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Anti-Nucleosome Antibody as a Marker of Systemic Lupus Erythematosus Activity

NADIA M. ABD EL-WAHAAB, M.D.*; SOHAIR K. SAYED, M.D.*; OMER HERDAN, M.D.**; MONA A. EL-ZOHRI, M.D.**; AYMAN M. MAHRAN, M.D.*** and HADIER M. HASSOUNA, M.Sc.*

The Departments of Clinical Pathology, Internal Medicine & Rheumatology** and Dermatology***, Faculty of Medicine, Assiut University*

Abstract

Background: Systemic Lupus Erythematosus (SLE) is a chronic multisystem autoimmune disease that is highly heterogeneous in its presentation. Effective SLE patient care relies on serological biomarkers. There is high interest in the identification of autoantibodies other than Antinuclear Antibodies (ANA) and anti-double stranded DNA (Anti-dsDNA).

Aim: To evaluate the use of antinucleosome (Anti-NCS) antibody as a single marker to detect disease activity and renal involvement in SLE patients.

Methods and Material: This case-control study was carried out in Clinical Pathology Department at Assiut University Hospital in the period from 2014 to 2017. It included 92 patients. Sixty-two patients were diagnosed as SLE. Thirty patients had connective tissue diseases other than SLE (non-SLE). Twenty apparently healthy subjects were taken as controls. All subjects were tested for ANA, anti-dsDNA antibody and Serum anti-NCS antibody on Alegria ® (ORGENTEC Diagnostic GmbH-Germany). Statistical analysis: Date entry and data analysis were done using SPSS Version 19, Chicago, USA (Statistical Package for Social Science).

Results: There was higher significant elevation in the level of anti-NCS antibodies than anti-dsDNA in SLE patients in comparison to those with non-SLE and control groups.In LN patients anti-NCS antibodies were highly elevated than anti-dsDNA comparing to non LN patients. Anti-NCS antibodies had a stronger correlation than anti-dsDNA antibodies with SLEDAI score. In 22 negative anti-dsDNA SLE patients, 13 (59.1%) were positive for anti-NCS. Anti-NCS antibodies showed higher sensitivity and specificity than anti-dsDNA in SLE and LN patients.

Conclusion: Anti-nucleosome antibodies is highly sensitive and specific in diagnosis of SLE, especially if anti-dsDNA antibodies are absent. Anti-NCS antibodies are good disease activity markers for the assessment of SLE and LN disease activity.

Correspondence to: Dr. Nadia M. Abd El-Wahaab,
The Department of Clinical Pathology, Faculty of Medicine, Assiut University

Key Words: *Systemic lupus erythematosus – Antinuclear antibodies – Anti-double stranded DNA – Anti-nucleosome antibodies – Lupus nephritis.*

Introduction

SYSTEMIC Lupus Erythematosus is an acquired, chronic, inflammatory, multiorgan, autoimmune disease **[1]** of unknown etiology **[2]**. The clinical spectrum of SLE is wide and ranges from benign easily treated disease with rash, arthritis, fatigue, to a very severe life threatening illness with progressive irreversible damage. The course of the disease is variable and is characterized by flares of inflammation that can threaten, in an unpredictable manner, almost any organ in the body **[3]**. Major organ involvement may lead to significant morbidity and mortality **[4]**. Abnormal activation of self-reactive T and B cells, self-antibodies and immune complex production were detected in SLE [5].Although clinical assessment is the cornerstone of SLE patient management, these evaluations are limited and require additional tests to confirm diagnosis and determine disease activity. Effective SLE patient care relies on serological biomarkers [6].Autoantibodies have been implicated with increased risk of organ involvement in SLE **[7]**. The most important diagnostic test for SLE is the detection of the presence of ANAs, however, they have low specificity for the diagnosis of SLE, because they are found in most systemic autoimmune diseases and even in healthy individuals **[8]**. It is well-known that patients continue to have disease activity 10 years after diagnosis **[9]** even with appropriate management, often involving new organ systems **[10]**. The assessment of disease activity in SLE depends on the use of standardized, reliable and validated indices **[11]**.

Anti-double stranded DNA antibodies serve as a diagnostic marker [6], and have been used as a marker for disease activity, especially in renal disease. But they are found only in 50% of SLE patients and do not always correlate with disease activity. Therefore, there is a great amount of interest in the identification of other autoantibodies that can be used in the diagnosis and assessment of disease activity in SLE patients [12].

Nucleosomes are basic elements of chromatin. They are the fundamental package unit of dsDNA and histones which is composed of approximately 146 base pairs of DNA wrapped twice around the protein core which is an octamer composed of two molecules and each of the molecule consists of the histones (H2A-H2B-H3-H4), histone H1 bound on the outside, similar to a finger on a knot to tighten the complex [12]. Nucleosomes are considered to be the major antigens in the pathophysiology of SLE [13]. These nucleosome-restricted or specific antibodies emerge before the occurrence of anti-dsDNA and antihistone antibodies and persist later in the course of the disease, along with the development of anti-dsDNA and antihistone antibodies [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-dsDNAcomplexes. The nucleosome has been identified as a major autoantigen responsible for generation of a number of antinuclear antibodies in SLE and LN [14]. Nucleosomes also play a pivotal role in the evolution of tissue lesions, especially glomerulonephritis. In LN, nucleosomes, anti-nucleosome antibodies, and nucleosome/ immunoglobulin complexes have been detected in the glomerular immune deposits, which support these findings [15].

Material and Methods

This case-control study included 92 patients attending the Rheumatology Unit, Department of Internal Medicine, Assiut University Hospital. Sixty two patients were diagnosed as SLE according to the criteria of American College of Rheumatology (ACR) for the classification of SLE [16]. Thirty patients had connective tissue diseases other than SLE (non-SLE); 15 patients Rheumatoid Arthritis (RA), 15 patients with other connective tissue diseases (Systemic Sclerosis (SSC), Spondyloarthritis (SPA), Behcet, ankylosing spondylitis and scleroderma). Twenty apparently healthy subjects were taken as controls. Thirty nine patients out of 62 SLE patients were diagnosed as lupus nephritis according to ACR criteria for diagnosis of LN [17,18]. The study was approved by the Ethical Committee of Faculty of Medicine, Assiut University. Written consents were taken from the patients before enrollment in this study.

Methods:

Five ml of venous blood were collected under complete aseptic conditions and divided into: Two ml of venous blood on EDTA tube for CBC and ESR and three ml were collected into plain tube. Blood was allowed to clot for 15 minutes at 37ºC and serum was separated by centrifugation at 1000 gx for 10 minutes. Separated serum was inspected to ensure it is clear and non-hemolyzed or lipaemic. Serum was divided into aliquots and were stored at –20ºC till time of assay. 24-hours urine was collected for measuring protein in urine. Creatinine clearance was calculated [19]. ANA, anti-dsDNA and anti-NCS were done on Alegria® (ORGENTEC Diagnostic GmbH-Germany), using ANA detect kit (ORG200-ORGENTEC-Germany) Lot.no. 1608420, anti-dsDNA screen kit (ORG204S-Orgentec-Germany) Lot.no.1615834 and anti-NCS kit (ORG228-ORGENTEC-Germany) Lot.no. 1614230 & 22851521.

Statistical analysis:

Date entry and data analysis were done using SPSS Version 19, Chicago, USA (Statistical Package for Social Science). Data were presented as number, percentage, mean, median and standard error. Chi-square test was used to compare between qualitative variables. Independent samples *t*-test was used to compare quantitative variables between two groups. Person correlation was done to measure correlation between quantitative variables. p-value considered statistically significant when $p<0.05$.

Results

Table (1) shows the demographic data of SLE, non-SLE patients and control group.

There was significant increase in the frequency of ANA in SLE and non SLE patients when compared to control group ($p=0.002$, $p=0.003$ respectively). In SLE patients there were significant increase in the frequency of anti-dsDNA and anti-NCS when compared to non-SLE and control groups ($p=0.000$) (Table 2).

Table (3) showed that the anti-dsDNA was significantly higher in SLE patients than in non-SLE patients and control group ($p=0.000$). The mean value of anti-NCS was significantly higher in SLE patients than in non-SLE patients and control group ($p=0.000$).

Among 22 SLE patients with negative anti-dsDNA, 13 (59.1%) patients had positive anti-NCS.

In SLE patients anti-dsDNA showed positive correlation with arthralgia (r=0.253, p=0.047) and SLEDAI score (r=0.262, p=0.040). Anti-NCS in SLE patients showed positive correlation with 24-hour protein, SLEDAI score, and anti-dsDNA (r= 0.485, p=0.000), (r=0.332, p=0.008), (r=0.324, p=0.010) respectively as shown in Figs. (1-3). There was no significant correlation between anti-dsDNA and anti-NCS antibodies in both non-SLE and control groups.

In LN patients there was significant positive correlation between anti-NCS level with 24-hour protein in urine, SLEDAI score and serum anti-dsDNA level (r=0.594, p=0.000), (r=0.341, p= 0.034), (r=0.335, p=0.037) respectively.

Table (4) showed significant increase in the frequency of anti-NCS antibodies in SLE patients with nephritis than SLE patients without nephritis (p=0.019).

In 39 LN patients, it was found that according to SLEDAI score [20], three patients were inactive (score=0) while 36 patients were active (score >4).

Anti-NCS test is more sensitive and specific in SLE and LN patients than anti-dsDNA. However by combining the results of the anti-dsDNA antibodies and anti-NCS antibodies in SLE patients it was found that the diagnostic sensitivity increased up to 85.48% and specificity was 78%. However by combining the results of the anti-dsDNA antibodies and anti-NCS antibodies in LN patients the diagnostic sensitivity was 84.7% and specificity was 53.5% as summarized in (Table 5).

Table (1): Demographic data of SLE, non-SLE patients and control group.

	SLE (n=62)		Non-SLE (n=30)		Control (n=20)		p-value 1	p-value 2	p-value 3
	No.	%	No.	%	No.	%			
Sex:									
Male	6	9.7	6	20.0	3	15.0	0.196	0.681	0.724
Female	56	90.3	24	80.0	17	85.0			
Age (years):									
Mean ± SE	35.13±0.57		34.37±1.23		35.90±1.46		0.679	0.991	0.552
Median (range)	36 (20-45)		35 (19-48)		34.5 (27-47)				
Duration of disease (years):									
Mean ± SE	3.39±0.38		3.64±0.46		–		0.365	–	–
Median (range)	2.5 (3m-13y)		3.0 (6m-9y)		–				
SLEDAI score:									
Mean ± SE	19.85±1.21		–		–		–	–	–
Median (range)	19 (4-42)		–		–		–	–	

Table (2): Frequency of ANA, anti-dsDNA and anti-NCS in all groups.

	SLE (n=62)		Non-SLE (n=30)		Control (n=20)		p-value 1	p-value 2	p-value 3
	No.	%	No.	%	No.	%			
ANA (U/ml):									
Negative	40	64.5	20	66.7	20	100	0.839	0.002*	0.003*
Positive	22	35.5	10	33.3	0	0			
Anti-dsDNA (U/ml):									
Negative	22	35.5	24	80	20	100	0.000*	0.000*	0.069
Positive	40	64.5	6	20	0	0			
Anti-NCS (U/ml):									
Negative	21	33.9	26	86.7	19	95	0.000*	0.000*	0.636
Positive	41	66.1	4	13.3	1	5			

*: Statistical significant difference (p<0.05).
1: Comparison between SLE and non-SLE groups.
2: Comparison between SLE and control groups.
3: Comparison between non-SLE and control groups.

Table (3): Comparison between the mean level of anti-dsDNA and anti-NCS in the studied groups.

	SLE (n=62)	Non-SLE (n=30)	Control (n=20)	*p*-value 1	*p*-value 2	*p*-value 3
Anti-dsDNA (U/ml):						
Mean ± SE	493.6±118.1	25.1±5.9	15.9±2.0	0.000*	0.000*	0.992
Median (range)	75.5 (6.3-297)	11.5 (3.2-150.0)	16 (2.0-29.0)			
Anti-NCS (U/ml):						
Mean ± SE	88.4±12.5	18.4±3.6	11.9±0.7	0.000*	0.000*	0.216
Median (range)	27.4 (3.6-6102)	14.1 (4.9-102.7)	11.3 (6.3-20.2)			

*: Statistical significant difference (p<0.05).
1: Comparison between SLE and non-SLE groups.
2: Comparison between SLE and control groups.
3: Comparison between non-SLE and control groups.

Table (4): Frequency of ANA, anti-dsDNA and anti-NCS in SLE patients with and without nephritis.

	SLE with nephritis (n=39)		SLE without nephritis (n=23)		*p*-value
	No.	%	No.	%	
ANA (U/ml):					
Positive	14	35.9	8	34.8	0.929
Negative	25	64.1	15	65.2	
Anti-dsDNA (U/ml):					
Positive	23	59.0	17	73.9	0.235
Negative	16	41.0	6	26.1	
Anti-NCS (U/ml):					
Positive	30	76.9	11	47.8	0.019*
Negative	9	23.1	12	52.2	

Table (5): Sensitivity and specificity of anti-dsDNA, anti-NCS and (anti-dsDNA + anti-NCS) in SLE and LN group.

Variable	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
SLE:						
Anti-dsDNA (U/ml)	64.52	53.85	87.0	24.1	62.7	0.592
Anti-NCS (U/ml)	66.13	90.00	89.1	68.2	76.8	0.781
Anti-dsDNA & Anti-NCS (U/ml)	85.48	78.00	82.8	81.2	82.1	0.817
LN:						
Anti-dsDNA (U/ml)	58.97	60.5	57.5	61.9	59.7	0.67
Anti-NCS (U/ml)	84.62	76.74	76.7	84.6	80.5	0.81
Anti-dsDNA & Anti-NCS (U/ml)	84.70	53.49	62.3	79.3	68.3	0.69

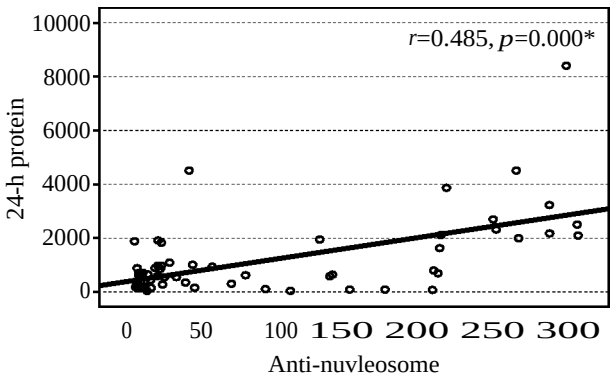

Fig. (1): Anti-nuvleosome.

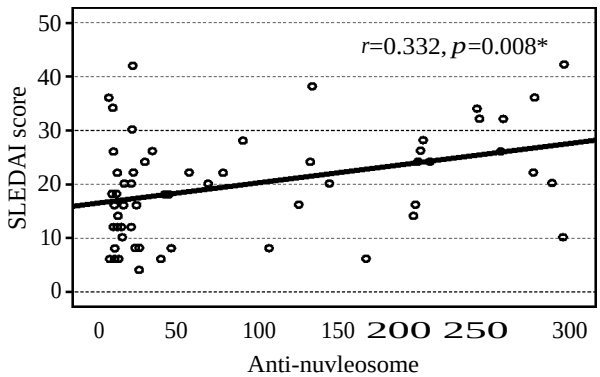

Fig. (2): Anti-nuvleosome.

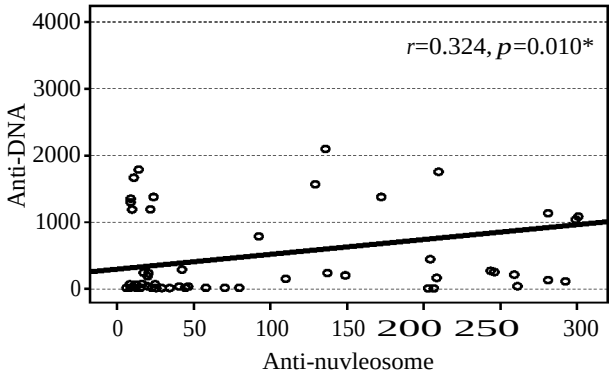

Fig. (3): Anti-nuvleosome.

Discussion

The present study included 92 patients; out of them, 62 patients were diagnosed as SLE: 56 (90.3%) of them were females and 6 (9.7%) were males. Couping with this result Zivkovic et al., [21] reported that females were higher than males. SLE had a strong female predominance because females with SLE presented with increased level of estrogens and reduced level of androgens, probably due to increased aromatase activity, an enzyme that converts androgens into estrogens [22,23]. In this study there was no significant difference between SLE, non-SLE and control groups in age and sex, and in disease duration between SLE and non-SLE patients.

Autoantibodies are important elements in both the diagnosis and monitoring of SLE, as some antibodies appear before the onset of clinical symptoms and others are associated with specific clinical manifestations [24]. Comparing anti-NCS with anti-dsDNA in SLE patients in the current study, the frequency of anti-NCS (66.1%) was higher than anti-dsDNA (64.5%). This result was in consistent with the results of the studies by Pradhan et al., [15] and Zivkovic et al., [21]. On the other hand, Min et al., [25] and Campos et al., [26] had reported lower frequency of anti-NCS than anti-dsDNA (76% & 79.6%-40% & 58.6% respectively). This difference from our study may be attributed to the difference in the number of studied cases and the sensitivity of the method used. In this study,out of 22 negative anti-dsDNA SLE patients, 13 (59.1%) patients had positive anti-NCS antibody. Similar to our result, Su et al., [27] reported that anti-NCS was present in 51.2% of anti-dsDNA negative SLE patients and in a Tunisian study, 23.8% had anti-NCS antibodies without anti-dsDNA Haddouk et al., [28]. These results supported that anti-NCS may be more sensitive in patients with SLE lacking of anti-dsDNA. Comparing anti-NCS with anti-dsDNA in non-SLE group in this study, the frequency of anti-dsDNA (20%) was higher than that of anti-NCS (13.3%). This result was agreed with the results of the study by saigal et al., [2]. On the contrary, Quattrocchi et al., [29] reported higher frequency of anti-NCS than anti-dsDNA.

This study demonstrated significant positive correlation between anti-NCS and anti-dsDNA in all SLE patients (r=0.324, p=0.010). Similar results were reported by Braun et al., [21] and Zivkovic et al., [30]. In the current study there was stronger correlation of anti-NCS than anti-dsDNA with SLEDAI score in SLE patients (r=0.332, p=0.008 and r=0.262, p=0.04) respectively. This is in agreement with the study by Amoura Z et al., [31], Simon et al., [32] and Suleiman et al., [33]. On the other hand, Saigal et al., [2] found a stronger correlation of anti-dsDNA than anti-NCS antibodies with SLEDAI score.

Comparing the sensitivity and specificity of anti-NCS with that of anti-dsDNA in SLE patients of this study, it was found that the sensitivity and specificity of anti-NCS (66.1% and 90% respectively) were higher than that of anti-dsDNA (64.5% and 53.8% respectively). Previous studies done by Abdel Gawad et al., [12] also reported higher sensitivity and specificity of anti-NCS than anti-dsDNA. On the contrary, Zivkovic et al., [21] reported that the sensitivity of anti-NCS antibodies for SLE was lower than the sensitivity of anti-dsDNA antibodies (82.35% vs. 87.06% respectively). This finding can be explained by antibody positivity cut-off values according to the ROC curve used in their study were lower than that recommended by the ELISA test manufacturer. On the other hand, Bizzaro et al., [34] reported that data from the meta analysis have shown that anti-NCS have equal specificity with anti-dsDNA antibodies in SLE. In our study by combining the sensitivity of anti-dsDNA and anti-NCS, the sensitivity became 85.5% which was higher than that of anti-dsDNA or anti-NCS alone. This result indicated that both of anti-dsDNA and anti-NCS together resemble the ideal tests for diagnosis SLE.

Out of 62 SLE cases of this study, 39 (63%) cases found to have LN. This result was in agreement with Cervera et al., [35] and Pradhan et al., [15] who detect nephritis in 52% and 44% respectively. ANA and anti-dsDNA frequencies in this study showed insignificant increase in LN patients [14 (35.9%) and 23 (59%) respectively] than in patients without nephritis [8 (34.8%) & 17 (73.9%) respectively]. The frequency of anti-NCS showed significant increase (p=0.019) in LN patients

(76.9%) than in non LN patients (47.8%). Su et al., **[27]** and Zivkovic et al., **[21]** also found that anti-NCS antibodies were associated with renal involvement.

In LN patients of this study, anti-dsDNA showed no significant correlation with any of the following investigations (ESR, CRP, urea, creatinine, creatinine clearance and 24-hour protein in urine). Also, anti-NCS showed insignificant correlation with (ESR, CRP, urea, creatinine, creatinine clearance) but showed significant positive correlation with 24-hour protein in urine (r=0.594, p=0.000). Saigal et al., **[2]** reported that anti-NCS antibodies showed significant correlation with various manifestations of LN, this suggested a possible role of anti-NCS in the pathogenesis of LN. Simon et al., **[32]** explained this association by the fact that histones which constitute part of the nucleosomes have a cationic charge, whereas the glomerular basement membrane has an anionic charge, which permits an interaction between them.

In this study in LN patients, there was no significant correlation between anti-dsDNA and SLEDAI score whereas anti-NCS and SLEDAI showed significant positive correlation (r=0.341, p=0.034). Therefore, serum anti-NCS antibody levels have value as diagnostic markers and in the assessment of disease activity especially active renal disease. Chen et al., **[36]** described anti-NCS as indicator of disease activity and lupus nephritis activity in adults. Significant positive correlation was detected in LN patients between anti-NCS and anti-dsDNA (r=0.335, p=0.037). Anti-nucleosome antibodies and anti-dsDNA antibodies are independent and complementary markers of SLE diagnosis and, therefore, are strongly suggested as combined tests **[37]**.

For LN in the current study it was found that the sensitivity and specificity of anti-NCS antibody (84.6% and 76.7% respectively) were significantly higher than that of anti-dsDNA (58.9% and 60.5% respectively). This result couping with the studies by Gutierrez-Adrianzen et al., **[38]** and Abd El-Gawad et al., **[12]**. On the other side, Saigal et al., **[2]** reported that anti-NCS antibodies were more sensitive but not more specific than anti-dsDNA antibodies in cases with nephritis. By combining the results of anti-dsDNA and anti-NCS in LN patients of this study, the sensitivity was (84.7%) higher than that of anti-dsDNA (58.9%) and nearly similar to that of anti-NCS (84.6%). So, combination of anti-dsDNA and anti-NCS were unnecessary in the diagnosis of LN.

Conclusion:

Results of this study may indicate that anti-NCS antibodies can be used as an additional marker for diagnosis and assessment of disease activity in SLE patients and especially LN patients. Anti-NCS antibodies may be a sensitive marker of SLE in the absence of anti-dsDNA antibodies. We recommended that Anti-NCS and anti-dsDNA should be done together for SLE patients as they provide more sensitivity than each parameter alone, while in LN patients this combination is unnecessary as the sensitivity of both together almost equal to the sensitivity of anti-NCS alone. We propose that the serological ACR criteria for the classification of SLE should be reconsidered and revised to include anti-NCS antibody as a criterion.

References

1- BAZZAN M., VACCARINO A. and MARLETTO F.: "Systemic lupus erythematosus and thrombosis." Thromb. J., 13: 6, 2015.

2- SAIGAL R., GOYAL L.K., AGRAWAL A., MEHTA A., MITTAL P., YADAV R.N., et al.: "Anti-nucleosome antibodies in patients with systemic lupus erythematosus: Potential utility as a diagnostic tool and disease activity marker and its comparison with anti-dsDNA antibody." J. Assoc. Physicians India, 61 (6): 372-7, 2013.

3- BICHILE L.S. and CHEWOOLKAR V.C.: Lupus flare: How to diagnose and treat. Medicine Update, 2011.

4- SHAIKH M.F., JORDAN N. and D'CRUZ D.P.: Systemic lupus erythematosus. Clin. Med. (Lond), 17 (1): p. 78-83, 2017.

5- ZHENG W., WU Y. and HUANG W.: "Down-regulation of nectin-4 inhibits apoptosis in systemic lupus erythematous (SLE) through targeting Bcl-2/Bax pathway." Int. J. Clin. Exp. Pathol., 8 (9): 10915-21, 2015.

6- LI T., PROKOPEC S.D., MORRISON S., LOU W., REICH H., GLADMAN D., et al.: "Anti-nucleosome antibodies outperform traditional biomarkers as longitudinal indicators of disease activity in systemic lupus erythematosus." Rheumatology (Oxford), 54 (3): 449-57, 2015.

7- WAN S., XIA C. and MOREL L.: "IL-6 produced by dendritic cells from lupus-prone mice inhibits CD4 + CD25 + T cell regulatory functions." J. Immunol., 178 (1): 271-9, 2007.

8- WANDSTRAT A.E., CARR-JOHNSON F., BRANCH V., GRAY H., FAIRHURST A.M., REIMOLD A., et al.: Autoantibody profiling to identify individuals at risk for systemic lupus erythematosus. J. Autoimmun., 27 (3): p. 153-6, 2006.

9- SWAAK A.J., VAN DEN BRINK H.G., SMEENK R.J., MANGER K., KALDEN J.R., et al.: "Systemic lupus erythematosus. Disease outcome in patients with a disease duration of at least 10 years: Second evaluation." Lupus, 10 (1): 51-8, 2001.

10- PETRI M.: "Hopkins Lupus Cohort. 1999 update." Rheum. Dis. Clin. North Am., 26 (2): 199-213, 2000.

11- TOUMA Z., UROWITZ M.B., TAGHAVI-ZADEH S., IBANEZ D. and GLADMAN D.D.: "Systemic lupus erythematosus disease activity Index 2000 Responder Index 50: Sensitivity to response at 6 and 12 months." Rheumatology (Oxford), 51 (10): 1814-19, 2012.

12- ABDEL GAWAD E.R., AI MANSOUR, ABDEL AZIZ Y.A., SOLIMAN A.F. and FAWZY R.M.: "Role of anti-nucleosome antibodies in the diagnosis of systemic lupus erythematosus and as a marker for lupus nephropathy." Egypt J. Immunol., 21 (1): 57-65, 2014.

13- GUPTA S., TATOULI I.P., ROSEN L.B., HASNI S., ALEVIZOS I., MANNA Z.G., et al.: Distinct Functions of Autoantibodies Against Interferon in Systemic Lupus Erythematosus: A Comprehensive Analysis of Anticytokine Autoantibodies in Common Rheumatic Diseases. Arthritis Rheumatol., 68 (7): p. 1677-87, 2016.

14- De ZUBIRIA SALGADO A. and HERRERA-DIAZ C.: Lupus nephritis: An overview of recent findings. Autoimmune Dis., p. 849684, 2012.

15- PRADHAN V.D., PATWARDHAN M.M. and 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., 76 (2): 145-9, 2010.

16- YU C., GERSHWIN M.E. and CHANG C.: Diagnostic criteria for systemic lupus erythematosus: A critical review. J. Autoimmun., 48-49: p. 10-3, 2014.

17- TAN E.M., COHEN A.S., FRIES J.F., MASI A.T., McSHANE D.J., ROTHFIELD N.F. et al.: The 1982 revised criteria for the classification of systemic lupus erythrematosus. Arthritis and Rheumatism, 25 (11): 1271-7, 1982.

18- PETRI M.: Systemic Lupus International Collaborating Clinic (SLICC). SLICC revision of the ACR classification criteria for SLE. Arthritis and Rheumatism, 60 (supplement 10): p. 895, 2009.

19- HAFEEZ A.R., IDREES M.K. and AKHTAR S.F.: Accuracy of GFR estimation formula in determination of glomerular filtration rate in kidney donors: Comparison with 24h urine creatinine clearance. Saudi J. Kidney Dis. Transpl., 27: 320-5, 2016.

20- POLACHEK A., GLADMAN D.D., SU J. and UROWITZ M.B.: Defining Low Disease Activity in Systemic Lupus Erythematosus. Arthritis Care Res (Hoboken), 69 (7): p. 997-1003, 2017.

21- ZIVKOVIC V., STANKOVIC A., CVETKOVIC T., MITIC B., KOSTIC S., NEDOVIC J., et al.: "Anti-dsDNA, anti-nucleosome and anti-C1q antibodies as disease activity markers in patients with systemic lupus erythematosus." Srp. Arh. Celok. Lek., 142 (7-8): 431-6, 2014.

22- KASSI E. and MOUTSATSOU P.: "Estrogen receptor signaling and its relationship to cytokines in systemic lupus erythematosus." J. Biomed. Biotechnol., 317-452, 2010.

23- WECKERLE C.E. and NIEWOLD T.B.: "The unexplained female predominance of systemic lupus erythematosus: Clues from genetic and cytokine studies." Clin. Rev. Allergy Immunol., 40 (1): 42-9, 2011.

24- CHING K.H., BURBELO P.D., TIPTON C., WEI C., PETRI M., SANZ I., et al.: Two major autoantibody clusters in systemic lupus erythematosus. PLoS One, 7 (2): p. e32001, 2012.

25- MIN D.J., KIM S.J., PARK S.H., SEO Y.I., KANG H.J., KIM W.U., et al.: Anti-nucleosome antibody: Significance in lupus patients lacking anti-double-stranded DNA antibody. Clin. Exp. Rheumatol., 20 (1): p. 13-8, 2002.

26- CAMPOS L.M., KISS M.H., SCHEINBERG M.A., MANGUEIRA C.L. and SILVA C.A.: Antinucleosome antibodies in patients with juvenile systemic lupus erythematosus. Lupus, 15 (8): p. 496-500, 2006.

27- SU Y., JIA R.L., HAN L. and LI Z.G.: "Role of anti-nucleosome antibody in the diagnosis of systemic lupus erythematosus." Clin. Immunol., 122 (1): 115-20, 2007.

28- HADDOUK S., BEN AYED M., BAKLOUTI S., HACHICHA J., BAHLOUL Z. and MASMOUDI H.: Clinical significance of antinucleosome antibodies in Tunisian systemic lupus erythematosus patients. Clin. Rheumatol., 24 (3): p. 219-22, 2005.

29- QUATTROCCHI P., BARRILE A., BONANNO D., GIANNETTO L., PATAFI M., TIGANO V., et al.: [The role of anti-nucleosome antibodies in systemic lupus erythematosus. Results of a study of patients with systemic lupus erythematosus and other connective tissue diseases]. Reumatismo, 57 (2): p. 109-13, 2005.

30- BRAUN A., SIS J., MAX R., MUELLER K., FIEHN C., ZEIER M., et al.: Anti-chromatin and anti C1q antibodies in systemic lupus erythematosus compared to other systemic autoimmune diseases. Scand. J. Rheumatol., 36 (4): p. 291-8, 2007.

31- AMOURA Z., KOUTOUZOV S., CHABRE H., CACOUB P., AMOURA I., MUSSET L., et al.: Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases: Antinucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. Arthritis Rheum., 43 (1): p. 76-84, 2000.

32- SIMON J.A., CABIEDES J., ORTIZ E., ALCOCER-VARELA J. and SANCHEZ-GUERRERO J.: "Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker." Rheumatology (Oxford), 43 (2): 220-4, 2004.

33- SULEIMAN S., KAMALIAH D., NADEEM A., NAING N.N. and CHE MARAINA C.H.: "Anti-nucleosome antibodies as a disease activity marker in patients with systemic lupus erythematosus." Int. J. Rheum. Dis., 12 (2): 100-6, 2009.

34- BIZZARO N., VILLALTA D., GIAVARINA D. and TOZZOLI R.: "Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis." Autoimmun. Rev., 12 (2): 97-106, 2012.

35- CERVERA R., KHAMASHTA M.A., FONT J., SEBASTIANI G.D., GIL A., LAVILLA P., et al.: Morbidity and mortality in systemic lupus erythematosus during a 10-year period: A comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore), 82 (5): p. 299-308, 2003.

36- CHEN D.Y., CHEN Y.M., WEN M.C., HSIEH T.Y., HUNG W.T. and LAN J.L.: "The potential role of Th17 cells and

Th17-related cytokines in the pathogenesis of lupus nephritis." Lupus, 21 (13): 1385-96, 2012.

37- INFANTINO M., GROSSI V., BENUCCI M., LI GOBBI F., DAMIANI A. and MANFREDI M.: "The impact of biological treatments on the anti-dsDNA and anti-nucleosome tests." Lupus, 27 (1): 40-8, 2018.

38- GUTIERREZ-ADRIANZEN O.A., KOUTOUZOV S., MOTA R.M., DAS CHAGAS MEDEIROS M.M., BACH J.F. and De HOLANDA CAMPOS H.: "Diagnostic value of anti-nucleosome antibodies in the assessment of disease activity of systemic lupus erythematosus: A prospective study comparing anti-nucleosome with anti-dsDNA antibodies." J. Rheumatol., 33 (8): 1538-44, 2006.

الآجسام المضادة للنيوكلوسومات كعلامة لنشاط مرض الذئبة الحمراء

من هذه الدراسة وجد آن هناك نتيجة متوافقة بين حساسية، دقة، القيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية بين الآجسام المضادة للنيوكلوسومات Anti-NCS ومرض الذئبة الحمراء. لذا يمكن الإعتماد على نتائج إختبار الآجسام المضادة للنيوكلوسومات فى مختبراتنا فى مستشفيات آسيوط الجامعية لآنها يمكن آن تساهم فى تشخيص وتحديد نشاط مرض الذئبة الحمراء ومرضى الإلتهاب الكلوى التناتج عن الذئبة الحمراء بحساسية ودقة آعلى من آى إختبار آخر.