Original Article

Anti-nucleosome antibodies as a disease marker in systemic lupus erythematosus and its correlation with disease activity and other autoantibodies

Vandana D. Pradhan, Manisha M. Patwardhan, Kanjaksha Ghosh

Department of Autoimmune Disorders, National Institute of Immunohematology, Indian Council of Medical Research, 13th floor, KEM Hospital, Parel, Mumbai - 400 012, India

Address for correspondence: Dr. Vandana D. Pradhan, Department of Autoimmune Disorders, National Institute of Immunohematology (ICMR), 13th floor, KEM Hosptial, Parel, Mumbai, India. E-mail: pradhanv69@rediffmail.com

DOI: 10.4103/0378-6323.60558

ABSTRACT

Background: Detection of anti-nucleosome antibodies (anti-nuc) in patients with systemic lupus erythematosus (SLE) has been well established and it is claimed that their presence is associated with disease activity. **Aims:** The aim of this study is to evaluate the incidence of anti-nuc antibodies and to correlate them with disease activity and its association with other autoantibodies like anti-nuclear antibodies (ANA), anti-double stranded DNA (anti-dsDNA), anti-histone antibodies (AHA), as well as autoantibodies to histone subfractions like H1, (H2A-H4) complex, H2B, and H3. **Methods:** This cross-sectional study included 100 SLE patients referred from the Rheumatology, Dermatology, and Nephrology Departments. SLE disease activity was evaluated by using SLE-Disease Activity Index (SLEDAI) score. A patient was defined as having active SLE when the SLEDAI score was more than 5.0. Fifty normal controls were also tested as a healthy control group. Anti-nuc antibodies, anti-dsDNA, and AHA were tested by Enzyme-Linked Immunosorbent Assay (ELISA) and ANA was detected by an indirect immunofluorescence test. **Results:** All patients studied were in an active stage of disease and were untreated, of which 44 patients had renal biopsy-proven kidney involvement, which was categorized as lupus nephritis (LN) and 56 patients did not show any renal manifestations (SLE without LN). Anti-nuc antibodies were positive in 88%, anti-dsDNA in 80%, and AHA in 38% of the cases. ANA was positive in all SLE patients studied. None of the normal controls was found to be positive for these antibodies. Although a slightly higher incidence of autoantibodies were noted in LN, there was no statistical difference noted between LN and SLE without LN groups for anti-nuc and anti-dsDNA antibodies ($p > 0.05$). A higher incidence of autoantibodies to ANA specificities were noted in anti-nuc positive cases, but there was no statistical difference between anti-nuc positive and anti-nuc negative cases for ANA specificities among LN and SLE without nephritis groups ($p > 0.05$). **Conclusions:** Anti-nuc antibody detection could be a better tool for the diagnosis of SLE. Although there was no significant difference in LN and SLE without LN groups, this study suggests that anti-nuc detection can be useful as an additional disease activity marker to other laboratory tests.

Key words: Systemic lupus erythematosus, anti-nucleosome antibodies, anti-double stranded DNA antibodies, anti-histone antibodies, lupus nephritis, SLE without nephritis

INTRODUCTION

Despite decades of research the cause(s) of systemic lupus erythematosus (SLE) still remains a 'jigsaw' puzzle. Various marker autoantibodies play an important role in the immunopathogenesis of this disease. Until now, more than 100 autoantigens have been identified, which are targeted by SLE autoantibodies.[1] The autoantigens that are targeted by autoantibodies vary among SLE patients. As autoantibodies to double stranded DNA (dsDNA) are found in nearly all patients with an active stage of the disease, they are considered as 'serological markers' for SLE. However, DNA is not immunogenic

How to cite this article: Pradhan VD, Patwardhan MM, Ghosh K. Anti-nucleosome antibodies as a disease marker in systemic lupus erythematosus and its correlation with disease activity and other autoantibodies. Indian J Dermatol Venereol Leprol 2010;76:145-9.

Received: April, 2009. **Accepted:** June, 2009. **Source of Support:** Nil. **Conflict of Interest:** None declared.

by itself. Only when bound to proteins (histones / viral proteins) it can induce anti-nucleosome (anti-nuc) or anti-DNA response. Therefore, DNA-histone complexes (nucleosome) are proposed to be major autoantigens. Since the last decade there were several reports on anti-nucleosome antibodies and its clinical association with the severity of the disease.[2-5]

The term 'nucleosome' defines a basic unit of chromatin. Each nucleosome consists of 146 base pairs of double stranded DNA, wrapped twice around a histone octamer, a protein core. A histone octamer consists of two molecules each of histones H2A, H2B, H3, and H4. In a chromatin, nucleosomes are connected by 15-80 base pairs of linker DNA, to which histone H1 is attached. Anti-dsDNA and anti-histone antibodies (AHA) belong to the nucleosome family as do anti-nuc specific antibodies, since nucleosomes share several common epitopes with dsDNA and histones. Nucleosome specific antibodies do not react with the individual components of the nucleosome, that is, DNA and histones, but recognize conformational epitopes resulting from interactions between the DNA and histone.[2]

Lupus Nephritis (LN) is one of the most serious complications of SLE, occurring in up to 60% of the patients with SLE. Traditionally it was thought that LN resulted from glomerular deposition of DNA / anti-dsDNA complexes. However, DNA / anti-dsDNA complexes are highly nephritogenic.[6] The nucleosome has been identified as a major autoantigen. Nucleosome specific autoantibodies have been found in a majority of SLE patients. In recent years, it has been observed that nucleosomes together with other lupus autoantigens cluster in apoptotic bodies at the surface of apoptotic cells. Systemic release of these autoantigens is normally prevented by the swift removal of apoptotic cells. Berden *et al*.,[7] have reported that if the rate of apoptosis overflows the removal capacity, and / or the cleaning machinery is impaired, nucleosomes are released. Furthermore, during apoptosis, autoantigens can be modified, which makes them more immunogenic. Nucleosomes also play a pivotal role in the evolution of tissue lesions, especially glomerulonephritis. In LN, nucleosomes, anti-nucleosome antibodies, and nucleosome / Ig complexes have been detected in the glomerular immune deposits, which support these findings.

There are various reports on the presence of anti-nuc antibodies in active SLE and their role in the evolution of disease activity in patients with SLE, suggesting that the determination of circulating anti-nuc antibodies could be a useful parameter for early diagnosis and follow-up of SLE patients.[8-11] In this study we have investigated the incidence of anti-nuc antibodies and their correlation with clinical manifestations as well as with other autoantibodies in LN and in SLE patients without nephritis, to evaluate the possible role of anti-nuc antibodies in the disease activity.

METHODS

This consecutive cross-sectional study was conducted in 100 SLE patients in the Rheumatology, Dermatology, and Nephrology Departments of the KEM hospital, Mumbai, for the period of three years (2005 – 2008). All these patients had recent onset of SLE (< 1 year since diagnosis) as diagnosed according to the American College of Rheumatology (ACR) criteria. A written consent was obtained from each patient and the study was carried out after obtaining the requisite Ethics Committee approval. The disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). We excluded pregnant and postmenopausal women, smokers, patients with diabetes, and patients with significant hyperlipemia. In addition we also excluded patients with a history of venous or arterial thrombosis. Fifty age-matched, healthy subjects that met the same exclusion criteria were used as healthy normal controls. Blood was collected and sera were stored in aliquots at -80^{0}C until tested. Renal biopsies of LN cases were examined by light microscopy, with hematoxylin and eosin (H and E) and periodic Schiff (PAS) staining, by immunofluorescence microscopy, using anti-IgG, anti-IgM, anti-IgA, anti-C3, anti-C4, and anti-fibrinogen fluorescein isothiocyanate conjugate (FITC).

Anti-nuclear antibodies (ANA) were tested using the Bio Rad kit, where ANA positivity was detected using HEP-2 cell line as a substrate. ANA slides were visualized using a fluorescence microscope (Nikon, Optiphot II). Some unusual and rare ANA patterns were confirmed using a Confocal Laser Scanning Microscope (Carl, Zeiss, LSM -510). Antibodies to various antigen specificities for all ANA-positive patients were further detected by ANA-BLOT using Euroimmune, Germany kit. Anti-nuc and anti-dsDNA were detected by ELISAs using the commercially

available Euroimmune, Germany kits. Anti-histone antibodies (AHA) were estimated by the ELISA technique.[12,13] In brief AHA were estimated by ELISA techniques using commercially available histones (whole molecular) as also their various subfractions such as H1, (H2A – H4) complex, H2B, and H3 from Sigma, USA. Commercial histone antibody standards (high, medium, and low) from Novamed, Israel, were used as standards in the assay. As indirect ELISA was standardized to quantitate the autoantibodies in the patients' sera, the results were expressed in units per milliliter with the help of a standard graph. All AHA positive cases were further tested for the presence of autoantibodies to histone subfractions. The laboratory was blinded to the disease status of patients and their visceral involvement and a double blinded study was conducted on the positive samples.

Statistical analysis

Continuous variables were expressed as mean $\pm$ SD. Pairs of groups were compared using student's 't' test for a normally distributed continuous distribution. The 'X^2' test was used for the categorical variables as needed. Statistical significance was set at $p \leq 0.05$.

RESULTS

We included 100 clinically diagnosed SLE patients (93 females, 7 males) with a mean $\pm$ SD age of 27.5 $\pm$ 9.52 and a mean $\pm$ SD SLE disease duration in months of 6.5 $\pm$ 3.0. The mean $\pm$ SD SLEDAI score at clinical evaluation was 5.8 $\pm$ 6.5. The most frequent clinical manifestations defined, according to ACR criteria, are summarized in Table 1. These patients were further categorized as lupus nephritis (LN) (n = 44) and SLE without nephritis (n = 56), based on their renal involvement. In the SLE patients studied, the prevalence of ANA, anti-nuc, anti-dsDNA, and AHA was 100, 88, 80, and 38%, respectively. Healthy controls did not show the presence of these antibodies. The distribution of these autoantibodies in LN and SLE without nephritis is shown in Table 2. There was no statistical difference noted between LN and SLE without nephritis groups ($p > 0.05$) for the presence of autoantibodies such as, ANA, anti-nuc, anti-dsDNA, and AHA. All ANA positive sera were further tested for autoantibodies to Sm, nRNP, SSA (Ro), SSB (La), Rib P, and ANCA specificities. The distribution of these autoantibody specificities in anti-nuc positive and anti-nuc negative patients in both LN and SLE without nephritis groups is shown in Table 3. It was observed that although anti-nuc positive patients showed a higher incidence for autoantibodies to ANA specificities, there was no statistical difference between anti-nuc positive and anti-nuc negative cases for ANA specificities among LN and SLE without nephritis groups ($p > 0.05$).

Table 1: Baseline characteristics of SLE patients studied (n = 100)

Characteristics	
Sex ratio (F:M)	14: 1 (28:2)
Mean age (years ± SD)	27.5 ± 9.52
SLE duration in months (Mean + SD)	6.5 ± 3.0
SLEDAI (Mean ± SD)	5.8 ± 6.5
Clinical manifestations (%)*	
Malar / Discoid rash	50 (50%)
Photosensitivity	38 (38%)
Oral ulcers	10 (10%)
Arthritis	57 (57%)
Serositis	10 (10%)
Renal involvement	44 (44%)
Neurological disorders	5 (5%)
Laboratory Characteristics (%)	
Hematological Abnormalities	14 (14%)
Immunological Abnormalities	100 (100%)
ANA	100 (100%)

According to ACR criteria

The distribution of autoantibodies to histone whole molecule and its subfractions indicated that 38 patients (38%) showed AHA, that is, autoantibodies to histone whole molecule, of which, 34 patients (89.5%) had anti-H1, 21 patients (55.3%) had anti-(H2A-H4), 26 patients (68.4%) had antibodies to H2B, and 14 patients (38.8%) had antibodies to H3 subfraction of histone, indicating that H1 is the most common antigenic subfraction for the development of AHA as shown in Table 4. Table 5 gives the association of clinical manifestations in anti-nuc positive and anti-nuc negative patients according to the ACR criteria. Although the severity and organ involvement is comparatively seen more in LN patients, there is statistically no significant difference noted when that is compared with SLE without nephritis in anti-nuc positive and anti-nuc negative patients.

DISCUSSION

There are various reports available in SLE patients, giving incidences of anti-nuc antibodies and its correlation with other antibodies, such as, ANA and AHA, where investigators have evaluated the possible role of anti-nuc antibodies with disease activity.

Table 2: Distribution of anti-nucleosome antibodies (Anti-nuc) and other autoantibodies in SLE patients (n = 100)

SLE type (n = 100)	ANA (n = 100)	Anti-nuc (n = 88)	Anti-dsDNA (n = 80)	AHA (n =38)
Lupus nephritis (n = 44)	44 (100%)	38 (86.4%)	35 (79.5%)	18 (40.1%)
SLE without nephritis (n = 56)	66 (100%)	50 (89.3%)	45 (80.4%)	20 (35.7%)

Table 3: Association of ANA specificities by ANA-BLOT

SLE type	Anti-nuc positives (n = 88)						Anti-nuc negatives (n = 12)					
	Sm	RNP	SS-A	SS-B	Rib-P	ANCA	Sm	RNP	SS-A	SS-B	Rib-P	ANCA
LN (n = 44)	25	28	4	9	5	16	3	3	1	2	1	3
SLE without LN (n = 56)	33	40	6	11	5	14	3	5	1	3	1	1
Total (n = 100)	58	68	10	20	10	30	6	8	2	5	2	4

Table 4: Distribution of autoantibodies to histone subfractions in anti-nuc positive and negative patients

SLE type	Anti-nuc positives (n = 88)				Anti-nuc negatives (n = 12)			
	H1	(H2A-H4)	H2B	H3	H1	(H2A-H4)	H2B	H3
LN (n = 44)	12	7	10	6	2	0	1	0
SLE without LN (n = 56)	18	12	12	8	2	2	3	0
Total (n =100)	30	19	22	14	4	2	4	0

Table 5: Association of clinical manifestations in anti-nuc positive and anti-nuc negative patients according to ACR criteria (n = 100)

ACR criteria	Anti-nuc positives (n = 88)		Anti-nuc negatives (n = 12)	
	LN (38)	SLE without LN (50)	LN (6)	SLE without LN (6)
Rash (Malar + Discoid)	16	28	2	4
Photosensitivity	16	18	2	2
Oral ulcers	6	4	0	0
Arthritis	28	18	6	5
Serositis	0	8	0	2
Renal disorders	38	0	6	0
Neurological disorders	0	3	0	2
Hematological disorders	8	2	2	2
Immunological disorders	38	50	6	6

Incidence of anti-nuc autoantibodies varies from 40 to 100%. Simon *et al*.,[11] have reported a 100% incidence of anti-nuc antibodies with an incidence of 63% for anti-dsDNA and 15% AHA in SLE, showing a positive correlation with SLEDAI and renal manifestations.

Campos *et al*.,[8] reported an incidence of 40% anti-nuc and 58.6% anti-dsDNA positivity among SLE patients studied. Min *et al*.,[14] reported an incidence of 76% for anti-nuc and 79.6% for anti-dsDNA antibodies. In a recent study by Bigler *et al*.,[9] 89% LN cases and 80% SLE without LN had anti-nuc antibodies, and 94.3 % LN and 84.5% SLE without LN had anti-dsDNA antibodies. Duzgun *et al*.,[16] reported that among 38.9% SLE patients with renal involvement, 74.5% had anti-nuc antibodies,78.4% had anti-dsDNA antibodies, and among anti-dsDNA negative patients, only 31.4% had anti-nuc antibodies. It was also reported that both anti-nuc and anti-dsDNA antibodies correlated with SLEDAI scores. Haddouk *et al*.,[10] reported an incidence of 78.6% positivity for anti-nuc antibodies and-reported an incidence of 23.8% for anti-nuc antibodies among anti-dsDNA negative patients. Among the 59.5% LN cases included in this study a positive correlation was reported between anti-nuc antibodies and SLEDAI scores, where hematological manifestations were seen in 80.1% cases and arthralgias and arthritis were found to be present in 79.8% cases.

Gomez Puerta,[20] reported that the presence of anti-chromatin (anti-nuc, anti-dsDNA, and anti-histone) could be used in conjunction with the clinical findings

and other laboratory tests, to help in the diagnosis of SLE.[17] It was also reported that the presence of anti-chromatin antibodies have been linked to glomerulonephritis in SLE patients. Yin *et al*.,[18] reported that 61.8% had anti-nuc antibodies and the levels of anti-nuc correlated with SLEDAI. It was also reported that among anti-dsDNA negative patients, 51.2% patients showed presence of anti-nuc antibodies where anti-nuc positivity was more common in patients with fever, skin rash, and arthralgias with lower C3, C4 levels.

In our study, anti-nuc antibodies were present in 88% of SLE patients, but there was no statistical difference in LN and SLE without nephritis. Anti-nuc antibodies seemed to be of limited help in the distinction between these two groups. This was in agreement with the findings reported by Bigler *et al*.,[9] and Quattrocchi *et al*..[19] Anti-dsDNA antibodies, which were the diagnostic marker antibodies for SLE, were found in 80% of the patients and there was no statistical difference observed in LN and SLE without LN groups. It seemed that anti-nuc antibodies did not provide any significant advantage over the existing tests from the SLE diagnosis point of view.

Our study also suggests that the presence of anti-nuc can be used in conjunction with the clinical findings and other laboratory tests, to help in the diagnosis and assessment of disease activity. We have detected AHA to whole molecule and autoantibodies, to different histone subfractions, to know its association with anti-nuc antibody development. More studies are required to evaluate their role and association with clinical manifestations in SLE, along with anti-nuc and anti-dsDNA autoantibodies, both in the anti-nuc positive and negative groups. These findings will throw light on the development of anti-chromatin antibodies, to describe the mechanisms leading to nucleosome production and anti-nucleosome autoimmunity-related, apoptosis-related mechanisms for better understanding of the immunopathogenesis of SLE.[20]

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