in healthy individuals in a significant proportion. The positive ANA alone cannot diagnose SLE disease. Meanwhile, the negative ANA makes it less likely to have this disease [7].

Anti-dsDNA antibodies are hallmark autoantibodies in SLE disease with specificity of up to 96%. They are the important immunological criteria according to the classification for SLE of Systemic Lupus International Collaborating Clinics 2012 (SLICC) and European League Against Rheumatism/American College of Rheumatology 2019 (EULAR/ACR). Deposition of anti-dsDNA to autologous nuclear antigens in the glomerulus and glomerular basement membrane causes kidney damage. Therefore, anti-dsDNA antibodies are also a valuable marker to predict the progression of lupus erythematosus nephropathy. Moreover, these antibodies strongly correlate with disease activity

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levels and can change over time. Anti-dsDNA may not be detected during treatment and increase during flare-ups, especially in the case of active lupus erythematosus nephropathy. Therefore, the sensitivity of anti-dsDNA in SLE diagnosis is low, estimated to be between 52% and 70% [8].

Fluorescence immunoassay (FIA) is a sensitive technique and has been the gold standard technique for ANA. However, this technique has not been used widely because of its complexity and high cost. Meanwhile, enzyme-linked immunosorbent assay (ELISA) is a simple method and is used more widely than FIA, even though its sensitivity for ANA and anti-dsDNA is low [6]. In Vietnam, tests for ANA and anti-dsDNA are mainly performed by ELISA technique. Furthermore, the results of ANA and anti-dsDNA in SLE patients in many studies are still inconsistent. To provide additional data about ANA and anti-dsDNA results using the ELISA method, we conducted this study to describe ANA and anti-dsDNA test results in patients with SLE and to investigate the association between these two antibody tests.

2. MATERIALS AND METHODS

2.1. Study design and population

Retrospective data and a cross-sectional descriptive study were conducted on patients who were already diagnosed with SLE based on EULAR 2019 criteria. They came for examination and treatment at Hue University of Medicine and Pharmacy Hospital from January 2018 to April 2023. This study recruited 147 SLE patients who were assigned to be tested for ANA and anti-dsDNA using the ELISA technique.

Patients without a confirmed SLE diagnosis, those with other autoimmune diseases such as rheumatoid arthritis, type 1 diabetes, and Grave disease; and those not indicated to be tested for both ANA and anti-dsDNA at the same time were excluded from this study.

2.2. Measurements

Demographic features of SLE patients including age and gender were recorded. Serum samples of patients with SLE were tested to detect the presence of ANA and anti-dsDNA by ELISA technique. The commercial ELISA kits were provided by DIA.PRO Diagnostic Bioprobes company, Italia. The ANA and anti-dsDNA were performed according to the manufacturer's instructions. The Tecan's Sunrise absorbance microplate reader, Australia, was used to measure optical density (OD) for each of these serum samples.

OD ratios of ANA below 0.8 were considered as negative; between 0.8 and 1.1 were considered indeterminate, and values above 1.1 were considered as positive. The levels of anti-dsDNA below 25.0 IU/ml were considered as negative and above 25.0 IU/ml were considered as positive. The serum samples of 147 patients with SLE were tested at the Department of Immunology, Hue University of Medicine and Pharmacy Hospital.

2.3. Data analysis

The collected data were analyzed according to medical statistical algorithms, using SPSS 20.0 software.

Variables were shown in numbers, percentages, mean, and standard deviation. The chi-square test was used to evaluate the association between anti-dsDNA and ANA test results as well as their associations with other variables. *p*-value <0.05 was considered significant in statistical analyses.

3. RESULTS

Table 1. Age characteristics of the study population

	Group	n	%
Age	< 20	23	15.6
	20 - <40	87	59.2
	≥ 40	37	25.2
	Total	147	100
	Mean (± SD)	32.2 (± 13.6)	
	Youngest age	7	
	Oldest age	69	

The mean age of the study population was 32.2 ± 13.6 years, with ages ranging from 7 to 69 years. The majority of SLE patients were aged between 20 and 40 years, comprising 87 (59.2%) patients.

Table 2. Gender characteristics of the study population

Group		n	%
Gender	Female	127	86.4
	Male	20	13.6
Total		**147**	**100**

The prevalence of females with SLE accounted for 86.4%, a female-to-male ratio was 6.35:1.

Table 3. ANA and anti-dsDNA characteristics of the study population

		n	%
ANA	Positive	84	57.1
	Negative	63	42.9
Anti-dsDNA	Positive	44	29.9
	Negative	103	70.1
Total		**147**	**100**

The prevalence of ANA and anti-dsDNA positivity were 57.1% and 29.9% of total SLE patients, respectively.

Table 4. Association of ANA test results with age and gender

		ANA				*p*-value
		Negative		Positive		
		n	%	n	%	
Age	< 20	9	39.1	14	60.9	0.282
	20 - < 40	34	39.1	53	60.9	
	≥ 40	20	54.1	17	45.9	
Gender	Male	15	75	5	25	0.002
	Female	48	37.8	79	62.2	
Total		**63**	**42.9**	**84**	**57.1**	

The difference was no statistically significant in ANA results among age groups ($p > 0.05$). However, the rate of positive ANA results in females was significant higher than in males ($p < 0.05$).

Table 5. Association of anti-dsDNA test results with age and gender

		Anti-dsDNA				*p*-value
		Negative		Positive		
		n	%	n	%	
Age	< 20	14	60.9	9	39.1	0.094
	20 - < 40	58	66.7	29	33.3	
	≥ 40	31	83.8	6	16.2	
Gender	Male	17	85	3	15	0.117
	Female	86	67.7	41	32.3	
Total		**103**	**70.1**	**44**	**29.9**	

There was no statistically significant association between anti-dsDNA results and age or gender groups ($p > 0.05$).

Table 6. Association between anti-dsDNA and ANA test results

		Anti-dsDNA				*p*-value
		Negative		Positive		
		n	%	n	%	
ANA	Negative	63	100	0	0	0.000
	Positive	40	47.6	44	52.4	
	Total	**103**	**70.1**	**44**	**29.9**	

A statistically significant association was found between anti-dsDNA and positive and negative ANA results ($p < 0.05$).

4. DISCUSSION

The incidence of SLE mainly occurs in young adults and adolescents, although it can happen at any age. It is generally observed that younger individuals with SLE tend to have higher disease severity and accumulate disease damage earlier than older individuals with the condition. In our study, the mean age of the study population was 32.2 ± 13.6 years and the youngest and oldest ages were 7 years and 69 years, respectively. The most common age group was between 20 and 40 years, comprising 87 (59.2%) of the total population (Table 1). Vo Tam et al., who conducted on 55 SLE patients, showed that the mean age of the study population was 33.2 ± 11.4 years [9]. Yamei Tang et al. on 140 SLE subjects recorded that the mean age of patients in the stable, mild, and severe disease level groups was 36.75 ± 11.42 years, 34.15 ± 9.84 years, and 39.88 ± 12.72 years [10]. Other studies, such as those by Magro-Checa et al. and Raafat et al., also found that the average ages of SLE patients were 35.4 ± 14.93 years and 32.52 ± 8.24 years, respectively [11], [12].

The prevalence of SLE is notably higher in females than in males, especially in the childbearing age. The changes in female sex hormones impact innate and adaptive immune responses, and dysregulation of these mechanisms contributes to the clinical manifestations of SLE. Progesterone and androgens function to fight autoimmune diseases, while estrogen is generally regarded as pathogenic, due to its immune-stimulatory effects. Therefore, estrogen is considered to contribute to predisposition to SLE [3]. In our study, it was found that 86.4% of total SLE patients were females, while only 13.6% were males (Table 2). Many studies also showed that the incidence of SLE primarily occurs in women. Ahmed et al., conducted on 150 SLE patients and showed that the incidence in females was 86.7% [13]. Vo Tam et al. found that the prevalence of SLE in females was up to 94.5%. Raafat et al. also found that the prevalence of SLE in females was 90.7% [12].

SLE is a complex autoimmune disorder with clinical heterogeneity and variable pathogenesis. The diagnosis of SLE is based on characteristic clinical findings as well as on serological parameters. ANA and anti-dsDNA are important immunological criteria for SLE diagnosis according to SLICC and EULAR/ACR. Detection of ANA and anti-dsDNA in the blood can be performed using a variety of techniques including ELISA, FIA, CIA (Chemiluminescence immunoassay) and RIA (Radioimmunoassay) [14]. Each method has a different sensitivity, specificity, and restrictive criterion. The FIA technique is a highly sensitive technique and is considered the gold standard in SLE diagnosis, especially testing for ANA. However, this technique is demanding on experimental conditions and experimenters and is affected by many factors such as temperature and microscope. Meanwhile, the ELISA technique is widely used in research laboratories and diagnostics due to its quick implementation time and simple steps [15] [16]. To provide additional data, we conducted a retrospective survey of the ANA and anti-dsDNA test result features in 147 SLE patients using the ELISA technique with commercial kits from DIA.PRO company, Italy according to the manufacturer's instructions. Our study results recorded that the prevalence of positive ANA and anti-dsDNA test results were 57.1% and 29.9%, respectively (Table 3). Most other studies reported higher rates of ANA positivity than those in our study; however, anti-dsDNA antibody results were inconsistent among the studies (Table 7).

Table 7. The prevalence of positive ANA and anti-dsDNA test results

No.	Author name (year of publication)	Study population (n)	ANA		Anti-dsDNA	
			Positive rate (%)	Detection method	Positive rate (%)	Detection method
1	Vo Tam et al. (2016) [9]	55	96.4	ELISA	89.1	ELISA
2	Magro-Checa et al. (2016) [11]	112	99.1	FIA	20.5	FIA
3	Qu et al. (2019) [18]	194	91.75	RIA	67.01	RIA
4	Moreno-Torres et al. (2022) [17]	77	88.0	FIA	38.0	ELISA
5	Li et al. (2022) [19]	617	97.89	FIA	69.2	RIA, CIA
6	Winikajtis-Burzy´nska et al. (2023) [20]	200	99	FIA	45.7	ELISA

Anti-dsDNA levels are commonly measured by the ELISA method because of its high sensitivity. The positive anti-dsDNA rate in our study (29.9%) is higher than in Magro-Checa et al. (20.5%). However, our anti-dsDNA positivity rate is lower than in other studies (Table 7). This situation could be explained by differences in the study population, study methods, and detection techniques, Moreover, anti-dsDNA levels also change following treatment or over time. There is much evidence to prove that anti-dsDNA levels have a significant association with clinical manifestation and laboratory parameters of SLE. Hence, anti-dsDNA is suggested to assess disease activity and predict flares of SLE.

Although the rate of positive ANA results was higher in SLE patients aged under 40 years compared to those aged over 40 years, this difference was not statistically significant ($p>0.05$) (Table 4). However, there was an association between ANA and gender. The prevalence of positive ANA test results was 62.2% in females, compared to 25% in males, with a statistically significant difference ($p<0.05$) (Table 4). Zakeri et al. also concluded that there was no relationship between ANA results and age. They found that the ANA positivity rate was predominantly observed in women, accounting for 85.4% [21]. Meanwhile, there was no relationship between anti-dsDNA test results and age or gender (Table 5).

We found a significant association between anti-dsDNA and ANA results ($p<0.05$). Specifically, 100% of SLE patients with negative ANA results also had negative anti-dsDNA test results. Meanwhile, the prevalence of positive anti-dsDNA results among SLE patients with positive ANA results was 52.2% (Table 6). A positive ANA test alone is not specific to SLE. However, both a positive anti-dsDNA test and a positive ANA can strongly suggest SLE, particularly when accompanied by clinical symptoms and other laboratory findings. A positive ANA test may prompt further testing, including the anti-dsDNA test, to support a diagnosis of SLE. Therefore, our research results provide additional data to guide appropriate requests for diagnostic tests in SLE.

Although the ELISA technique is not the gold standard for ANA testing in SLE diagnosis, it is commonly used to quantify autoantibodies. Quantification of anti-dsDNA using an indirect ELISA technique is often indicated to monitor disease activity and predict progress [22]. However, our study has several notable limitations. It was based on the retrospective data, thus we did not investigate the correlation of ANA and anti-dsDNA test results with clinical manifestations or disease activity according to the SLEDAI scale (Systemic Lupus Erythematosus Disease Activity Index). Moreover, ANA detected by ELISA has limited accuracy. Moving forward, we will focus on evaluating the relationship between anti-dsDNA and ANA and their associations with clinical and paraclinical characteristics. We aim to confirm their roles in the diagnosis and prognosis of SLE using the ELISA technique.

5. CONCLUSIONS

Our study on 147 SLE patients revealed that the mean age of the study subjects was 32.2 ± 13.6 years, with the majority being female, constituting 127 (86.4%) of the total patients. The prevalence of positive ANA and anti-dsDNA test results detected by the ELISA technique was 57.1% and 29.9%, respectively. We found a statistically significant relationship between anti-dsDNA with ANA results

($p<0.05$). Specifically, the positive anti-dsDNA rate was significantly higher in ANA-positive individuals (52.4%) compared to ANA-negative counterparts (0.0%). These results contribute additional data on ANA and anti-dsDNA results detected by the ELISA method in SLE. However, given the limitations of our study, further research is needed to evaluate the associations of ANA and anti-dsDNA with clinical characteristics and other laboratory parameters in SLE.

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