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Original article

Antinucleosome antibodies as early predictors of lupus nephritis

Background: The role of the nucleosome in the induction of antibody response in lupus mediated tissue damage especially glomerulonephritis, may provide a new insight in the early diagnosis and alternative therapeutic developments in systemic lupus erythematosus (SLE).

Objectives: To evaluate the frequency and specificity of antinucleosome antibody expression in SLE patients in relation to disease activity. Also, to assess their predictive value in subclinical lupus nephritis.

Methods: This study included 26 patients with SLE and 52 control subjects (26 were healthy and 26 had juvenile rheumatoid arthritis "JRA"). Among lupus patients, 15 had clinical evidence of renal involvement. After clinical evaluation to calculate the SLE disease activity index (SLEDAI), measurements of urinary microalbumin and serum antinucleosome antibodies (antinucleosome specific, antihistone and anti ds-DNA antibodies by ELISA) were performed. Patients without clinical evidence of renal involvement were followed up for one year and measurement of urinary microalbumin was repeated at the end of the study period. Those who later developed microalbuminuria were categorized as patients with subclinical lupus nephritis.

Results: The expression of the 3 studied antinucleosome antibodies was significantly higher among lupus patients as compared to JRA patients and healthy controls. Seropositivity for one or more antinucleosome antibodies was elicited in 84.5% of lupus patients. Serum levels of the 3 antinucleosome antibodies were significantly higher among lupus patients with clinical nephritis than those without nephritis. ANSAb had higher sensitivity, specificity and positive and negative predictive values for subclinical lupus nephritis (100%) than antihistone and anti ds-DNA antibodies (43%, 100%, 100% and 50% respectively for either antibodies). All patients with lupus nephritis were seropositive for at least one of the antinucleosome antibodies, while those without clinical or subclinical nephritis were seronegative for the 3 antinucleosome antibodies. In 27.3% of patients with lupus nephritis, ANSAB was positive while both antihistone and ds-DNA antibodies were negative. Antinucleosome antibodies correlated positively with SLEDAI and cumulative steroid dose and negatively with corrected creatinine clearance.

Conclusions: The observed sensitivity and specificity of antinucleosome specific antibodies as early indicators of subclinical lupus nephritis appear encouraging and deserve further analysis on a large scale in order to confirm their validity, especially in the anti ds-DNA seronegative lupus patients.

Key words: antinucleosome antibodies, antinucleosome specific antibodies, anti ds-DNA antibodies, antihistone antibodies, SLE, lupus nephritis.

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INTRODUCTION

Evidence accumulated in recent years suggests that the nucleosome, the fundamental unit of chromatin and normal product of cell apoptosis, plays a key role in murine and human lupus as a major target autoantigen for autoantibody mediated tissue lesions¹. The broad antinucleosome antibody family

include; the nucleosome specific antibodies (antinucleosome antibodies without anti ds-DNA and antihistone reactivities), the antinucleosome antibodies with anti ds-DNA reactivity and the antinucleosome antibodies with antihistone reactivity². Anti ds-DNA antibodies account for a minor part (<30%) of the serum antinucleosome reactivity in SLE patients and nucleosome specific

autoantibodies are in large excess over anti ds-DNA in SLE³.

Antinucleosome specific antibodies have different genetic origin and pathogenic consequences compared with histone and ds-DNA specific antibodies⁴. In murine lupus, antinucleosome specific antibodies arise before the emergence of anti ds-DNA and antihistone antibodies and may be detected long before lupus-prone mice produce pathogenic autoantibodies (Pre-autoimmune mice)⁵.

Lupus nephritis is an immune complex disease. Nucleosomes and possibly complement factor C1q may be the major players in its pathogenesis. Antinuclear antibody (ANA) complexed to nucleosomes can bind to heparan sulphate in the glomerular basement membrane via the histone part of the nucleosome⁶. Understanding the key role of the nucleosome will likely make possible new therapeutic interventions in SLE, such as tolerance induction to the subnucleosomal particles⁷.

This study aimed to evaluate the frequency and specificity of antinucleosome autoantibodies in SLE patients and to study their relationship with disease activity and renal involvement.

METHODS

Study population:

This case-control study was conducted on 26 patients with SLE (22 females and 4 males) aged between 9 and 16 years (mean±SD: 13.5±2.5 years). They were enrolled from the Pediatric Allergy and Immunology Unit of the Children's Hospital of Ain Shams University, meeting at least 4 of the American Rheumatism Association Revised Criteria for diagnosis of SLE⁸. These patients were classified into two groups, the first group included fifteen patients with clinical renal involvement in the form of persistent proteinuria (more than 0.5 gm/day or more than 3+ by dipstick test) and/or cellular casts in urine (red blood cell, haemoglobin, granular, tubular or mixed)⁹. The second group comprised 11 patients with no clinical evidence of renal involvement based on normal results of urine analysis, serum creatinine and creatinine clearance. Patients without clinical renal involvement (group II) were followed up for one year by monthly microscopic urine analysis and 24 hours urinary protein. Measurement of urinary microalbumin was repeated at the end of the study period and patients who had microalbuminuria were categorized as patients with subclinical lupus nephritis. All lupus patients underwent detailed history and clinical examination to calculate the total SLE activity score (SLEDAI)¹⁰.

Twenty five out of the studied lupus patients (26) were receiving oral prednisolone (0.5-2 mg/kg/day) either as monotherapy (n=15) or in combination with other immunosuppressive drugs, such as intravenous pulsed cyclophosphamide (n=7), or oral azathioprine (n=3). Only one patient was receiving azathioprine alone without prednisone. The cumulative dose of steroid therapy during the whole duration of the disease was calculated (from the patients' records). The dose of intravenous pulsed steroids was added. The average dose of steroids per square meter body surface area was calculated.

In addition, 52 age- and sex- matched subjects were studied as a control group (group 3). They included 26 patients with juvenile rheumatoid arthritis "JRA" (22 females and 4 males), regularly followed-up at the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University (group 3A) after fulfillment of the American College of Rheumatology criteria for diagnosis of JRA¹¹. Their ages ranged between 9 and 16 years (mean±SD = 13.5±2.4 years). Group 3B included 26 healthy children (22 females and 4 males) with no personal or family history of collagen vascular disease or renal disorders. Their ages ranged between 9 and 16 years (mean±SD = 13.6±2.5 years).

Study measurements:

Sampling

Six milliliters of venous blood were collected. Four milliliters were transferred into a clean dry tube and left to clot. Prompt separation of serum was carried and used for direct assay of urea, creatinine, ANA, C3 and part of serum was stored at -20°C until assayed for of antinucleosome antibodies. The other two milliliters were collected on sodium citrate for erythrocyte sedimentation rate (ESR) measurement by Westergren method.

Twenty-four hours urine was collected for determination of urinary creatinine, total protein and microalbumin. The urine was collected in sterile containers with no preservatives. It was stored at 2-8°C. On the other hand, complete urine analysis was done on a freshly collected sample.

Assessment of serum and urinary creatinine:

This was carried out on synchron CX7 autoanalyzer (Beckman instruments, Brea, California, USA) by modified rate Jaffe method¹². The corrected creatinine clearance, according to the surface area, for every patient was then calculated as a percentage of the lowest normal value for age and sex.

Assessment of serum antinuclear antibodies (ANA) and C3:

Serum ANA was detected by indirect immunofluorescence (IMMCO Diagnostics, USA)¹³. Estimation of serum C3 was done by quantitative determination by turbidimetry (Turbiquant C3, Behringwerke Diagnostics, Marburg, Germany)¹⁴.

Determination of urinary total protein:

Urinary protein was assayed applying timed end point method. Protein in the sample reacted with the pyrogallol red molybdate to form purple colour complex that had a maximum absorbance at 600 nm. The assay was carried out using an automated analyzer (Synchron Cx7 system)¹⁵.

Determination of urinary microalbumin:

This was carried out using the turbidimetric analyzer. An undiluted urine sample was added to a buffer containing antibody specific for human serum albumin. The absorbance of the resulting turbid solution was proportional to the concentration of albumin in the sample urine. By constructing a standard curve from the absorbances of standards, the albumin concentration of the sample was determined¹⁶.

Determination of antinucleosome specific antibodies:

This was performed by the ELISA technique (ORGENTEC, Carl-Zeiss-Strasse Mainz)¹⁷. Antibodies to highly purified human nucleosomes (antinucleosome specific antibodies), if present in diluted serum, would bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically binds to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing substrate in the presence of bound conjugate is hydrolyzed to form a blue colour. The addition of an acid stops the reaction forming a yellow end product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample. Determination of antihistone antibodies and anti ds-DNA antibodies was based on the same principle¹⁷.

Interpretation of serum antinucleosome antibodies results

As data distribution was non-parametric, serum antinucleosome antibodies levels were considered

to be elevated if their levels were above the 95th percentile of the healthy control values (33.95 U/ml for antinucleosome specific antibodies, 39.3 U/ml for antihistone antibodies and 34.3 U/ml for anti ds-DNA antibodies).

Statistical analysis

The results were analyzed by commercially available software package (StatView, Abacus concepts, Inc., Berkeley, CA, USA). The data were presented as mean and standard deviation, in addition to median and interquartile ranges (IQR). Non parametric data were analyzed by the Mann Whitney (z) test and the relationship between numeric variables was determined using the Spearman correlation coefficient (r) test Chi-square test was used to compare qualitative variables. For all tests, a probability (p) of less than 0.05 was considered significant.

RESULTS

Serum concentration of antinucleosome antibodies in the studied population and their relation to clinical lupus nephritis

The three studied antinucleosome antibodies were significantly higher among lupus patients as compared to patients with JRA and healthy children ($p < 0.05$ for the 3 antibodies). Meanwhile, patients with JRA had significantly higher values of both serum antinucleosome specific and anti ds-DNA antibodies than healthy controls. Although serum levels of antihistone antibodies of JRA patients were higher than that of healthy controls, the difference was statistically insignificant. Serum antinucleosome antibodies were significantly higher in lupus patients with clinical nephritis than patients without clinical nephritis ($p < 0.05$ for the three antibodies). Table (1).

Microalbuminuria in SLE patients without clinical renal involvement and its relation to antinucleosome antibodies seropositivity

Initially, 4/11 (36.4%) of patients without clinical renal involvement had microalbuminuria. After a year of follow up, three more patients developed microalbuminuria as well. The 7 patients (63.6%) with microalbuminuria were considered to have subclinical lupus nephritis. All of them were seropositive for antinucleosome specific antibodies and three only were seropositive for antihistone and anti ds-DNA antibodies (Fig. 1).

Frequency of the antinucleosome antibodies among patients with SLE and JRA (pathological controls):

The group of lupus patients as a whole and the subgroups with and without clinical renal involvement had significantly higher percentage of elevated antinucleosome specific antibodies than those with JRA (80.8%, 93.3% and 63.6% respectively vs 3.8%) (Fig. 2A). Meanwhile, only lupus patients with clinical renal involvement had significantly higher percentage of elevated antihistone antibodies (80%) than controls with JRA (46.2%). Results of the SLE patients compiled in one group (57.7%) and those of SLE patients who had no clinical renal involvement (27.3%) were comparable with JRA controls (Fig. 2B). On the other hand, the whole group of SLE (50%) and subgroup with clinical renal involvement (66.7%) had significantly higher percentage of elevated anti ds-DNA antibodies than subjects with JRA (7.7%). Results of lupus patients who had no clinical renal involvement (27.3%) were comparable to the JRA controls (Fig. 2C).

Sensitivity, specificity and predictivity of antinucleosome antibodies for diagnosis of SLE and subclinical lupus nephritis:

Antinucleosome specific antibodies had the highest sensitivity, specificity and predictive values for SLE. Anti ds-DNA antibodies had higher specificity and predictive values than antihistone antibodies for SLE, while antihistone antibodies had higher sensitivity for SLE than anti ds-DNA

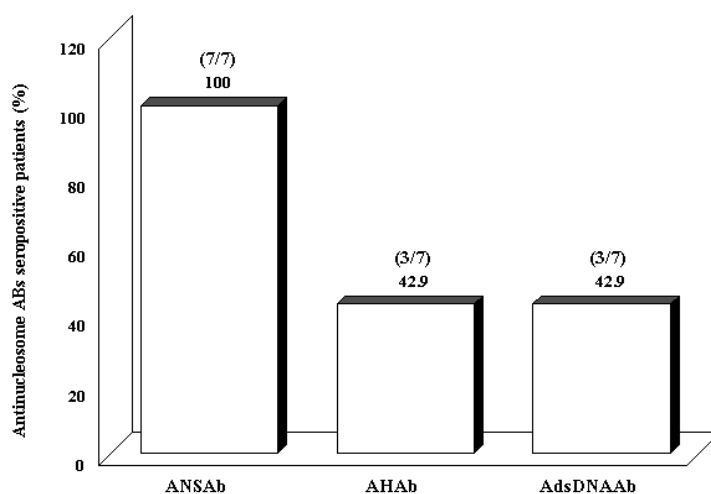
antibodies. On the other hand, antinucleosome specific antibodies had higher sensitivity, specificity and predictive values for early diagnosis of subclinical renal involvement in SLE than antihistone and anti ds-DNA antibodies (table 2).

Renal involvement in relation to antinucleosome antibodies' seropositivity:

All seropositive patients for one or more of the antinucleosome antibodies had lupus nephritis (either clinical or subclinical). On the other hand, lupus nephritis (either clinical or subclinical) was diagnosed in 20%, 63.5% and 69.2% of antinucleosome specific, antihistone and anti ds-DNA seronegative patients respectively. All anti ds-DNA and antihistone seronegative lupus patients with nephritis were seropositive for anti nucleosome specific antibodies (table 3).

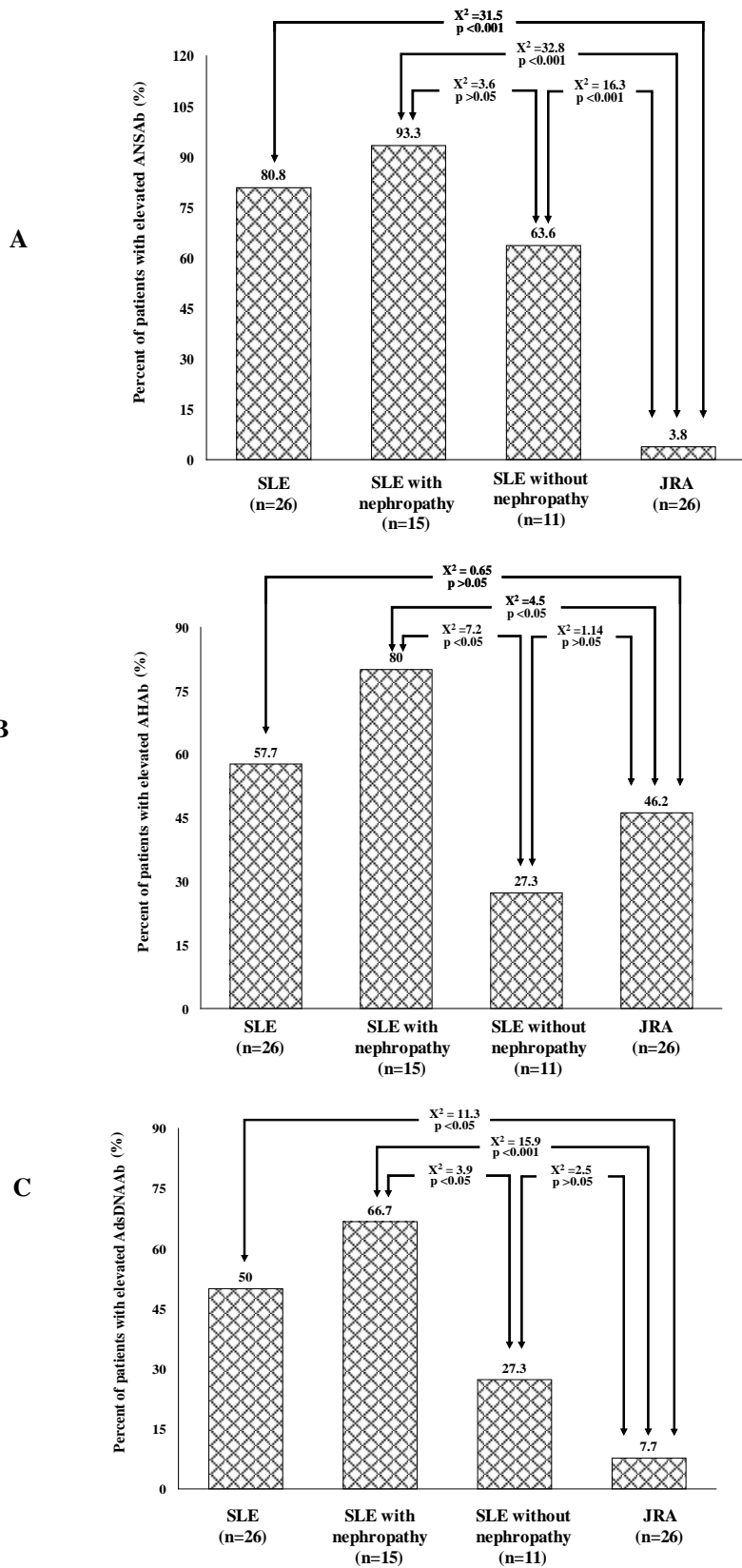
Correlation analyses

The recorded values of SLEDAI of SLE patients correlated positively to serum ANSAb, serum AHAb and serum anti ds-DNA antibodies ($r = 0.97, 0.99$ and 0.97 , respectively, $p < 0.05$). Also, the recorded cumulative steroid doses of SLE patients showed significant positive correlations with serum ANSAb, serum AHAb and serum anti ds-DNA antibodies ($r = 0.5, 0.6$ and 0.6 , respectively, $p < 0.05$). Also, values of creatinine clearance of SLE patients showed significant negative correlations with serum ANSAb, serum AHAb and serum anti ds-DNA antibodies ($r = 0.87, 0.85$ and 0.82 , respectively, $p < 0.05$).



ANSAb: antinucleosome specific antibodies, AHAb: antihistone antibodies, AdsDNAAb: antidouble stranded DNA antibodies.

Figure 1. Percentage of elevated antinucleosome antibodies among the seven patients with subclinical nephritis.



ANSAb: antinucleosome specific antibodies, AHAb: antihistone antibodies, AdSDNAAb: antidouble stranded DNA antibodies.

Figure 2. Percentage of patients with elevated antinucleosome antibodies in the studied groups

Table 1. Comparison between lupus patients and controls in serum levels of antinucleosome antibodies

	ANSAb (U/mL)			Antihistone Ab (U/ml)			Anti ds-DNA Ab (U/ml)		
	mean±SD	Median (IQR)	z (p)	mean±SD	Median (IQR)	z (p)	mean±SD	Median (IQR)	z (p)
All lupus patients	102.31 ±74.69	80 (135)	5.16	94.46 ±80.04	85 (143.75)	2.79	91.08 ±85.39	47 (138.5)	3.36
versus	vs	vs	(<0.05)	vs	vs	(<0.05)	vs	vs	(<0.05)
healthy controls	22.31 ±6.92	23 (10.25)		27.5 ±7.09	26 (9.5)		22.19 ±6.34	24 (8.5)	
All lupus patients	102.31 ±74.69	80 (135)	4.2	94.46 ±80.04	85 (143.75)	2.79	91.08 ±85.39	47 (138.5)	3.36
versus	vs	vs	(<0.05)	vs	vs	(<0.05)	vs	vs	(<0.05)
JRA controls	28.15 ±3.15	28 (4)		40.77 ±27.61	30.5 (43)		30.46 ±7.29	29 (2.5)	
JRA controls	28.15 ±3.15	28 (4)	3.49	40.77 ±27.61	30.5 (43)	2.77	30.46 ±7.29	29 (2.5)	4.33
versus	vs	vs	(<0.05)	vs	vs	(>0.05)	vs	vs	(<0.05)
Healthy controls	22.31 ±6.92	23 (10.25)		27.5 ±7.09	26 (9.5)		22.19 ±6.34	24 (8.5)	
Lupus with clinical nephritis	137.47 ±79.54	120 (139)	2.62	134.07 ±81.86	110 (158)	3.25	127.33 ±93.54	118 (170)	2.91
versus	vs	vs	(<0.05)	vs	vs	(<0.05)	vs	vs	(<0.05)
Lupus without clinical nephritis	54.36 ±26.59	56 (54)		40.45 ±32.75	28 (67)		41.64 ±36.33	22 (63)	

ANSAb = antinucleosome specific antibodies, IQR = interquartile range, p < 0.05 = significant

Table 2. Sensitivity, specificity and predictive values of antinucleosome antibody family for diagnosis of SLE and subclinical lupus nephritis.

	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
	SLE	Subclinical nephritis	SLE	Subclinical nephritis	SLE	Subclinical nephritis	SLE	Subclinical nephritis
ANSAb	80.8%	100%	96.2%	100%	95.4%	100%	83.3%	100%
AHAb	57.7%	43%	53.8%	100%	55.6%	100%	56%	50%
AdsDNAAb	50%	43%	92.3%	100%	86.6%	100%	64.8%	50%

ANSAb = antinucleosome specific antibodies, AHAb = antihistone antibodies, AdsDNAAb = anti double stranded DNA antibodies

Table 3. Renal involvement in relation to antinucleosome antibodies seropositivity

	ANSAb		AHAb		AdsDNA Ab	
	Seropositive (n=21)	Seronegative (n=5)	Seropositive (n=15)	Seronegative (n=11)	Seropositive (n=13)	Seronegative (n=13)
Renal involvement (clinical or subclinical)	21/21 (100%)	1/5 (20%) clinical nephritis	15/15 (100%)	7/11 (63.5%) clinical nephritis (3) subclinical nephritis (4)	13/13 (100%)	9/13 (69.2%) clinical nephritis (5) subclinical nephritis (4)

ANSAb = antinucleosome specific antibodies; AHAb = antihistone antibodies, AdsDNAAb = anti double stranded DNA antibodies.

DISCUSSION

Nucleosomes are generated *in vivo* by the process of apoptosis which is disturbed in SLE. Nucleosomes are the major target autoantigens for autoantibodies mediating tissue lesions, especially glomerulonephritis in SLE¹⁸. In the present study, all SLE patients had significantly higher serum levels of the three antinucleosome antibodies (antinucleosome specific, antihistone and anti ds-DNA antibodies) than both healthy controls and patients with JRA. Seropositivity for at least one of the three studied members of the antinucleosome antibody family was elicited in 84.6% of our studied SLE patients. These results are in accordance with previous studies which have reported that antinucleosome antibody reactivity is a very sensitive marker of SLE^{3,19,20,21}. In addition, 27.3% of our 26 lupus patients had elevated antinucleosome specific antibody level only. Similarly, it has been reported that 30% of SLE patients with high antinucleosome specific antibody reactivity have little, if any, anti ds-DNA or antihistone reactivity²¹.

The mechanisms that lead to the induction of antinucleosome autoantibodies in SLE remain obscure. In view of the prominence of nucleosomes which circulate at high levels in approximately one third of patients with SLE²², it has been speculated that highly accelerated rates of apoptosis²³, and/or abnormal sites or abnormal processing of apoptotic cells could lead to autoantibody production²⁴. Also, nucleosomes may elicit the production of interleukin-6 and stimulation of lymphoproliferation and IgG synthesis by splenic B cells. This could result in a polyclonal activation that triggers both a specific (nucleosome-driven) and non specific antibody production²². An important clue for the etiopathogenic role of antinucleosome antibodies in SLE is our finding of a significant positive correlation between SLEDAI and the 3 antinucleosome antibodies, this denotes that serum antinucleosome antibodies; reflect disease activity

in SLE patients. Amoura and associates³ have reported that antinucleosome antibody titres were positively correlated closely with the SLEDAI score.

In the present study, the antinucleosome specific antibodies had a sensitivity of 80.8% for the diagnosis of SLE. In previous studies, the reported sensitivities were 71.7% and 76% respectively^{3,25}. We also found that the antinucleosome specific antibodies had an excellent specificity for the diagnosis of SLE (96.2%). Bruns and associates²⁶ assumed that antinucleosome specific antibodies were highly specific for the disease (97%) and superior to anti ds-DNA antibodies. Antihistone antibodies had lower sensitivity (57.7%) and specificity (53.8%) for SLE than antinucleosome specific antibodies. This low specificity might be due to the presence of high percentage of antihistone antibody seropositivity among JRA patients (46.2%) while 3.8% and 7.7% only of JRA patients were seropositive for antinucleosome specific and anti ds-DNA antibodies respectively. In addition, our data revealed that anti ds-DNA antibodies had a much lower sensitivity (50%) for SLE than both antinucleosome specific and antihistone antibodies. In contrast, it had a better specificity (92.3%) for SLE. In other reports^{27,28}, the sensitivity for anti ds-DNA antibodies in SLE ranged between 50% and 70%.

Renal disease is a common manifestation of SLE. Although the multiple immunologic abnormalities of lupus can affect any organ system, involvement of the kidneys is often a major source of patient's morbidity and mortality²⁹. In the present study, lupus patients with clinical nephritis had significantly higher levels and seropositivity percentage of the three antinucleosome antibodies than patients without clinical nephritis. These findings indicate that antinucleosome antibodies might be reliable indicators of lupus nephritis. This assumption is supported by the results of Amoura and associates³ who revealed that patients with

active lupus nephritis had significantly higher percentage of antinucleosome antibody seropositivity than patients with active SLE but without nephritis.

It has long been known that in absence of clinical evidence of renal disease, excess urinary excretion of microalbumin could indicate subclinical nephropathy and its measurement may have a role in the early diagnosis and subsequent monitoring of renal disease in SLE³⁰. In the present work, 7 out of the 11 studied patients without clinical renal involvement had microalbuminuria i.e. subclinical nephritis and were antinucleosome specific antibody seropositive. In contrast, seropositivity for antihistone and anti ds-DNA antibodies was demonstrated in 3 patients only. Therefore, both antihistone and anti-ds DNA antibodies had a much lower sensitivity (43%) than antinucleosome specific antibodies (100%) for early diagnosis of subclinical lupus nephritis. This notion is supported by the presence of high levels of antinucleosome specific antibodies among patients who were initially free of microalbuminuria and were seronegative for both antihistone and anti-dsDNA antibodies. Actually, positive results for antinucleosome specific antibodies preceded the occurrence of microalbuminuria in those patients. Previous studies showed that antinucleosome specific antibodies appear before anti ds-DNA and antihistone antibodies and persist later in the course of the disease, along with the development of anti ds-DNA and antihistone antibodies⁷. These data are in agreement with current finding of an excellent sensitivity, specificity and predictivity for antinucleosome specific antibody in the early diagnosis of subclinical lupus nephritis (100%). Also, our results showed that 9 out of the 13 (69%) anti ds-DNA seronegative lupus patients were seropositive for antinucleosome specific antibodies, and 7 out of the 11 (63.6%) antihistone antibodies seronegative patients were seropositive for antinucleosome specific antibodies. Interestingly, all patients who were seropositive for antinucleosome specific antibodies and in the same time seronegative for either antihistone or anti ds-DNA antibodies showed some evidence of nephritis whether clinical or subclinical. This might suggest that the detection of anti-nucleosome specific antibodies could be a useful predictor of renal involvement specially among anti ds-DNA seronegative lupus patients.

An interesting observation was the absence of renal involvement, whether clinical or subclinical, among the 4 lupus patients who were seronegative for the three antinucleosome antibodies during one

year of follow up. It is hypothesized that when one or more of the antinucleosome antibodies is positive, this may be an index of renal involvement but when the 3 antinucleosome antibodies are negative, lupus nephritis may not be expected. The findings are of course limited by the sample size. Large scale studies are needed to verify this observation.

In the present work, there was a significant negative correlation between the three antinucleosome antibodies and creatinine clearance and a significant positive correlation between them and the cumulative dose of steroids. This means that the extent of the elevation of serum antinucleosome antibodies was closely linked to the severity of lupus nephritis as assessed by creatinine clearance and cumulative steroid dose. The relationship of antinucleosome antibodies to the severity of lupus nephritis is possibly a causal one in which these antibodies might not only play a role in the pathogenesis of lupus nephritis but also determine its clinical severity.

In conclusion, the observed sensitivity and specificity of antinucleosome specific antibodies as early indicators of subclinical lupus nephritis, especially in anti ds-DNA seronegative lupus patients, appear encouraging and deserve further analysis on a large sample of lupus patients in order to confirm the validity of these antibodies as early indicators of subclinical nephritis.

REFERENCES

1. **AMOURA Z, PIETTE JC, BACH JF, KOUTOUZOV S.** The key role of nucleosomes in lupus. *Arthritis Rheum* 1999; 42(5): 833-43.
2. **AMOURA Z, PIETTE JC.** Role of the nucleosome in the physiopathology of systemic lupus erythematosus [Abstr]. *Ann Med Interne (Paris)* 2003; 154 (1): 25-32.
3. **AMOURA Z, KOUTOUZOV S, CHABRE H, GAGOUB P, AMOURA I, MUSSET L, ET AL.** Presence of antinucleosome antibodies in a restricted set of connective tissue diseases: antinucleosome antibodies of IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. *Arthritis Rheum* 2000; 43 (1): 76-84.
4. **MOHAN C, LIU F, XIE C, WILLIAMS RC JR.** Anti-subnucleosome reactivities in systemic lupus erythematosus (SLE) patients and their first degree relatives. *Clin Exp Immunol* 2001; 123 (1): 119-26.
5. **SUENAGA R, ABDU NL.** Anti-(DNA-histone) antibodies in active lupus nephritis. *J Rheumatol* 1996; 23: 279-84.

6. **VAN BRUGGEN MCJ, KRAMERS C, WALGREEN B, ELEMA JD, KALLENBERG CG, VAN DEN BORN J.** Nucleosomes and histones are present in glomerular deposits in human lupus nephritis. *Nephrol Dial Transplant* 1997; 12 (1): 57-66.
7. **AMOURA Z, KOUTOUZOV S, PIETTE JC.** The role of nucleosomes in lupus. *Curr Opin Rheumatol* 2000; 12 (5): 369-73.
8. **TAN EM, COHEN AS, FRIES JF, MASI AT, MCSHANE DJ, ROTHFIELD NF, ET AL.** The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-7.
9. **DAVIS ID, AVNER ED.** Glomerulonephritis associated with systemic lupus erythematosus. In: Behrman RE, Kliegman RM, Jenson HB, editors. *Nelson Textbook of Pediatrics*. 17th ed. Philadelphia: WB Saunders; 2004. p. 1743-4.
10. **BOMBARDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH.** Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
11. **CASSIDY JT, LEVINSON JE, BASS JC, BAUM J, BREWER EJ, FINK GW, ET AL.** A study of classification criteria for diagnosis of JRA, a subcommittee of the diagnostic and therapeutic criteria and committee of the American Rheumatism Association. *Arthritis Rheum* 1986; 29: 274-81.
12. **BOWERS LD.** Kinetic serum creatinine assays. The role of various factors in determining specificity. *Clin Chem* 1980; 26(5): 551-4.
13. **TAN EM, CHAN KF, SULLIVAN K, ROBIN RL.** Determination of ANA and anti-double stranded DNA by immunofluorescent technique, clues towards the understanding of systemic autoimmunity. *Clin Immunol Immunopathol* 1988; 97: 121-5.
14. **JOHNSON AM.** A new international reference preparation for proteins in human serum. *Arch Pathol Lab Med* 1993; 117(1): 29-31.
15. **CANNON DC.** Principles and techniques. In: Henry RJ, Cannon DC, Winkelman JW, editors. *Clinical chemistry textbook*. 2nd ed. New York: Harper and Row Company; 1974. p. 422-31.
16. **MANCINI G, CARONARA AD, HERMANUS JF.** Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 1965; 2(3): 235-54.
17. **BURLINGAME RW, RUBIN RL.** Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. *J Immunol Methods* 1990; 134(2): 187-99.
18. **BERDEN JH, LICHT R, VAN BRUGGEN MC, TAX WJ.** Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis. *Curr Opin Nephrol Hypertens* 1999; 8 (3): 299-306.
19. **BURLINGAME RW, BOCY ML, STARKEBAUM G, RUBIN RL.** The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J Clin Invest* 1994; 94: 184-92.
20. **WALLACE DJ, LIN HC, SHEN GQ, PETER JB.** Antibodies to histone (H2A-H2B)-DNA complexes in the absence of antibodies to double-stranded DNA or to (H2A-H2B) complexes are more sensitive and specific for scleroderma-related disorders than for lupus. *Arthritis Rheum* 1994; 37(12): 1795-7.
21. **CHABRE H, AMOURA Z, PIETTE JC, GODEAU P, BACH JF, KOUTOUZOV S.** Presence of nucleosome-restricted antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 1995; 38(10): 1485-91.
22. **AMOURA Z, PIETTE JC, CHABRE H, CACOUB P, PAPO T, WECHSLER B, ET AL.** Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus: Correlation with serum antinucleosome antibody titers and absence of clear association with disease activity. *Arthritis Rheum* 1997; 40 (12): 2217-25.
23. **LORENZ HM, GRUNKE M, HIERONYMUS T, HERRMANN M, KUHNEL A, MANGER B, ET AL.** In vitro apoptosis and expression of apoptosis-related molecules in lymphocytes from patients with systemic lupus erythematosus and other autoimmune diseases. *Arthritis Rheum* 1997; 40 (2): 306-17.
24. **HERRMANN M, VOLL RE, ZOLLER OM, HAGENHOFER M, PONNER BB, KALDEN JR.** Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. *Arthritis Rheum* 1998; 41 (7): 1241-50.
25. **MIN DJ, KIM SJ, PARK SH, SEO YI, KANG HJ, KIM WU, ET AL.** Antinucleosome antibody: significance in lupus patients lacking anti-double stranded DNA antibody. *Clin Exp Rheumatol* 2002; 20 (1): 13-8.
26. **BRUNS A, BLASS S, HAUSDORF G, BURMESTER GR, HIEPE F.** Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. *Arthritis Rheum* 2000; 43 (10): 2307-15.
27. **MOKHTAR GM, MOSTAFA GA, ABD EL-HALIM AZ, KHALIFA SO.** Carnitine in pediatric patients with systemic lupus erythematosus. *Egypt J Pediatr* 2002; 19 (4): 627-42.
28. **BENSELER SM, SILVERMAN ED.** Systemic lupus erythematosus. *Pediatr Clin North Am* 2005; 52 (2): 443-67.
29. **APPEL GB, D'AGATI V.** Renal involvement in multisystem diseases: Lupus nephritis. In: Massry SG, Glassock RJ, editors. *Textbook of nephrology*. 4th ed. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 777-87.
30. **GUY JM, BRAMMAH TB, HOLT L, BERNSTEIN RM, MCMURRAY JR, TIESZEN K, ET AL.** Urinary excretion of albumin and retinol binding protein in systemic lupus erythematosus. *Ann Clin Biochem* 1997; 34 (Pt 6): 668-74.