

## Immunodiagnosis of Systemic Lupus Erythematosus (SLE) in a Tertiary Care Hospital

Latha Ragunathan<sup>1\*</sup>, Anandi Chidambaram<sup>2</sup>, Kavitha Kannaiyan<sup>1</sup> and Chidambaram N<sup>3</sup>

<sup>1</sup>Dept Of Microbiology, Aarupadai veedu medical college & hospital, Pondicherry India

<sup>2</sup>Dept Of Microbiology, Vinayaka Missions Medical College & Hospitals – Karaikal India

<sup>3</sup>Dept of Medicine, Rajah Muthiah Medical college, Chidambaram India

### ABSTRACT

**Objectives:** To diagnose patients with Systemic lupus erythematosus clinically and compare various immunological tests available to detect their specificity and sensitivity of the various parameters employed.

**Methods:** It was hospital based cross sectional study. All patients satisfying the revised American College of Rheumatology criteria (1982) for SLE were included in the study over a period of 3 years. A total of two hundred and four individuals were included. The patients were divided into three groups- (Group I- Confirmed SLE patients-32, Group II- Incomplete SLE patients- 60, and Group III -patients with other autoimmune disorders- 42) and 70 persons were included as controls

**Results:** The patients were divided into three groups I,II,III and their clinical & laboratory features were compared. Females (90.6%) were more affected than males (9.4%) and 69% were in the age group of 20-40 years. The average age of onset of disease was 24.4 years. Predominant clinical features were arthritis (81.25%), skin rashes (72%), myalgia (68.75%), alopecia (68.75%), fever (65%) and oral ulcers 18(56.25%). Anti-ds-DNA test and ANA test were positive in all the patients and the anti-DNP test was positive in 59.3% patients.

Females were more affected (81.7%) than males (18.3%). 85% were between 21-50 years The average age of patients was 30.16 years with a range of 10-70 years. Patients were regularly followed up and 32 patients (26.67%) developed complete SLE. The most common manifestations were arthritis (88.3%), fever (43.3%) skin rashes (68.3%). 10.34% of ILE patients developed full SLE and all were females. Average age of disease onset was 24years. Anti-ds-DNA antibodies was detected in 28 patients (23.4%), ANA in (25%) patients and Anti-DNP antibodies in 12 (10%) patients.

Females (81%) were affected than males (19%).86% were between 21-50 years. The average age of patients was 33.24 years. The predominant clinical features were arthritis (78.6%) and myalgia (76.2%). Anti-ds-DNA antibodies were detected in 6%, ANA in 35.7% patients & Anti-DNP antibodies in 3.6%. Antibodies to ds-DNA were present in low titers in this group.

**Conclusion:** A clear separation between SLE and other autoimmune disorder was found with the anti-ds-DNA test. It has a high specificity for the differential diagnosis of SLE. Our study, comparing three assays with respect to their ability to predict disease activity, indicated that ds- DNA ELISA is the best method.

**Keywords:** Autoantibodies, Systemic Lupus Erythematosus, SLE, ANA, Ds-DNA, Lupus

### Introduction

Systemic lupus erythematosus (SLE) is an immunological disorder with multi system involvement. It is a disease of unknown etiology in which tissues and cells are damaged by autoantibodies and immune complexes. SLE primarily affects young women and is estimated to occur in as much as 0.1% of the population.<sup>[1]</sup>

The discovery of LE cell by Hargreaves et al (1948)<sup>[2]</sup>, Antinuclear antibody (ANA) by Meischer et al (1953)<sup>[3]</sup> and antibody to double stranded deoxyribonucleic acid (ds-DNA) by Cepellini R, et al 1957<sup>[4]</sup>, Robbins WC, et al 1957<sup>[5]</sup> led to a greater recognition of the milder cases and to a marked increase in its reported frequency.

ANA's are family of autoantibodies, which may be directed against nuclear antigens like double stranded-DNA (ds-DNA), nuclear RNA, extractable nuclear antigens (ENA) and histones.<sup>[6]</sup> ANA is a good screening test for SLE because 95% of cases show a high titre (1:80 or more) of this autoantibody<sup>[6]</sup>. ANA may be positive in other rheumatic disorders such as systemic sclerosis, Sjogren's syndrome, overlap syndrome, antiphospholipid syndrome, polymyositis and rheumatoid arthritis.<sup>[6]</sup>

Antibodies to ds-DNA are found in the sera of SLE patients, and are considered as a marker of disease activity.<sup>[7]</sup> These antibodies are considered to be of diagnostic significance and also of great prognostic value<sup>[8]</sup> especially during

clinical follow up. The ds- DNA antibodies level tend to fluctuate with the course of the disease<sup>[9]</sup> and may act as a predictor of disease activity and exacerbation. <sup>[10]</sup> The present study was to diagnose patients with SLE at Rajah Muthiah Medical College and Hospital, Chidambaram clinically and compare with various immunological tests available and also to detect their specificity and sensitivity of the various parameters employed.

## Materials and Methods

The study was conducted in Department of Microbiology in collaboration with Department of Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram. The study period was spanned for a period of twenty two months. A standardized proforma was followed regarding the clinical history, general physical examination and laboratory tests done during the study period. A total of one hundred and thirty four individuals were included in this study (confirmed SLE-32, Incomplete SLE- 60, and other autoimmune disorders- 42) and 70 persons were included as controls at Rajah Muthiah Medical College & Hospital, Tamilnadu, India.

The patient group was further divided into **Group I**-Sera from 32 patients with SLE (All patients met the 1982 American College of Rheumatology for SLE (updated 1997), fulfilling at least 4 of the 11 ARA criteria's).<sup>[11]</sup> **Group II**- consists of 60 clinically suspected SLE patients who didn't fulfill at least 4 of the 11 ARA criteria's. **Group III**- 42 individuals having autoimmune disorders other than SLE. Patients having Rheumatoid arthritis (RA), Mixed Connective Tissue Disorders (MCTD), Scleroderma (Scl), Chronic Active hepatitis (CAH) were included in this group.

**Clinical Examination:** The diagnosis of SLE was made in patients because of the presence of at least four of the eleven criteria of the American College of Rheumatology (ACR). The Dermatologists, Physicians, carried out clinical examination and the clinical findings were recorded. The inpatient and outpatient medical records were carefully reviewed for other details such as age, sex, occupation, and socioeconomic status. Examinations of the patients were done according to the criteria's given in the 1997 revised ACR criteria for classification of systemic lupus erythematosus. The criteria included: mucocutaneous lesions (malar rash, discoid rash, photosensitivity, and oral ulcers); inflammatory arthritis; serositis (pleuritis; pericarditis, peritonitis); nephritis; neuropsychiatric disorders (seizure, psychosis, organic brain syndrome, and chorea), blood changes, immunological changes, abnormal titre of antinuclear antibody (ANA). At least 4 of 11 criteria must be present to diagnose SLE clinically.

Blood was collected from patients and controls and serum was separated as by the standard procedure and aliquoted into 1 ml sterile plastic vials and stored at -20° C. Laboratory investigations were done which included immunological assays- anti-ds-DNA test, ANA, anti-DNP test, LE cell test, Rheumatoid factor (RF), C-reactive protein (CRP), full blood cell count, coagulation tests, routine biochemical and lipid profile, urine analysis, and 24 hour proteinuria. The following immunoassays were performed using commercially available kits:

Anti-ds-DNA antibodies test by ELISA, Antinuclear antibodies (ANA) by ELISA and Anti-deoxyribonucleoprotein (Anti-DNP), Rheumatoid factor (RF), C-reactive protein (CRP) by slide test by latex agglutination method.

**Statistical analysis:** Sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) was calculated by using standard formulae.

## Results

The age and the sex distribution of the patient groups are given in the table 1.

And the clinical and the laboratory features in the patient groups were classified according to the 1982 revised criteria and are shown in the table 2 and the results of the immunodiagnosics tests in the patient groups and the controls are shown in the table 3

## Discussion

The importance of antibodies to ds-DNA in the diagnosis and management of SLE has been universally recognized and used in daily clinical practice. The regional variation in the presentation of SLE is related to different genetic and environmental influences.<sup>[12]</sup>

**Immunological Profile in Controls:** In our study all the controls were negative for anti ds-DNA antibodies; 6% were positive for ANA and 5.7% were positive for anti-DNP antibodies which was higher than as reported by Burlingame RW and Cervera R. 2002.<sup>[13]</sup>

Studies have shown different prevalence of auto antibodies in healthy populations and they are always found in lower titers than those detected in autoimmune diseases. <sup>[14, 15,16]</sup> Sera of healthy adults especially females display a high frequency of ANA.<sup>[17]</sup>

**In Group-I :** Females (90.6%) were more affected than males (9.4%) and 69% were in the age group of 20-40 years. The average age of onset of disease was 24.4 years with a range of 10-56 years. The female to male ratio was 9.6:1. Masi et al 1978 <sup>[18]</sup> reported an average male to

**Table 1: Age and Sex distribution of patient groups.**

Patient group	Total number	Av Ds onset yrs	M:F ratio	Age group (Yrs)	M	F	Total
I	32	24.4	1:9.6	10-20	1	2	3(9%)
				21-30	0	13	13(41%)
				31-40	0	9	9(28%)
				41-50	1	3	4(13%)
				51-60	1	2	3(9%)
				<b>Total</b>	<b>3</b>	<b>29</b>	<b>32</b>
II	60	30.16	1:4.5	10-20	2	4	6(10%)
				21-30	3	11	14(23%)
				31-40	5	16	21(35%)
				41-50	1	15	16(27%)
				51-60	0	3	3(5%)
				<b>Total</b>	<b>11</b>	<b>49</b>	<b>60</b>
III	42	33.24	1:4.25	10-20	1	1	2(5%)
				21-30	2	7	9(20%)
				31-40	2	12	14(33%)
				41-50	3	11	14(33%)
				51-60	1	2	3(9%)
				<b>Total</b>	<b>9</b>	<b>33</b>	<b>42</b>

The results of the comparison of clinical features of patients group I, II, III are given in the table 2.

**Table 2: Comparison of Clinical features of patients group I, II, III.**

Clinical features	Group I (%) N=32	Group II (%) N= 60	Group III (%) N= 42
Arthritis	81.25	88.3	78.6
Skin rashes	72	68.3	9.5
Photosensitivity	43.75	10	05
Alopecia	68.75	20	12.5
Oral ulcers	56.25	6.67	-
Fever	65.6	43.3	12.5
Lymphadenopathy	37.5	35	22.6
Cardiac involvement	12.5	2.5	1.2
Pulmonary involvement	18.75	05	22.6
Raynaud phenomenon	-	-	4.8
Myalgia	72	54.2	76.2
Neuropsychiatric manifestations	12.1	1.7	-

**Table 3: Results of immunodiagnostic tests.**

Patients, Controls	Anti ds-DNA		ANA test		Anti-DNP	
	Positive (%)	N Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Controls	0(0)	70(100)	4(6)	66(94)	6(4)	66(94)
Group-I	32(100)	0	32(100)	0	19(59.3)	13(40.7)
Group-II	28(23.4)	92(76.6)	30(25)	90(75)	12(10)	108(90)
Group-III	5(6)	79(94)	30(35.7)	54(64.3)	3(3.6)	81(96.4)

female ratio of 1:5.5, Paul et al 2003<sup>[19]</sup> had reported male to female ratio of 1:19 and Malaviya et al 1988<sup>[20]</sup> had reported a male to female ratio of 1:8.

The peak incidence was seen in the third decade. Similar observations were made by Malaviya et al 1988<sup>[20]</sup> and Vaidya et al 1997<sup>[21]</sup> but Masi and Kaslow 1978<sup>[22]</sup> observed a median age of disease onset at 31 years. In a study conducted by Paul et al 2003<sup>[19]</sup> in Kerala median age of disease onset was 21.6 years. Predominant clinical features were arthritis (81.25%), skin rashes (72%), myalgia (68.75%), alopecia (68.75%), fever (65%) and oral ulcers 18(56.25%) patients.

In our study Anti-ds-DNA test and ANA detected antibodies in all the patients who were clinically diagnosed as SLE according to the ARA criteria and the anti-DNP test detected only in 59.3% patients.

Detection of ANA is a fundamental laboratory test for diagnosing systemic autoimmune diseases. Currently the method of choice is indirect immunofluorescence (IIF) on Hep-2 cell substrate.<sup>[23]</sup> but now ELISA is also used as an alternative-screening test to IIF for detection of ANA. ANA-EIA is not only sensitive and specific compared to IIF using Hep-2 cells.<sup>[24]</sup> but also objective, easy to perform and also less labor intensive when screening a large number of clinical specimens for ANA, but none of them are 100% sensitive.<sup>[24]</sup>

Antibodies to ds-DNA are considered as a marker of disease activity for SLE<sup>[7]</sup>, they are not only considered to be of diagnostic significance and also of prognostic value.<sup>[8]</sup> The detection of anti ds-DNA is one of the diagnostic criteria for SLE according to the American College of Rheumatology (ACR). Measurement of anti-DNA antibody activity has become a routine laboratory procedure of value in diagnosing and managing patients with SLE. Fluctuations in antibody level tend to correlate with course of the disease.<sup>[9]</sup> Several reports have also studied the changes in anti-ds-DNA antibody level instead of absolute values and find that increases in anti-ds-DNA antibody level may act as a predictor of disease activity and exacerbation.<sup>[10]</sup> Patients with significant rise in anti-ds DNA antibodies at were more likely to have renal disease than those who did not.<sup>[25]</sup> In our study all the patients in this group had high titres of anti ds-DNA antibodies. Anti-deoxyribonucleoprotein (anti-DNP) antibodies are found in approximately 75% of people with systemic lupus erythematosus.<sup>[13]</sup>

**Group II of suspected SLE cases:** Females were more affected (81.7%) than males (18.3%). 85% were between 21-50 years. The average age of patients was 30.16 years

with a range of 10-70 years. The female to male ratio was 4.5:1.

Incomplete lupus erythematosus (ILE) patients were regularly followed up and out of these only 32 patients (26.67%) developed complete SLE. This is much higher than the study done by Greer and Panush in 1989<sup>[26]</sup>, who noted only 5% of the ILE patients, developed complete SLE.

Arthritis (88.3%) was seen as the commonest manifestation similar to the observation made by Swaak et al 2001.<sup>[27]</sup> This is much higher than that reported by Greer and Panush (47%). Fever was present in 43.3% of patients in our study. Dermatological manifestations like skin rashes, photosensitivity, oral ulcers and alopecia was noted in 68.3% of patients. This is almost same as reported by Greer and Panush (61%).<sup>[26]</sup>

In our study the most common haematological abnormality was anaemia (38.3%) and most of them were normocytic normochromic anaemia. Lymphadenopathy was seen in 35% of the patients.

When compared with group I- confirmed SLE patients, ILE patients had fewer systemic manifestations. Clinical and serological manifestations of ILE patients were studied by Vila et al 2000<sup>[28]</sup> and according to them only 8.5% of the ILE patients when followed up developed complete SLE, but none with major organ damage but in our study 26.7% developed complete SLE. Malar rash and oral ulcers are less frequently seen ILE patients and they have less chances of developing photosensitivity and antibodies to ds-DNA.<sup>[28]</sup> Patients with ILE had more of skin and musculoskeletal involvement and 18% of ILE patients developed full SLE.<sup>[27]</sup> ILE patients have better prognosis than complete SLE.<sup>[27]</sup> 10.34% of ILE patients developed full SLE and all were females. Average age of disease onset was lower in full SLE (24 years) than those remained as ILE (34 years).<sup>[28]</sup>

In our study, in suspected SLE patients; anti-ds-DNA antibodies was detected in 28 patients (23.4%) patients, ANA in (25%) patients and Anti-DNP antibodies in 12 (10%) patients.

When this group was followed during our study period, only 32 patients developed complete SLE and out of these 32 patients, 28 patients had positivity for anti-ds-DNA, 30 patients had positivity for ANA and 12 patients were positive for anti-DNP. Rest of the patients' signs and symptoms simulating SLE disappeared during the course of the treatment given.

**Group III of Autoimmune Disorders Other than SLE Cases:** Females (81%) were affected than males



(19%). 86% were between 21-50 years. The average age of patients was 33.24 years with a range of 10-70 years. The female to male ratio was 4.25:1.

The predominant clinical features were arthritis (78.6%) and myalgia (76.2%). In this group anti-ds-DNA antibodies were detected in 6% of the patients. ANA was detected in 35.7% patients. Anti-DNP antibodies were detected in 3.6%. ANA are diagnostic markers for a variety of autoimmune diseases including rheumatoid arthritis, scleroderma, Sjogren's syndrome, SLE, autoimmune hepatitis.<sup>[17]</sup>

In SLE, antibodies to ds-DNA are present in high titers but they may be present in low titers in other rheumatic diseases.<sup>[29]</sup> In our study, all the five patients who were anti-ds-DNA positive were having low titers suggesting high specificity and discriminating power of this immunological test for SLE.

Comparison of clinical features of group I with group II and group III: In our study, females were more affected than males (1:9). Patients with renal disease had high titres of anti-ds-DNA levels and also had active disease. In group II, the age of disease onset is more (34 years) as compared to confirmed SLE (24.4 years). Multiple organ involvement was less commonly seen and male to female ratio was 1:4.5. The course of disease is mild as compared to group I and most of the patients responded to non-steroidal anti-inflammatory drugs (NSAID).

## Conclusion

In the present study a clear separation between SLE and other autoimmune disorder was found with the anti-ds-DNA test. It has a high specificity for the differential diagnosis of SLE. Due to availability of laboratory investigation there has been increased report of prevalence of SLE throughout the world. ELISA assay has been introduced as a sensitive method for detection of anti-ds-DNA Abs. This is based on the finding that by this technique both low and high avidity antibodies are detected. Our study, comparing three assays with respect to their ability to predict disease activity, indicated that ds-DNA ELISA is the best method. Advanced renal disease is now less common due to recognition of milder forms of the disease. Thus, the potential benefits of making the correct diagnosis of SLE at an early stage are obvious. We therefore recommend the measurement of anti ds-DNA antibodies wherever clinically appropriate regardless of the presence or absence of ANA.

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**\*Corresponding author:**

**Dr. R. Latha**, Dept of Microbiology Aarupadai Veedu Medical College & Hospital, Pondicherry- 607403. INDIA

**Phone:** +91 9894155330

**Email:** latha.ragunathan@avmc.edu.in

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