ORIGINAL ARTICLE

Anti-nucleosome antibodies: A potential surrogate marker for renal affection in lupus patients with insignificant proteinuria

Samah A. El Bakry a,*, Amina Bader El Din a, Al Hussein M. El Dakrony b, Nouran M. Abaza c, Rania A. Abo-Shady d, Nesrine A. Mohamed d, Ola H. Nada e

a Internal Medicine and Rheumatology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
b Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University, Cairo, Egypt
c Rheumatology and Rehabilitation Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
d Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
e Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

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KEYWORDS
Anti-nucleosome antibodies; SLE; Lupus nephritis; SLEDAI

Abstract Background: Lupus nephritis (LN) is a serious manifestation of systemic lupus erythematosus (SLE). Clinical renal involvement is present in about two-thirds of lupus patients and more patients would have morphologic evidence of renal disease without clinical manifestations.

Aim of the work: To investigate serum anti-nucleosome antibodies role as a biomarker for renal affection in lupus patients with insignificant proteinuria.

Patients and methods: Twenty-four lupus patients with proteinuria < 500 mg/d (group-A), 30 patients with established lupus nephritis (group-B) and 15 controls were included. Systemic lupus erythematosis disease activity index (SLEDAI), anti-nucleosome, anti-dsDNA antibodies and renal biopsy were assessed in all patients.

Results: Serum anti-nucleosome antibodies were significantly higher in all lupus patients than control ($P < 0.001$) and showed significant positive correlation with SLEDAI score. SLE patients
1. Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects mostly all organs of the body [1]. Lupus nephritis (LN) is one of the most serious manifestations in SLE and it occurs in about 60% of patients. Patients with LN have increased risk of progressive deterioration of kidney function as well as increased mortality rates [2]. The most important feature in LN is immune complex deposition in renal glomeruli which produces glomerular inflammation. The site and the amount of these immune deposits determine the degree and class of LN [3].

Given the heterogeneity of the disease, many mechanisms have been postulated for formation of glomerular immune complexes [4–6]. One of our interests is the presence of antichromatin autoantibodies (anti-nucleosome, anti-histone and anti-DNA) which are present in 75% of patients with SLE and up to 100% of patients with drug-induced lupus [7]. Nucleosomes, released by apoptosis, bind to the mesangial matrix and glomerular basement membranes and may represent a relevant source of autoantigens. Thus, these apoptotic nucleosomes may serve both as an inducer and a major target for nephritogenic autoantibodies in SLE [8].

Several studies have discussed the role of anti-nucleosome antibodies (ANuA) in the diagnosis and assessment of disease activity in SLE, as well as its role in evaluating severity of SLE renal disease requiring transplantation [9–11]. In a systematic review and meta analysis, Bizzaro and colleagues showed that the overall sensitivity of the ANuA assay is 61% and the specificity is 94%. This suggests that antibodies have equal specificity but higher sensitivity and prognostic value than anti-dsDNA antibodies in the diagnosis of SLE [10]. As well, ANuA have been investigated as a biomarker for the evaluation of active proliferative LN. It was found that those antibodies have a high prevalence in patients with severe lupus nephritis but its role in differentiating patients with active LN from those with inactive renal disease is still questionable [12,13].

Significant renal affection in SLE can occur before impairment of kidney function or changes in laboratory parameters. Christopher-Stine et al., studied the renal biopsies of 21 lupus patients with low levels of proteinuria and found significant renal involvement (Class III, IV, or V LN) in SLE patients with proteinuria with or without hematuria [14].

Unfortunately, the use of conventional clinical parameters such as creatinine clearance, proteinuria, urine sediments, anti-dsDNA and complement levels did not help much in the detection of ongoing disease activity in the lupus kidneys or diagnosis of early relapse of nephritis. Although renal biopsy is important for the diagnosis of histological class of LN, yet it has its limitations and contraindications. Thus, new biomarkers are needed to improve the diagnostic accuracy and sensitivity of lupus renal disease, monitoring of treatment response, and detection of early renal flares [15].

In this study, we investigated the potential role of serum anti-nucleosome antibodies as a marker for the detection of renal involvement in SLE patients with insignificant proteinuria and its correlation with the WHO pathological classes.

2. Patients and methods

2.1. Study design

This is a cross sectional-observational study.

2.2. Clinical evaluation

A total of 54 Egyptian lupus patients were consecutively enrolled along with 15 healthy subjects (age and sex matches) who served as a control group for serum anti-nucleosome antibody. All lupus patients were recruited from the rheumatology clinic-internal medicine department-Ain Shams University, rheumatology and rehabilitation clinic-Cairo University and rheumatology and rehabilitation clinic-Ain Shams University. All patients fulfilled 1997 revised criteria of the American College of Rheumatology (ACR) for SLE [16]. Patients were recruited between October 2011 and December 2012. All participants gave written informed consent to participate in the study, which was approved by our local Ethics Committee.

Twenty-four lupus patients with proteinuria less than 500 mg/24 h and normal urine analysis were set for group A. Thirty lupus patients with clinical renal involvement were set for group B. All patients were subjected to full medical history and thorough clinical examination (general, systemic and musculoskeletal). Assessment of SLE disease activity was done by using the systemic lupus erythematosus disease activity index (SLEDAI) [17].

2.3. Laboratory assessment

Venous blood (8 ml) was withdrawn from each patient where, 5 ml was placed in EDTA tube for performing complete blood count (CBC) and erythrocyte sedimentation rate (ESR) and
3 ml of blood was collected in plain vacutainers for analysis of ANA, anti-dsDNA and anti-nucleosome antibodies. Serum samples were stored at -20°C until the time of assay. CBC was done using Coulter counter (T6600), ESR was done by the Westergren method. ANA and anti-dsDNA were performed by indirect immunofluorescence assay using IMMCO Diagnostics, USA (ANA on Hep-2 substrate and anti-dsDNA on Crithidia luciliae substrate). ANuA were assayed using the QUANTA Lite Chromatin ELISA Kit (INOVA Diagnostics, Inc. USA) according to the manufacturer’s instructions where serum values <20 U/ml were considered negative, values between 20 and 60 were considered moderately positive and values >60 U/ml were considered strongly positive.

2.4. Renal histopathological assessment for LN

The renal tissue was obtained from all lupus patients and evaluated by light microscopy by one pathologist. The biopsies were graded according to the classification of lupus nephritis by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) [18] in which normal glomeruli are designated as class I lupus nephritis while mesangial hypercellularity represents class II and a state of lupus nephritis showing focal or diffuse segmental or global endo- or extracapillary glomerulonephritis, with or without mesangial alterations is classified as class III (focal lupus nephritis) and class IV (Diffuse lupus nephritis), respectively. Membranous nephritis is categorized as class V and class VI is characterized by advanced sclerosis. Activity and chronicity scores are used according to Austin et al. [19].

Statistical methods: Statistical analysis was performed using IBM SPSS statistics (V. 21.0, IBM Corp., USA, 2012). Data were expressed as Mean ± SD for quantitative parametric measures in addition to median percentiles for quantitative non-parametric measures and both number and percentage for categorized data. Wilcoxon Rank Sum test was used for comparison between two independent groups for non-parametric data. Wilcoxon signed rank test was used for comparison between two dependent groups for non-parametric data. Comparison between pathological LN groups for non-parametric data was done by using the Kruskall Wallis test. Ranked Spearman correlation test was used to study the possible association between each of the two variables among each group for non-parametric data. Chi-square test was used to study the comparison between patients with and those without LN as regards the categorized data. P value < 0.05 indicated statistical significance while P < 0.01 and 0.001 indicated high statistical significance.

3. Results

3.1. Clinical and laboratory data of SLE patients

Twenty-four lupus patients with proteinuria less than 500 mg/24 h and normal urine analysis were included in group-A (20 females and 4 males) and their mean age was 24.54 ± 3.93 years. Thirty lupus patients with established renal disease were included in group-B (all females) and their mean age was 29.4 ± 5.8 years. Other characteristics of both groups are displayed in Table 1. Serum levels of ANuA were significantly higher in lupus patients, both groups A and B, than the control group (78.3 ± 43.6 and 86.2 ± 40.3 versus 14.6 ± 8.8 U/ml, respectively; P < 0.001).

3.2. Correlation between anti-nucleosome antibodies and lupus disease activity

Using ranked Spearman test (Table 2), serum ANuA levels in group-A patients showed a significant correlation with ESR and highly significant correlation with the SLEDAI, while in group-B the levels showed highly significant correlation with the SLEDAI and both pathological activity and chronicity indices whereas there was a significant negative correlation with hemoglobin levels. In group-A, 20 patients had positive and 4 had negative anti-dsDNA antibodies, while it was 15 and 15, respectively in group-B (data not shown). Using Wilcoxon signed rank test (Table 3), we found that patients with positive anti-dsDNA antibodies had more active disease presented by higher SLEDAI and levels of ANuA than those with negative anti-dsDNA antibodies. This was found in both groups.

3.3. Relation of anti-nucleosome antibodies and renal histopathological changes

In group-A, only 20 patients agreed to do renal biopsy, eight (40%) had class II LN and 12 (60%) had class III. Patients with class II LN had statistically higher levels of ANuA than the controls (52.1 ± 5.5 and 14.6 ± 8.8 U/ml, respectively; P < 0.001). Moreover, levels of ANuA were much higher in patients who had class III LN than those with class II LN (116.1 ± 23.5 and 52.0 ± 5.5 U/ml, respectively; P < 0.001) (Fig. 1). In group-B, eight patients (26.7%) had class II LN, eight (26.7%) had class III and 14 patients (46.6%) had class IV. Patients with class II LN had statistically higher levels of ANuA than the controls (35.6 ± 7.5 and 14.6 ± 8.8 U/ml, respectively; P < 0.001). As well, levels of ANuA were much higher in patients with class III and IV than those with class II LN (100.6 ± 38.4 and 106.8 ± 25.4 vs. 35.6 ± 7.4 U/ml).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Laboratory data and pathological data of the systemic lupus erythematosus patients with proteinuria less than 500 mg/24 h (group-A) and clinical renal involvement (group-B).</th>
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</thead>
<tbody>
<tr>
<td>Parameter (mean ± SD)</td>
<td>Systemic lupus erythematosus patients</td>
</tr>
<tr>
<td>Group-A (n = 24)</td>
<td>Group-B (n = 30)</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>9.3 ± 1.2</td>
</tr>
<tr>
<td>WBCs (×10^3)</td>
<td>5.9 ± 3.0</td>
</tr>
<tr>
<td>Platelets (×10^9)</td>
<td>188.3 ± 62.6</td>
</tr>
<tr>
<td>ESR (mm/1st hr)</td>
<td>103.3 ± 32.5</td>
</tr>
<tr>
<td>ANA</td>
<td>858.0 ± 334.1</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>12.0 ± 4.1</td>
</tr>
<tr>
<td>Activity index</td>
<td>78.3 ± 43.6</td>
</tr>
<tr>
<td>Chronicity index</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Prot/cr (gm/ml)</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>ANuA (U/ml)</td>
<td>78.3 ± 43.6</td>
</tr>
<tr>
<td>SLEDAI score, systemic lupus erythematosis disease activity index.</td>
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</tbody>
</table>

Hb, hemoglobin; WBCs, white blood cells; ESR, erythrocyte sedimentation rate; ANA, anti nuclear antibodies; ANuA, serum anti-nucleosome antibodies; SLEDAI score, systemic lupus erythematosis disease activity index.
respectively; \( P = 0.001 \) (Fig. 2). Yet, there was no significant difference in anti-nucleosome levels between patients who had class III LN and those with class IV LN \( (Z = -1.059 \) and \( P = 0.289 \)).

### 4. Discussion

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with multi-organ affection and diversity of clinical presentations as well as unpredictable course [20]. Kidney disease is a central and serious complication with glomerulonephritis as a major cause of morbidity and mortality in patients with SLE [21]. The most important mechanism in developing LN is the formation of immune complexes. Deposition of circulating immune complexes or its formation in situ will produce glomerular inflammation which will depend on the site and amount of theses immune deposits [3]. In SLE, DNA is a major component of the serum nucleosome and its potential contribution is through apoptosis, necrosis and Neutrophil extracellular traps’ induced apoptosis (NETosis) [22]. Serum nucleosomes released by apoptotic cells can serve as major auto-antigens for T and B cells. Those nucleosomes recognize heparin sulfate/collagens components of the glomerular basement membrane and facilitate binding of anti-nucleosome and other nephritogenic antibodies [23,24]. Electron microscopic examination of renal biopsies from patients with lupus nephritis showed that auto-antibodies attached the extracellular deposits of chromatin, confirming the suggestion that intraglomerular membrane-associated nucleosomes are targeted by nephritogenic antibodies [25].

In this study serum levels of ANuA were significantly higher in lupus patients (both groups A and B) than the controls. Several studies have discussed role of ANuA in the diagnosis of SLE as well as its role in monitoring lupus disease activity [26–28]. Testing for ANuA may serve as a sensitive and specific tool in the diagnosis of SLE, especially when the anti-dsDNA antibodies are absent [25].

We have demonstrated that the higher the serum level of ANuA, whether there is renal involvement or not, the more active the disease is. Moreover, we found that patients with positive anti-dsDNA antibodies had higher levels of serum ANuA than those with negative anti-dsDNA antibodies. Similarly, Sardeto et al., in his study of Brazilian SLE population, found a positive relationship between serum level of ANuA and disease activity measured by SLEDAI and anti-dsDNA presence [28].

Clinical renal involvement is present in about two-thirds of lupus patients and it carries significant morbidity and mortality [29]. Despite the improvement of treatment protocols, the outcome of lupus nephritis is still not rewarding and about 25% of lupus patients will have end stage renal disease within 10 years of diagnosis [30]. The frequency of end stage renal disease in patients with proliferative nephritis is much higher than those with other classes of nephritis [31]. Moreover, many studies have suggested that a higher percentage of lupus patients would have morphologic evidence of renal disease without clinical manifestations. This condition has been referred to as silent lupus nephritis and it was only diagnosed by renal biopsy [32–35]. Wakasugi et al. studied the renal biopsies of 195 lupus patients including 86 with no clinical data of renal involvement and found that 58% of lupus patients with no clinical renal affection had class I nephritis while 15% of this group had classes III and IV nephritis [36]. In the current study, 54 lupus patients were included, 24 patients with no clinical manifestations of renal affection, group-A, and 30 patients with clinical lupus nephritis (group-B). In group-A, 40% had class II nephritis, while 60% had class III. In group-B, 53.4% had classes II and III nephritis and 46.6% had class IV.

The relationship between ANuA and the degree of renal disease activity has been investigated by many [12,13,37]. Bigler et al. measured levels of ANuA in 35 patients with active

<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Group-A ( (n = 24) )</td>
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<td></td>
<td>( r )</td>
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<tr>
<td>Hb (gm/ml)</td>
<td>0.172</td>
</tr>
<tr>
<td>ESR (mm/1sthr)</td>
<td>0.413</td>
</tr>
<tr>
<td>SLEDAI score</td>
<td>0.545</td>
</tr>
<tr>
<td>Activity index</td>
<td>0.046</td>
</tr>
<tr>
<td>Chronicity index</td>
<td>-0.043</td>
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</tbody>
</table>

Hb, hemoglobin; ESR, erythrocyte sedimentation rate; SLEDAI score, systemic lupus erythematosis disease activity index. *Significant. **Highly significant.

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<thead>
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<tbody>
<tr>
<td></td>
<td>Group-A</td>
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<tr>
<td></td>
<td>( Z )</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>-3.191</td>
</tr>
<tr>
<td>ANuA (U/ml)</td>
<td>-3.101</td>
</tr>
</tbody>
</table>

class III and IV lupus nephritis and compared them with 59 controls of SLE who had inactive or no nephritis and found that ANuA have a high prevalence in patients with severe lupus nephritis but are of limited help in the distinction between patients with active proliferative lupus nephritis and those without active renal disease [12]. In another recent study, ANuA were found to be correlated with the activity index of proliferative LN but not with the chronicity index [13]. In our study, we have demonstrated that serum levels of ANuA correlated with the degree of renal affection, as in all studied lupus patients, serum level of ANuA was statistically higher in patients with mesangioproliferative nephritis than the controls. Moreover, it was statistically higher in patients with proliferative nephritis than those with mesangioproliferative nephritis. Yet, in both groups of lupus patients, anti-nucleosome was not useful in differentiating active from chronic renal affection.

In conclusion, serum levels of ANuA are associated with active lupus disease and correlate with the degree of renal affection. Moreover, in patients with insignificant proteinuria, serum levels of ANuA were elevated and were related to the degree of renal affection. The data presented here suggest that ANuA may be used as a surrogate marker for early renal affection in lupus patients with insignificant proteinuria. However, more follow up studies on larger number of patients are needed to confirm this and to study the correlation with other disease parameters such as the presence of antiphospholipid syndrome and damage index as well as the response to therapy.

**Conflicts of interest**

The authors declare no conflict of interest.

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References