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Study of Anti-nucleosome Antibodies as A Predictor of Early Renal Affection in Systemic Lupus Erythematosus Patients

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ABSTRACT

Background: Anti-nucleosome antibodies are a wide group of autoantibodies targeting the native nucleosome, which contribute to systemic lupus erythematosus (SLE) development.

Objective: This study aimed to assess the anti-nucleosome antibodies as a diagnostic predictor to lupus nephritis (LN), to evaluate its sensitivity and specificity, and changes in titers with LN treatment.

Patients and methods: The current research was conducted at Zagazig University Hospitals, Internal Medicine Department. A total of 60 SLE patients were involved in this survey. They were split into two groups: Group I consisted of 30 SLE patients without renal disease, and group II comprised of 30 individuals with lupus nephritis (LN). Group I was subdivided according to results of renal biopsy into (group Ia) with free kidney biopsy (n=17) and (group Ib) with class II/III LN (n=13).

Results: SLE cases with pathological abnormalities in kidney biopsy, including those with (group II) and without clinical LN (group Ib or silent LN) showed significantly higher anti-nucleosome antibody titers. After therapy, there was a significant drop in group II's anti-nucleosome antibody titer and 24-hour urine proteins. Anti-nucleosome antibodies sensitivity for prediction of abnormal renal biopsy was 95.3%, specificity was 94.5%, while for prediction of proteinuria sensitivity was 80%, and specificity was 80%.

Conclusion: Anti-nucleosome antibodies were more specific and sensitive than anti-dsDNA antibodies for diagnosing LN and early prediction of renal affection. Anti-nucleosome antibodies have valuable importance in following the response to treatment in LN.

Keywords: Anti-dsDNA antibodies, Anti-nucleosome antibodies, Systemic lupus erythematosus, Lupus nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder due to abnormal immune system that induced the generation of harmful autoantibodies, which have a role in diagnosis and are linked in systemic affection [(1)].

Lupus nephritis (LN) is common reason for death and morbidity. LN patients have mortality rates that are nearly six times greater [(2)]**.** Abnormal activation of self-reactive T and B cells, auto-antibodies, and immune complex production were detected in SLE [(3)].

Although, clinical evaluation is the cornerstone of managing SLE, this evaluation has limitations and need to be supplemented with other tests in order to confirm the diagnosis and assess the severity of the disease. Serological biomarkers are essential for SLE patient treatment [(4)].

Autoantibodies have been implicated with an increased risk of organ involvement in SLE [(5)]. Antinuclear antibodies (ANA) are the most important diagnostic serology marker for SLE, however because they are present in the majority of systemic autoimmune illnesses and even in healthy people, they have low specificity for SLE diagnosis [(6)].

Fundamental components of chromatin are nucleosomes. Histones are an essential component of double-stranded DNA (dsDNA), yet they are made of about 146 base pairs of DNA that are twice wrapped around a protein core that is an octamer made of two molecules. Histone H1 is connected to the outside of the complex to further bind the molecules together [(7)].

Nucleosomes are thought to be the primary antigens in the pathophysiology of SLE [(8)]. These nucleosome-specific antibodies show up earlier in the course of the disease than anti-dsDNA and anti-histone antibodies [(9)]**.**

PATIENTS AND METHODS

This current work was done at Nephrology Unit, Internal Medicine Department, Zagazig University Hospitals to evaluate the anti-nucleosome antibodies as a diagnostic marker in LN and to evaluate its sensitivity and specificity in comparison with anti-dsDNA antibodies, and evaluate changes in anti-nucleosome antibodies titer with treatment of LN.

This study comprised a total of 60 SLE patients. They were divided into 2 groups, group I involved 30 SLE individuals without manifestations of renal affection and group II contained 30 LN individuals. Group I was subdivided according to results of renal biopsy into group Ia with free kidney biopsy (n=17) and group Ib with class II/III LN (n=13). SLE cases were diagnosed using the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) (EULAR/ACR) criteria for the classification of SLE [(10)].

LN was diagnosed according to the American College of Rheumatology (ACR) criteria [(11)]. All participants were submitted to thorough history taking with stress on arthritis or arthralgia, fever, vasculitic changes, loin pain, dysuria, seizures, headache or psychosis.

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All patients were clinically examined with stress on: general examination, joint examination, skin rash, cardiovascular, chest, neurological examination, oedema of lower limb and lymphadenopathy.

All patients were exposed to laboratory testing: Complete blood count (CBC), complete urine analysis (RBCs, hyaline, WBCs, granular casts), erythrocytic sedimentation rate (ESR), C-reactive protein (CRP), 24 hours proteins in urine, kidney function tests, ANA, Anti-dsDNA antibody titer, C3 and C4 levels, liver function tests, and anti-nucleosome antibodies titer by Enzyme-linked immunosorbent assay(ELISA).
Renal biopsy was done for all cases.

Ethical approval:

The research received approval from Zagazig University's Ethics Board (ZU-IRB #9788), and each subject provided signed informed permission. This research has been done in conformity with the Declaration of Helsinki, the World Medical Association's ethical guidelines for research trial.

Statistical Analysis

The statistical testing was done utilizing the SPSS program (Statistical Package for Social Science version 26 and NCSS 12, LLC, USA). Qualitative variables were provided as frequencies and percentages, and quantitative variables were given as the median and range or means and standard deviations.

For evaluating descriptive data, the chi-square analysis and the fisher exacts analysis were utilised, while the independent t test and the Mann-Whitney analysis was used for contrasting quantitative data. ROC Curve, Spearman's correlation, Mann- Whitney's, logistic and multiple regression analysis and Chi square assessment were utilized as tests of significances. When $P- \leq 0.05$, the assessed value is considered significant.

RESULTS

In this study, 60 SLE patients, 30 SLE patients in group I without renal affection, and 30 patients with LN in group II. 93.3% of group II individuals were of female gender, with a mean age of 40.37 years, compared to 96.7% of group I patients who were females with a mean age of 27.83. Regarding age (older in group II), serum albumin (lower in group II), C3 (lower in group II), C4 (lower in group II), serum creatinine, 24 hour urine proteins (greater in group II), and eGFR (lower in group II), there was a statistically significant distinction between the examined classes (Table 1).

Anti-nucleosome antibodies and anti-dsDNA antibodies titer between the included groups differed significantly. SLE instances with pathologic abnormalities in kidney biopsy, comprising SLE patients without clinical symptoms (group Ib or silent LN) as well as those with clinical LN (group II), had significantly higher anti-nucleosome antibody titers (Table 2).

Both the titer of anti-nucleosome antibodies and the 24 hour urine protein decreased significantly in group II individuals after six months of immunosuppressive therapy (Table 3).

Anti-nucleosome antibodies had a sensitivity of 95.3%, and a specificity of 94.5% for detecting abnormal renal biopsy. Anti-nucleosome antibodies had a sensitivity of 80%, and a specificity of 80% for detection of proteinuria (Table 4 and figures 1 & 2).

Anti-dsDNA autoantibodies had sensitivity of 80% and specificity of 76.7% for detecting proteinuria. Anti-dsDNA autoantibodies had a sensitivity of 93%, and a specificity of 94.5 for detecting an abnormal kidney biopsy (Table 4 and figures 3 & 4).

Hemoglobin, serum albumin, and WBCs all displayed a statistically significant negative correlation with anti-dsDNA antibody titers (Table 5).

Significantly negative correlations were found between anti-nucleosome antibodies and haemoglobin, serum albumin, eGFR, C3, C4, and platelet counts. However, serum creatinine, ESR, and anti-dsDNA titer all significantly correlated positively with one another (Table 5).

Table (6) showed clinical and renal biopsy results of SLE patients**.**

Table (1): Baseline and laboratory variables between the examined groups

Parameter	Group		Test	P
	group I	group II		
	Median (IQR)	Median (IQR)		
Age (year)	27.83 ± 7.41	40.37 ± 10.63	-5.299¥	<0.001**
Female gender	29 (96.7%)	28 (93.3%)	Fisher∞	>0.999
Duration (m)	11 (5 – 12)	10 (7.5 – 12)	-0.023§	0.982
Hemoglobin	10.67 ± 1.67	10.74 ± 1.54	-0.179§	0.859
Serum albumin	4.35 ± 0.51	3.25 ± 0.46	8.881¥	<0.001**
WBCs	4.15 (3.2 – 7.175)	5.5 (3.19 – 8.55)	-0.776§	0.437
Platelet	232 (189 – 367)	237.5 (195 – 355)1	-0.274§	0.784
ALT	23 (15.75 – 31.13)	25.5 (16 – 32.25)	-0.614§	0.539
AST	26 (20 – 32.25)	27 (22.5 – 35.25)	-0.733§	0.463
ESR	31 (14.75 – 75)	36 (17.75 – 80)	-0.599§	0.549
CRP	5.5 (2.3 – 9)	6.8 (2.58 – 9.5)	-0.577§	0.564
Serum creatinine	0.62 (0.52 – 0.82)	0.85 (0.64 – 1.31)	-2.955§	0.003*
eGFR	122.5 (110.78 – 129.1)	88.4 (52.98 – 104.93)	-4.369§	<0.001**
24 urinary protein	51.5 (35 – 81.5)	3825 (3325 – 5005.5)	-6.655§	<0.001**
Anti-nucleosome antibodies	21.65 (12.52 – 43.81)	24.22 (15.32 – 41.04)	-1.375§	0.169
Anti-dsDNA antibodies	44.5 (10.6 – 111.57)	184.81 (119.13 – 200)	-2.877§	0.004*
C3	106.17 ± 4.46	42.69 ± 6.25	9.097¥	<0.001**
C4	17.93 ± 3.69	5.9 ± 1.01	6.667¥	<0.001**

IQR= interquartile range *p<0.05 considered statistically significant **p≤0.001 considered statistically highly significant ¥ quantitative parametric data are represented as mean and standard deviation and compared using independent sample t test § quantitative non parametric data are represented as median and interquartile range and comparing using Mann Whitney test ∞ descriptive data are represented as frequency and percentage and compared using Fisher exact test

Table (2): Anti-nucleosome and anti-dsDNA antibodies of cases

Parameter	group I		group II	Test	P
	Median (IQR)		Median (IQR)		
	group Ia (n=17)	group Ib (n=13)	Class II/III/IV (n=30)		
Anti-nucleosome antibodies	21.65 (12.52 – 43.81)		24.22 (15.32 – 41.04)	-1.375§	0.169
	12.54(10.76 – 19.55)	44.89(32.32 – 54.78)		29.664¥	<0.001**
Pairwise	P_1 0.003*	P_2 0.116	P_3 <0.001**		
Anti-dsDNA antibodies	44.5 (10.6 – 111.57)		184.81 (119.13 – 200)	-2.877§	0.004*
	32.65(15-119.39)	134.6(80.4-150)		10.77¥	0.006*
Pairwise	P_1 0.538	P_2 0.491	P_3 0.005*		

§Mann Whitney test ¥KW Kruskall Wallis test *p<0.05 is statistically significant Mann Whitney test KW Kruskall Wallis test IQR= interquartile range **p≤0.001 is statistically highly significant **p1** disparity between group Ia and group Ib. **p2** disparity between group Ib and group II **p3** disparity between group Ia and group II

Table (3): Change in anti-nucleosome antibodies and proteinuria after treatment

Parameter	Treatment		Wx	P
	Before	After		
	Median (IQR)	Median (IQR)		
Proteinuria	3825 (3325 – 5005.5)	756.5 (562.25 – 977)	-4.782	<0.001**
Anti-nucleosome antibodies	180.63 (15.44 – 200)	24.22 (18.45 – 41.04)	-5.978	<0.001**

**p≤0.001 is statistically highly significant, IQR= interquartile range, Wx Wilcoxon signed rank analysis.

Table (4): Performance of anti-nucleosome antibodies and ant-dsDNA antibodies in diagnosis of lupus nephritis

	Cutoff	AUC	Sensitivity	PPV	Specificity	NPV	Accuracy	P
Anti-nucleosome antibodies	≥81.601	0.898 (0.82 –0.98)	80%	80%	80%	80%	80%	<0.001**
Anti-dsDNA antibodies	≥111.715	0.857 (0.76 – 0.95)	80%	77.4%	76.7%	79.3%	78.3%	<0.001**
Abnormal renal biopsy								
Anti-nucleosome antibodies	≥28.68	0.99 (0.974 – 1)	95.3%	97.6%	94.5%	88.9%	93.3%	<0.001**
Anti-dsDNA antibodies	≥59	0.966 (0.923 – 1)	93%	97.6%	94.5%	84.2%	93.3%	<0.001**

Table (5): Relationship between the variables under investigation and anti-nucleosome and anti-dsDNA antibodies

Parameter	Anti-dsDNA antibodies		Anti-nucleosome antibodies	
	R	P	R	P
Age (year)	0.173	0.185	0.11	0.403
Duration (m)	0.027	0.839	0.157	0.231
Hemoglobin	-0.381	0.003*	-0.376	0.003*
Serum albumin	-0.294	0.023*	-0.268	0.038*
WBCs	-0.29	0.025*	-0.147	0.263
Platelets count	-0.054	0.68	-0.275	0.034*
ALT	-0.085	0.518	-0.071	0.589
AST	0.289	0.025*	0.234	0.073
ESR	0.221	0.09	0.284	0.028*
CRP	0.253	0.051	0.367	0.004*
Serum creatinine	0.103	0.432	0.255	0.049*
eGFR	-0.173	0.186	-0.316	0.014*
C3	-0.253	0.054	-0.391	0.002*
C4	-0.279	0.031*	-0.475	<0.001**
Proteinuria	0.396	0.002*	0.238	0.067

*p<0.05 is statistically significant, r Spearman rank correlation coefficient

Table (6): Assessment of the clinical manifestations between the examined groups

Parameter	Group			χ^2	P
	group I		group II		
	N=30 (%)		N=30 (%)		
Fever	9 (30%)		7 (23.3%)	0.341	0.559
Fatigue	22 (73.3%)		25 (83.3%)	0.884	0.327
Arthralgia	26 (86.7%)		26 (86.7%)	0	>0.999
Arthritis	12 (40%)		14 (46.7%)	0.271	0.602
Myalgia	24 (80%)		24 (80%)	0	>0.999
Alopecia	7 (23.3%)		8 (26.7%)	0.089	0.766
Photosensitivity	12 (40%)		12 (40%)	0	>0.999
Oral ulcers	18 (60%)		20 (66.7%)	0.287	0.592
Malar rash	17 (56.7%)		14 (46.7%)	0.601	0.438
Discoid rash	4 (13.3%)		5 (16.7%)	Fisher	>0.999
Skin rash	3 (10%)		4 (13.3%)	Fisher	>0.999
Purpura	3 (10%)		3 (10%)	0	>0.999
DVT	0 (0%)		3 (10%)	Fisher	0.237
Vasculitis	2 (6.7%)	4 (13.3%)	Fisher	0.671	
Raynaud's	8 (26.7%)	8 (26.7%)	0	>0.999	
Lymphadenopathy	2 (6.7%)	3 (10%)	Fisher	>0.999	
LL edema	1 (3.3%)	24 (80%)	36.274	<0.001**	
Seizures	2 (6.7%)	4 (13.3%)	Fisher	0.671	
Psychosis	1 (3.3%)	6 (20%)	Fisher	0.103	
Visual	3 (10%)	4 (13.3%)	Fisher	>0.999	
Headache	7 (23.3%)	9 (30%)	0.341	0.559	
Myositis	2 (6.7%)	3 (10%)	Fisher	>0.999	
Pleurisy	8 (26.7%)	11 (36.7%)	0.693	0.405	
Pericarditis	2 (6.7%)	6 (20%)	Fisher	0.254	
SLEDA score: Mild Moderate High	 17 (56.7%) 12 (40%) 1 (3.3%)	 18 (60%) 11 (36.7%) 1 (3.3%)	0.052¥	0.819	
Renal biopsy Free Class II Class III Class IV	 17 (56.7%) 6 (20%) 7 (23.3%) 0 (0%)	 0 (0%) 6 (20%) 10 (33.3%) 14 (46.7%)	29.489¥	<0.001**	

¥Chi square for trend test, χ^2chi square test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

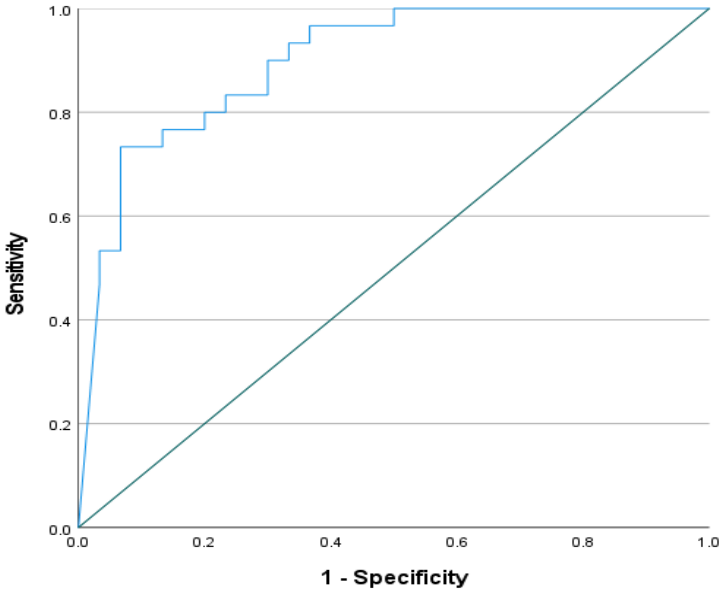

Figure (1): ROC curve showing performance of anti-nucleosome antibodies in diagnosis of proteinuria.

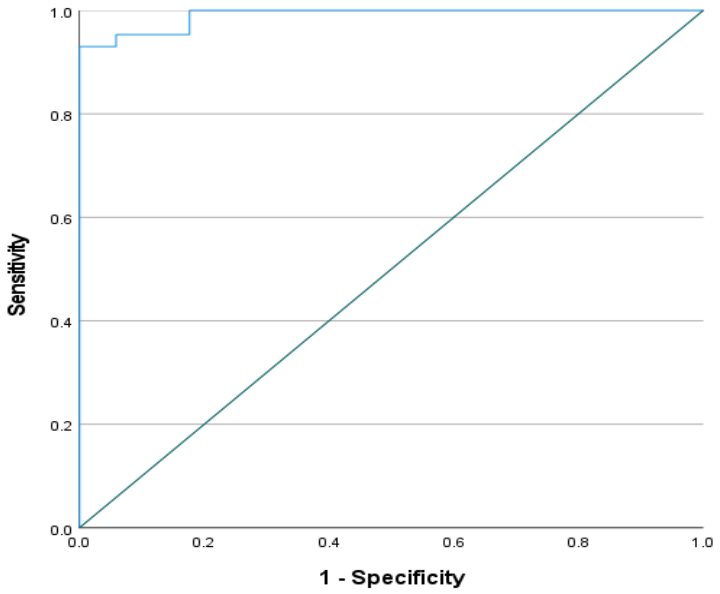

Figure (2): ROC curve showing performance of anti-nucleosome antibodies in diagnosis of abnormal renal biopsy.

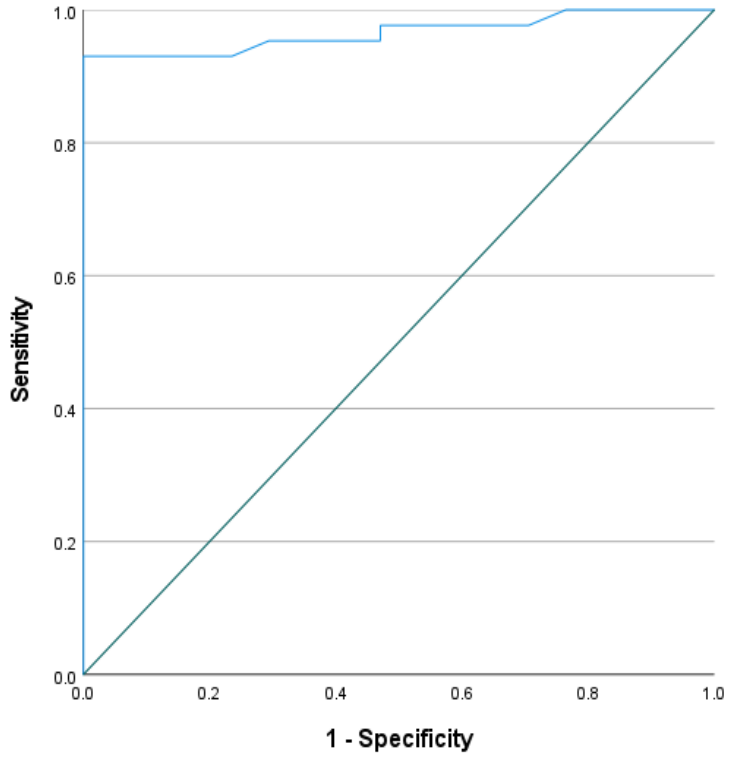

Figure (3): ROC curve showing performance of anti-dsDNA antibodies in diagnosis of proteinuria

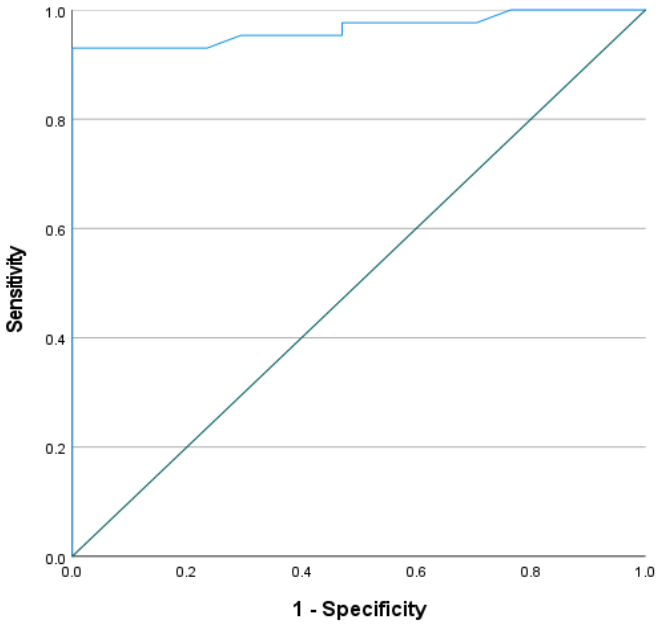

Figure (4): ROC curve examining performances of anti-dsDNA antibodies in diagnosis of abnormal renal biopsy.

DISCUSSION

The goal of this research was to investigate anti-nucleosome antibodies as a diagnostic marker for LN, to compare their sensitivity and specificity to anti-dsDNA antibodies, and to assess how treatment for LN affected the anti-nucleosome antibodies' titer. The anti-nucleosome antibody levels between LN cases and SLE cases without renal affection were evaluated in the current investigation.

Regarding patient demographics, this study discovered that LN patients had older mean ages than SLE patients without renal affection, which were respectively 40.37 ± 10.63 and 27.83 ± 7.41. Accordingly, this can be explained by the possibility that older people have more problems. In both groups, almost all individuals (93.3%) and (97.6%) were of female gender, with no discernible difference between the two groups. These findings are comparable with the results of **Sagial *et al.*** [9] who found that the majority of participants were of female gender, with a mean age of 30.92 ± 10.56 [9]. In contrast to LN individuals, we looked at SLE patients without renal affection as a control group in our research.

We discovered statistical insignificant differences in the examined groups regarding haemoglobin, WBCs, platelet counts, AST, ALT, ESR, CRP, gender, and disease duration. However, due to the presence of active LN in this cohort, there were statistically significant differences between the analyzed groups in terms of age (higher in LN group), serum albumin (lower in LN group), serum creatinine, 24 hour urinary proteins (higher in LN group), C3 (lower in group II), C4 (lower in group II), and eGFR (lower in LN group).

With the exception of LL edema, which was present in the LN group, there was insignificant difference between the included groups regarding clinical presentation or SLEDA score. 43.3% (13/30) of the participants in group I had renal biopsy pathological changes without LN clinical affection. Numerous studies have demonstrated that morphologic renal affection without clinical manifestations can occur in a higher percentage of lupus individuals. The only method of diagnosis for this condition, known as silent LN, was a kidney biopsy [12, 13]. **Wakasugi *et al.*** [14] examined the renal biopsies of 195 SLE individuals, there was no clinical proof of renal involvements in 86 of these people. 58% of these individuals had class I nephritis, and 15% additionally had classes III and IV LN. Participants with pathological abnormalities in their renal biopsy, both those with and without clinical symptoms (group Ib or silent LN), had considerably higher anti-nucleosome antibody titers. This clarifies the significance of anti-nucleosome antibodies in the early detection of LN prior to the onset of clinical symptoms or the use of renal biopsy for diagnosis. These findings are going with many studies [15-18]. This clarifies why the titer of both anti-nucleosome and anti-dsDNA antibodies rises when LN activities rise.

In our study, we sought to determine the correlation between anti-nucleosome antibody titers and disease activities. We discovered that active LN patients had high anti-nucleosome antibody titers, and we also sought to determine the relationship between anti-nucleosome antibody titers after immunosuppressive medications and the improvement of disease activity by monitoring 24 hour urinary proteins and anti-nucleosome antibody titers. To the best of our knowledge, there haven't been many research showing anti-nucleosome antibodies as a sign of therapeutic response. According to **Grootscholten *et al.*** [19], cyclophsophamide or azathioprine therapy could lower serum levels of anti-nucleosome antibodies. **Infantino *et al.*** [20] observed increased affinity to both anti-ds-DNA and anti-nucleosome antibodies in responding to biological therapy. According to **Rodriguez-Jimenez *et al.***, [17] although they did not

measure anti-nucleosome post-treatment, those with positive anti-nucleosome antibodies had a significant probability of renal recurrence by at least three times than that of remittent SLE cases who were negative for anti-nucleosome antibodies. Additionally, our study discovered that anti-nucleosome antibodies had higher sensitivity and specificity for detecting proteinuria than anti-dsDNA antibodies, but when it came to detecting abnormal kidney biopsy, anti-nucleosome antibodies had higher sensitivity but similar specificity to anti-dsDNA antibodies. These findings are similar to previous studies; **AbdEl-Wahab *et al.*** [21] found that for lupus nephritis individuals, anti-nucleosome antibodies had higher sensitivity and specificity (84.6% and 76.7%, respectively) than anti-dsDNA antibodies (58.9% and 60.5%, respectively) [21].

Li *et al.* [4] compared to anti-dsDNA antibodies, which properly recognised 67% and 50% of inactive lupus individuals, respectively, it was discovered that anti-nucleosome antibodies worked better than traditional markers, recognising > 80% of inactive lupus cases (sensitivity = 55%, specificity = 83%). **Suliman *et al.*** [22] revealed that although anti-dsDNA antibodies had a sensitivity of 61% and a specificity of 84% for the identification of active SLE, anti-nucleosome antibodies had a sensitivity of 98% and a specificity of 86%. **Elsayed *et al.*** [15] discovered that anti-nucleosome antibodies and anti-dsDNA antibodies both had high sensitivity and specificity for the diagnosis of LN. Anti-dsDNA antibodies, however, had lower sensitivity (83.3%) and specificity (93% and 94.4%) than anti-nucleosome antibodies (91.5%). **Sagial *et al.*** [9] stated that anti-nucleosome antibodies had a specificity of 69.59%, which was also lower than anti-dsDNA antibodies' (82.60%) and sensitivity of 70.59% and 64.70%, correspondingly. **Cervera *et al.*** [23] revealed that anti-nucleosome antibodies were more sensitive to the diagnosis of lupus nephritis than anti-dsDNA antibodies (81% and 75%, respectively), and that anti-nucleosome antibodies were more specific to the condition (39% and 63%, respectively). Finally, **Simon *et al.*** [24] observed that anti-nucleosome antibodies were more specific (100%) than anti-dsDNA antibodies (78.57%) and more sensitive (90% and 72.58%, respectively) than anti-dsDNA antibodies. On contrary, **Zivkovic *et al.*** [25] reported that Anti-nucleosome antibodies were less sensitive for SLE than anti-dsDNA antibodies (82.35% vs. 87.06%, correspondingly). This difference could be attributed to the fact that their survey's ROC curve employed antibody positivity cut-off ratios that were lower than those advised by the ELISA test manufacturers.

Leucocyte count was not associated with anti-nucleosome antibodies. In comparison with **Abdel Gawad *et al.*** [16] who discovered a negative association with WBCs, which can be attributed to the fact that leucopenia is a diagnostic indicator of SLE and a measure of the severity of the illness. While **Yang *et al.*** [26] discovered a positive association between white blood cell count and infections, which is connected to immunosuppression in lupus patients and can be attributed with this conclusion.

In line with **Simon *et al.*** [24], we discovered that the severity of the disease was not linked with either anti-nucleosome or anti-dsDNA antibodies. On the reverse **Rodriguez Jimenez *et al.*** [17] discovered a favourable association with the severity of the illness [17]. Additionally, it was shown that anti-nucleosome antibodies had a reverse correlation with haemoglobin level and this finding goes with **Abdel Gawad *et al.*** [16]**.** Consequently, it can be concluded that low haemoglobin is a pathologic and persistent indicator of lupus erythematosus. Consistent with earlier researches [16, 21, 24], a strong positive correlation between anti-nucleosome antibodies and ESR and CRP, however, they did not correlate with anti-dsDNA antibodies.

CONCLUSION

Anti-nucleosome antibodies were more sensitive and specific than anti-dsDNA antibodies for the detection of LN. Before clinical symptoms or assessment with renal biopsy and evaluation of disease activity, anti-nucleosome antibodies are a useful biomarker for early identification of renal affections in SLE patients. Anti-nucleosome antibodies are crucial for monitoring how immunosuppressive therapy is working for LN individuals.

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Conflict of interest: None.

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