Original Article

Anti-nucleosome antibodies – a valuable test in systemic lupus erythematosus

Ahmed Ali Abid* MBChB
Aida R.AL-Derzi* MSc, FICMS/Path
Khudhayer Z.Albidri** FICMS Medicine, FICMS Rheumlogy &medical Rehabilitation

Summary:

Background: Nucleosomes are fundamental units of chromatin released by internucleosomal cleavage during cell apoptosis, and nucleosomal material has been demonstrated in the surface blebs of apoptotic cells. Recent studies have shown the presence of antinucleosome antibodies in systemic lupus erythematosus (SLE).

Patients & Methods: We included 40 consecutive patients (36 female & 4 male) with SLE (four or more ACR criteria). As control groups we included 40 healthy blood donors and 48 patients with systemic autoimmune diseases (SADs). Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). A venous blood sample was drawn to measure anti-nucleosome (anti-NCS), anti-double-stranded DNA (anti-dsDNA) by enzyme-linked immunosorbent assay.

Results: When SLE and SADs patients were compared the sensitivity of anti-NCS, anti-dsDNA antibodies for SLE diagnosis was 90%, and 75% respectively and the specificity was 83.3%, and 85.4%. Anti-NCS antibodies were not useful in differentiating between SLE and scleroderma patients. Anti-NCS antibodies showed the highest correlation with disease activity ($r=0.58$, $P<0.001$). Anti-NCS antibodies also showed strong association with renal damage ($P=0.024$).

Conclusion: anti-NCS antibodies could be a useful tool in the diagnosis and assessment of disease activity in SLE patients, especially in patients who are negative for anti-dsDNA antibodies.

Keywords: Systemic lupus erythematosus (SLE), Anti-nucleosome antibodies,anti-dsDNA.

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Introduction:

Systemic lupus erythematosus is an autoimmune rheumatic disease with great diversity of clinical manifestations. More than 100 different autoantibodies have been identified in the sera of patients with SLE (1). The most prominent of these autoantibodies, anti–double-strandedDNA (anti-dsDNA) antibodies, are present in 40–80% of patients with SLE. For more than 35 years, the anti-dsDNA antibody has been utilized as a marker for disease activity, especially in renal disease. (2,3) Recently, newer assays detecting antinucleosome (anti-NCS) antibodies have shown promise in assessing disease activity, especially when anti-dsDNA antibodies are negative (4). Compelling data have shown that antinucleosome antibody reactivity is a very sensitive marker of SLE: ;70–90% of SLE patients . have been found to be positive, and among them, ;30% have high antinucleosome antibody activity with little, if any, anti-dsDNA or antihistone reactivity(5). The level of the anti-nucleosome antibody titer correlates with the level of disease activity.

This finding was also observed in SLE patients negative for anti-dsDNA antibodies. Anti-nucleosome antibodies are also associated with lupus nephritis, which is not surprising considering the bulk of evidence suggesting that they contribute to the pathogenesis of lupus nephritis (6). An increase in IgG3 anti-nucleosome titers was observed during SLE flare-ups, and this increase was found to be closely associated with active nephritis. IgG1 anti-nucleosome antibody titers tended to correlate inversely with SLE disease activity. One group found an association between anti-nucleosome antibodies and lupus psychosis (6).

Patients and methods:

A total of 40 patients (4 males &36 females) with SLE were included in this study. Their ages ranged between (12-61) years. The patients were diagnosed clinically by specialists and they were among patients who attending the rheumatology department of Baghdad Medical City Teaching Hospital & Al-Husain Teaching Hospital/Karbala during the period of November 2009 through July 2010. As control groups we included (i) 40 healthy blood donors and (ii) 48 clinically diagnosed with systemic autoimmune diseases (SAD),

* *Dept. of Microbiology/Collage of medicine/Baghdad University.*
** *Dept. of Rheumatology/ Baghdad Teaching Hospital.*

Table (1): Validity parameters for studied antibodies in differentiating SLE from other SADs

				PPV at pretest probability =		NPV at pretest probability =
	Sensitivity	Specificity	Accuracy	50%	90%	10%
AntidsDNA	75	85.4	80.7	83.7	97.9	96.8
Anti-NCS	90	83.3	86.4	84.3	98.0	98.6

including Rhreumatoid arthritis, scleroderma, polymyositis &, ankylosing spnondylitis). Anti-chromatin antibodies were determined by enzyme-linked immunosorbent assay, according to the manufacturer's recommendations [Orgentec Diagnostika (Germany) anti-NCS and anti-dsDNA antibodies]. Briefly, plates coated with purified human nucleosomes, dsDNA were incubated with 100 µl of 1:100 serum dilutions for 30 min at room temperature. Each plate included a calibration curve of six points, a positive control and a negative control. Plates were washed three times with the wash solutions of the kits. An anti-human IgG horseradish peroxidase conjugate (100 µl /well) was pipetted into the wells of the microplate and incubated for 15 min at room temperature. After three washes, 100 µl of substrate was added and incubated for 15 min at room temperature. Colour release was stopped by the addition of 100 µl of stop solution. Plates were read at 450nm.

Results:

The prevalence of anti-NCS, anti-sDNA antibodies in SLE patients was 90%,75% respectively; whereas in SADs was 16.6%, 14.6% respectively. Comparing SLE patients with SADs controls, anti-NCS, antidsDNA antibodies had a sensitivity of 90% &75% respectively, specificity of 83.3, 85.4%, positive predictive value (pretest probability 90%) of 98&97.9%, negative predictive value of 98.7&96.8% respectively for the diagnosis of SLE (Table 1). Both anti-NCS & antids-DNA antibodies correlated with SLE activity (Fig. 1). The correlation coefficient was stronger for anti-NCS antibodies ($r=0.58$, $P < 0.001$) than anti-dsDNA antibodies $r=0.444$, $P=0.004$). There were significant associations between the level of anti-NCS Abs with renal involvement ($p=0.024$). Both anti-NCS & anti-dsDNA were associated with proteinurea($p=0.009$)($p=0.01$) respectively.

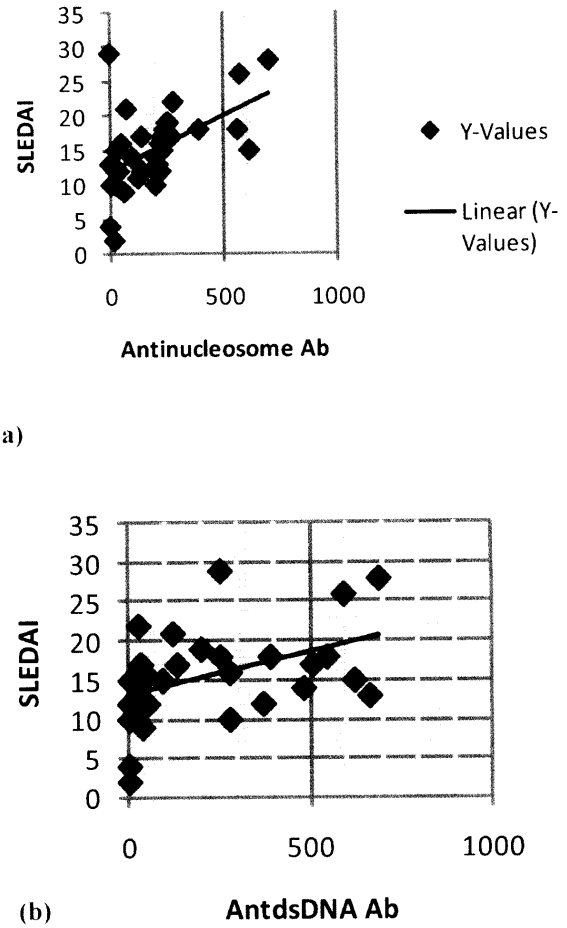

Fig. 1. (a) The correlation coefficient of the anti-dsDNA $r=0.444$, $P=0.004$ and (b) the anti-NCS $r=0.58$, $P < 0.001$ with disease activity index(SLEDAI) .

Discussion:

Systemic autoimmune diseases, including SLE, share several clinical and biological features that make their differential diagnosis difficult, especially in the early stages. Traditional tests used in the diagnosis of SLE, such as antinuclear antibodies, have low specificity because they identify antibodies against nuclear antigens generically (7). Because the nucleosomes have been considered the principal antigen in the pathophysiology of SLE (8-12), the present work investigated the prevalence of anti-NCS antibodies in SLE patients. In this study serum levels of anti-nucleosome antibodies correlate with anti ds-DNA antibodies, which is a recognized test for SLE and also one of the ACR criteria for the disease. Nevertheless, there is no complete overlap of positivity for these two tests. High levels of anti-nucleosome antibodies have been found in 20% sera

with no anti-ds-DNA reactivity, however, only 5% in vice versa. Antinucleosome specific antibodies represent the early marker of SLE recognizing conformational epitopes shared by the native nucleosome molecule(13). Later, the autoimmune response can diversify to the components of the nucleosomes, DNA and histones as a part of the inter-molecular spreading. Anti nucleosome antibodies are also relatively specific for SLE, besides SLE they were detected just in the mixed connective tissue disease (MCTD) and, as in this study, systemic scleroderma (5). They were absent in patients with inflammatory myopathies, primary Sjögren's giant cell arteritis,Takayasu arteritis, relapsing polychondritis, sarcoidosis, syndrome, RA, primary antiphospholipid syndrome, Wegener's granulomatosis, Behcet's disease and hepatitis C virus infection (5). The high sensitivity of anti-NCS antibodies for SLE diagnosis in this study is consistent with findings in human and murine models of lupus, in which it has been demonstrated that the development of anti-NCS antibodies occurs before other antichromatin antibodies (14). Interestingly, Simo´n et al., (2004), stated that the sensitivity of antinucleosome reaches 100% in patient with SLE of recent onset, and anti-NCS Abs were present in all patients who were negative for antidsDNA (15). Some reports have previously shown that both antinucleosome and anti-dsDNA IgG antibody activities correlated with scores on the SLEDAI (16). Simon and colleagues (2004) demonstrated that the correlation coefficient with disease activity was stronger for anti-NCS antibodies ($r=0.45$, $P < 0.0001$) than anti-dsDNA antibodies ($r=0.25$, $P=0.03$)(15). In the present study, anti nucleosome has a stronger correlation($r =0.58$, $P<0.001$) with SLEDAI than antidsDNA ($r=0.444$, $P=0.004$) which support the previous reports. These results suggest that anti-NCS antibodies, besides being potentially useful in SLE diagnosis, could be a biological marker of disease activity. In this study, anti-NCS antibodies were associated with renal damage, which suggests a possible role in the pathogenesis of lupus nephritis (17,18). A positive association of anti-NCS antibodies with renal damage has been demonstrated previously, both in murine models of lupus and in SLE (8, 9,17,18). This association seems to depend on a complex interaction between charges associated with the quaternary structure of the nucleosomes and epitope targets in renal tissue. That is, the histones that constitute part of the nucleosomes have a cationic charge, whereas the glomerular basement membrane has an anionic charge, which permits an interaction between them (15). In conclusion it should be emphasized that the determination of anti-nucleosome as the earliest antibody in SLE contributes to the diagnosis and is a test in differential diagnoses of connective tissue diseases and it seems to be especially valuable in anti-dsDNA negative lupus. Use of anti-nucleosome antibody measurement as a marker of disease activity deserves further evaluation.

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