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# Role of Anti-Nucleosome Antibodies in Diagnosis and Evaluation of both Disease Activity and Response to Therapy in Lupus Nephritis

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**Abstract**

The nucleosome plays a role in the pathogenicity of systemic lupus erythematouses. Antinucleosome antibodies are large family of autoantibodies react exclusively to nucleosomes and not to individual histones or native non-protein-complexed DNA .

***Purpose:*** Assessment of the role of anti-nucleosome antibodies in diagnosis and disease activity of lupus nephritis and their therapeutic response.

***Methods:***This is an observational prospective cohort study carried out at Baghdad Teaching Hospital, from first of January 2016 till December 2016. Fifty four patients their ages were 16-51years and there were 49 females and 5 males diagnosed as systemic lupus erythematouses and included into two groups: 27 patients in first group without renal involvement and 27 patients in second group with lupus nephritis (approved by biopsy). Serum anti-nucleosome and other biomarkers were measured to these two groups. The re-evaluation was assessed for each patient after three months period.

***Results:*** Anti-nucleosome antibody is high in the two groups but it is highest with lupus nephritis group (0.964) than second group (0.947) .Also anti-nucleosome antibody had the strong correlation between disease activity score for both groups. Find cut-off points of anti-nucleosome antibody is (>32.1) in lupus nephritis group which is lower than that of second group (>76.1).Patients using Mycophenolate mofetil had the lowest mean reduction in anti-nucleosome antibody (48.81) while patient taking Cyclophosphamide had the highest mean reduction (69.43) and both are significant.

***Conclusion:***Anti-nucleosome antibody is an excellent test of predicting disease activity,And could be a capable parameter to evaluate the effect of medications on disease activity.

**Keywords:** Systemic lupus erythematouses; lupus nephritis; anti-nucleosome antibodies

## INTRODUCTION

Systemic lupus erythematouses (SLE) is a chronic disease caused by an aberrant autoimmune response, which by various mechanisms leads to loss of self-tolerance causing inflammation and multiorgan dysfunction (1).This disease diversely affects multiple end-organs, including heart, joints, liver and kidneys by hyper activation of autoantibodies against cell nuclei antigens leads to the deposition of immune complex in end organs (2).

Lupus nephritis (LN) is a frequent and potentially serious complication to systemic lupus erythematouses (SLE) , in which autoantibodies combine with self-antigens to produce circulating immune complexes that deposit in the glomeruli, activate complement and provoke an inflammatory response(3). All renal parts including glomerular, tubulointerstitial, and vascular components may be affected by the disease; however, the term “lupus nephritis” is mainly used to define the immune complex-mediated glomerulonephritis (4).

Overall survival in patients with SLE is approximately 95% at 5 years after diagnosis and 92% at 10 years after diagnosis. The presence of lupus nephritis (LN) significantly reduces survival to approximately 88% at 10 years (5).

The antigens causing T-cell and B-cell stimulation in patients with SLE can be attributed to the inappropriate disposal of apoptotic cells. During the process of cellular death, pieces of cellular material form into the surface of the dying cell. Antigens that are normally absent from the surface of the cellular material but instead are embedded within, are now present on the cell surface (6). Nucleosomes and anionic phospholipids are examples of antigens that have been identified in patients with SLE, and they have the potential to trigger an immune response. It is believed that the removal of these apoptotic cells is compromised because of the impaired functioning of phagocytic cells, resulting in suboptimal disposal of dying cells and antigen recognition in patients with SLE. SLE is thought to develop when a T-lymphocyte to an antigen- presenting cell (APC) is introduced. The T-cell receptor binds to the major histocompatibility complex (MHC) portion of the APC, which may lead to cytokine releases, inflammation, and B-cell stimulation. Stimulation of B-cell division and the production of immunoglobulin G (IgG) autoantibodies that can cause tissue damage also occur . Unlike the situation in healthy adults, autoantigen-specific T cells and B cells may also interact and produce harmful autoantibodies (6,7).

Antinucleosome antibodies (AnuA) are a large family of autoantibodies directed to histone epitopes exposed to chromatin, against dsDNA and against conformational epitopes created by the interaction between dsDNA and core histones (8,9).Anti-nucleosomes antibodies react exclusively to nucleosomes and not to individual histones or native non-protein-complexed DNA .They detected by ELISA and represent the first serological marker for SLE described. Recently, nucleosomes are considered a major autoantigens in SLE in which they are positive about 85% of patients and probably play an important pathogenetic role (9).

Although anti-nucleosome antibodies may be also found in other autoimmune diseases such as systemic sclerosis, the prevalence of these antibodies in sera of SLE patients is higher. They are considered a more sensitive marker compared to dsDNA antibodies. Anti-nucleosomes may play an important role in SLE through the induction to a T cell mediated response by the hapten carrier-like system to raise several autoantibodies (9,10). At the

same time there exists evidence that nucleosomes are the central renal targets for nephritogenic autoantibodies since they are found in electron dense structure (EDS) in murine and human lupus nephritis (9,11). Also, some investigators reported that anti¬nucleosome antibodies could be found in patients that consistently tested negative for anti-dsDNA antibodies and that a significant fraction of those patients indeed had renal disease, suggesting that antinucleosome antibodies may serve as a sensitive marker for renal involvement in the absence of anti-dsDNA(9,12).

Aim of the study

To assess role of anti-nucleosome antibodies in diagnosis and evaluation of both disease activity and response to therapy in lupus nephritis.

## METHODS

***Study design:*** This study is an observational prospective cohort study.

***Setting and duration:*** It was carried out at the departments of Rheumatology and Nephrology at Baghdad Teaching Hospital, Medical City Complex, Baghdad, Iraq, from first of January 2016 till the December 2016.During that period fifty four patients was followed up with diagnosis of SLE and they were receiving treatment. Full medical history and complete physical examination were done for all patients.All patients have informed medical consent according to Helsenki laws .The study was approved by the ethical committee of Iraqi board of clinical pharmacy.

***Inclusion criteria:*** Patients with SLE (without renal involvement), Patients with LN (approved by biopsy) and Patients with age range from 16 to 51 years old.

***Exclusion criteria*** by history and clinical examination: Patients with concurrent infection or illness as tuberculosis and liver disease (who they have renal complications),Patients with renal impairment secondary to systemic disease as diabetes mellitus, vasculitis and systemic sclerosis,Pregnants and Patients with already immunological disease as asthma and inflammatory bowel disease (who they are taking immunosuppressant).

***Sample size and sampling technique :***

The sample size was sixty patients at beginning of study, then four patients withdrawn and two patients died, remnant fifty four patients included into two groups, each group contain 27 patients. First group SLE without renal involvement while second group of LN (approved by biopsy). They were 49 females (91%) and 5 males (9%). Their ages ranged between 16 and 51 years. All of them met the revised criteria of ACR/SLICC for diagnosis of SLE (2015).Disease activity scores was evaluated by using systemic lupus activity questionnaire (SLAQ) as showing in appendix 3.

***Methods***

The information data was taken from each patient include the following: name, age, weight, height, gender, BMI and therapy as showing in appendix 4. The re-evaluation and disease activity score were assessed for each patient after three months period according to SLAQ and serum anti-nucleosome antibodies titer which measured to these two groups. Also all patients were subjected to: Blood pressure,Laboratory investigations:Erythrocyte sedimentation rate (ESR) ,White blood cell count (WBC), Serum albumin,Anti-dsDNA antibodies,Complement 3 (C3),Complement 4 (C4) and Renal biopsy for LN patients .

Anti-nucleosome antibody was analyzed for two groups by enzyme- linked immunosorbent assay (ELISA) (Anti-Nucleosome ORG 228, Orgentec Diagnostika, Germany) according to the manufacturer's instructions. The Alegria® assay features barcoded 8-well-microstrips, called Alegria® Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria® Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and a test specific control. Moreover each strip has two antigen-coateds wells which serves as reaction wells for one control and one patient sample. The determination is based on an indirect enzyme linked immune reaction to the following steps: Antibodies present in positive samples bind to the antigen coated on the surface of the two reaction wells forming an antibody antigen complex. After incubation, a first washing step removes unbound and unspecific bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen complex. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolysation and color development during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nms. The Alegria® Test Strip is based on the proprietary SMC®-Technology (Sensotronic Memorized Calibration). The Alegria® Test Strip can be used with the diagnostic instrument Alegria® - a fully automated Random Access Analyser. Anti-dsDNA antibodies were detected by ELISA BioTek® instrument.WBC was measured by Ruby Cell-DYN Hematology Autoanalyzer while serum albumin is measured by Abbott® C8000 Autoanalyzer.C3 and C4 were detected by gel diffusion technique.

***Materials***

Five milliliters of venous blood were drawn from each patient, by sterile disposable syringe G23. 3 ml blood was transferred into disposable gel tube. Samples were allowed stand to 15 minutes to clot and then separated by centrifugation at 3000 rpms for 5 minutes to serum. 0.5 ml from serum was collected in plain tubes and kept frozen for measuring titer of anti-nucleosome antibodies. The remaining blood and serum was used to measure other tests.

***Instruments*** : Vortex mixer,Pipettes for 10 microliter,Deep freeze (Froilabo),Centrifuge (Rotofix 32A),ELISA Alegria®, ELISA BioTek®, Biochemistry Abbott® C8000 Auto-analyzer,Ruby Cell-DYN Hematology Auto-analyzer , ESR Mixrate X20 (Vital) ® & ESR 1.28 ml tube Antonio Serio (Sandonaci) and Sphygmomanometer 300-1(ITO) .

***Specimen collection and storage :***

The collection of whole blood specimens was using acceptable medical techniques to avoid hemolysis. Test serum should be clear and non-hemolyzed. Specimens of AnuA were refrigerated stored at -20°C up to six months and then analyzed at Al Nadhayir Al Mushah private laboratory, Baghdad, Iraq while other tests analyzed at laboratories of Baghdad Teaching Hospital. All materials were prepared for room temperature (20-28°C) prior to use.

***Statistical analysis:***

Continuous data presented using their mean and standard deviation if follow normal distribution, and their median and interquartile range (IQR: 25% - 75% of the data), for discrete variables their number and percentage used.Independent t test used to compare the significance of two means, while Mann Whitney U test used to compare the median between 2 groups. Chi square test used to compare the significance of distribution of different discrete groups, if sample size less than 20, or 2 or more expected frequency less than 5 Fisher exact used.Repeated measure two ways, analysis of variance (ANOVA) used to analyze the significance between 2 groups through 2 time intervals (1 month to 3 months) ,p value of interaction was calculated to see which of LN or SLE is better.

Linear regression analysis used to determine the degree of correlation between different continuous variables by correlation coefficient (r),if it is positive mean direct relationship, negative mean inverse relationship; 0 – 0.25 weak, 0.25 – 0.5 mild, and >

0.5 mean strong correlation.Receiver operator curves (ROC) used to see the validity of different parameters in separating cases of control and area under the curve i.e. AUC and its p value prescribe this validity (if AUC ≥ 0.9 mean excellent test, 0.8 – 0.89 means good test, 0.7 – 0.79 fair test otherwise unacceptable). Trapezoidal method used for calculating the curve.

In an ROC the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points. Each point of the ROC represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test of perfect discrimination (no overlap in the two distributions) has an ROC that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC is to the upper left corner, the higher the overall accuracy of the test.

All data were analyzed using SPSS20 program and Minitab version 17 software.

***Interpretation of Results :***

The cut-off values of anti- nucleosome antibodies is 20 U/ml .The normal value is < 20 U/ml while elevated value is ≥ 20 U/ml. Anti- dsDNA antibodies cut-off value is 25 U/ml and raised to value ≥ 25 U/ml .

## RESULTS

Fifty four Iraqi patients were enrolled in this study and baseline details for all patients are given in Table I . Medications at times of registration are shown in Figure I . Hydroxychloroquine was taken by 25 SLE patients while LN patients were 12 and its usage is significantly higher (*p* value <0.001) in SLE patients compared to LN. Mycophenolate mofetil was used by two patients of SLE and 9 patients of LN and its usage is significantly higher (*p* value <0.018) in LN compared to SLE patient whereas IV cyclophosphamide was taken by 18 LN patients only (no used by SLE patients). Cyclophosphamide usage is significantly higher (*p* value <0.001) in LN compared to SLE patients. In both groups Anti-nucleosome antibodies were significantly reduced (*p* value <0.001) from baseline, however there was no significant difference between LN and SLE in the reduction of anti-nucleosome antibodies Table II. In both SLE and LN groups, the reduction of the disease activity score was significant, however; the reduction of the disease activity score of LN was less than SLE.

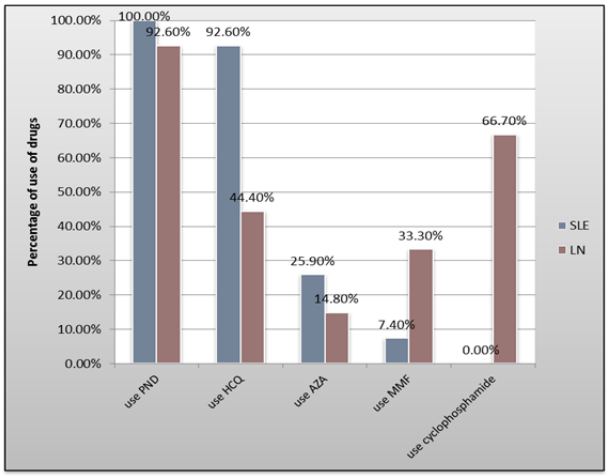


Figure I: percentage of the use of drugs for patients

***Correlation between disease activity and various variables: At baseline :*** The correlation coefficient of Anti-nuA is high in the two groups but it is the highest with LN (0.964) than SLE group . Also Anti-nuA had the strongest correlation between disease activity score .Anti-dsDNA had less correlation than Anti-nuA in both Table III .

***After 3 months:*** In LN group the correlation coefficient is high with anti-nuA (0.907) and in SLE group also anti-nuA had strongest correlation with disease activity score after three months. Anti-dsDNA after 3 months lost high correlation between SLAQ which indicates Anti-dsDNA with time loss high predictive ability with disease activity, while anti-nuA remains positivity correlated after 3 months Table IV .

**Table I: Demographic Data of Patients divided by Diagnosis**

| | | SLE | LN | All | P value |
|---|---|---|---|---|---|
| **Number** | | 27 | 27 | 54 | - |
| **Age** | | 32.0 ± 8.7 | 33.1 ± 10.1 | 32.6 ±9.3 | 0.677[a] |
| **Gender** | Female | 26 | 23 | 49 | 0.351[b] |
| | | 96.3% | 85.2% | 90.7% | |
| | Male | 1 | 4 | 5 | |
| | | 3.7% | 14.8% | 9.3% | |
| **Weight** | | 68.0 ± 10.4 | 67.5 ± 12.7 | 67.7 ± 11.5 | 0.889[a] |
| **Height** | | 159.9 ± 7.3 | 160.4 ± 7.5 | 160.1 ± 7.4 | 0.799[a] |
| **BMI** | | 26.8 ± 4.6 | 26.3 ± 4.7 | 26.5 ± 4.6 | 0.693[a] |

**a:Independent T test, b: Fisher exact test , SLE: Systemic lupus erythematosus , LN: Lupus nephritis, p value <0.05 is significant**

**Table II: Baseline Laboratory Variables for both Groups**

| | SLE | LN | P value |
|---|---|---|---|
| **Anti-dsDNA** | 59.6 ± 30.4 | 76.1 ± 46.4 | 0.130 |
| **C3** | 92.7 ± 18.6 | 83.6 ± 23.3 | 0.118 |
| **C4** | 23.1 ± 12.4 | 26.4 ± 17.0 | 0.422 |
| **WBC** | 7.8 ± 3.4 | 8.3 ± 3.3 | 0.604 |
| **ESR** | 52.7 ± 36.5 | 64.0 ± 36.9 | 0.260 |
| **Serum Albumin** | 3.9 ± 0.5 | 2.7 ± 0.9 | <0.001 |
| **Anti-nuA** | 129.7 ± 82.3 | 141.8 ± 87.4 | 0.606 |

**SLE: Systemic lupus erythematosus , LN: Lupus nephritis, p value <0.05 is significant**

**Table III: Correlation between Various Variables and Disease Activity Score at Baseline**

| Variable | SLE | | LN | |
|---|---|---|---|---|
| | r | P value | r | P value |
| **Age** | -0.397 | 0.040 | -0.051 | 0.801 |
| **BMI** | -0.487 | 0.010 | -0.132 | 0.512 |
| **Anti-dsDNA** | 0.560 | 0.002 | 0.602 | 0.001 |
| **$C_3$** | 0.077 | 0.704 | -0.396 | 0.041 |
| **$C_4$** | 0.230 | 0.248 | -0.213 | 0.287 |
| **WBC** | -0.404 | 0.036 | -0.016 | 0.936 |
| **ESR** | 0.357 | 0.068 | 0.345 | 0.078 |
| **Albumin** | 0.377 | 0.052 | -0.284 | 0.151 |
| **Anti-nuA** | 0.947 | <0.001 | 0.964 | <0.001 |

**SLE: Systemic lupus erythematosus , LN: Lupus nephritis, BMI:Body mas index, ESR: Erythrocyte sedimentation rate, WBC:White blood cell,p value <0.05 is significant, r: Correlation coefficient**

**Table IV: Correlation between Various Variables and Disease Activity Score after 3 months**

| Variable | SLE | | LN | |
|---|---|---|---|---|
| | r | P value | r | P value |
| **Age** | -0.300 | 0.128 | -0.070 | 0.727 |
| **BMI** | -0.338 | 0.085 | -0.100 | 0.619 |
| **Anti-dsDNA** | 0.309 | 0.117 | 0.289 | 0.143 |
| **$C_3$** | 0.097 | 0.629 | -0.076 | 0.707 |
| **$C_4$** | -0.196 | 0.326 | -0.160 | 0.426 |
| **WBC** | -0.402 | 0.037 | -0.206 | 0.302 |
| **ESR** | 0.429 | 0.025 | 0.153 | 0.445 |
| **Serum Albumin** | 0.184 | 0.357 | -0.003 | 0.987 |
| **Anti-nuA** | 0.929 | <0.001 | 0.907 | <0.001 |

**SLE: Systemic lupus erythematosus , LN: Lupus nephritis, BMI: Body mas index, ESR: Erythrocyte sedimentation rate, WBC:White blood cell,p value <0.05 is significant, r: Correlation coefficient**

**Table V : ROC analysis of the validity of baseline Anti-Nucleosome for predicting final SLAQ**

| | AUC | 95%CI | Optimal Cut off | Sensitivity | Specificity | P value |
|---|---|---|---|---|---|---|
| **LN** | 0.937 | 0.772 – 0.994 | >32.1 | 100% | 83.3% | <0.001 |
| **SLE** | 1.0 | 0.872 – 1.0 | >76.1 | 100% | 99.6% | <0.001 |

**SLE: Systemic lupus erythematosus , LN: Lupus nephritis, ROC: Receiver operator curve, CI: Confidence Interval , SLAQ: Systemic lupus activity questionnaire , AUC: Area under the curve , P value <0.05 is significant**

**Table VI : Correlation between Anti-Nucleosome and therapy for LN patients**

| Drugs | | Baseline | | 3 months | | Mean difference | P value |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | | |
| **Hydroxychlo-roquine** | Used | 132.78 | 99.17 | 82.34 | 68.65 | 50.43 | 0.018 |
| | Unused | 148.81 | 79.69 | 75.80 | 51.24 | 73.00 | <0.001 |
| **Azathioprine** | Used | 135.10 | 85.94 | 68.10 | 35.04 | 67.00 | 0.084 |
| | Unused | 142.83 | 89.56 | 80.55 | 62.04 | 62.28 | <0.001 |
| **Mycophenola-te mofetil** | Used | 162.64 | 74.62 | 113.83 | 62.25 | 48.81 | 0.013 |
| | Unused | 131.21 | 93.41 | 61.14 | 49.25 | 70.06 | <0.001 |
| **Cyclophosph-amide** | Used | 133.93 | 90.31 | 64.51 | 48.93 | 69.43 | <0.001 |
| | Unused | 157.19 | 84.35 | 107.11 | 68.27 | 50.08 | 0.023 |

**P value <0.05 is significant**

***Diagnostic accuracy of Anti-nucleosome antibody for predicting disease activity***

Anti-nuA is an excellent test of predicting SLE disease activity. The Optimal (founded) cut-off values of anti-nucleosome antibodies is lower for predicting disease activity in LN patients (>32.1U/ml) compared to SLE patients (>76.1U/ml) as show in Table V.

Patients using MMF had the lowest mean reduction in serum anti-nuA (48.81) while patient taking IV Cyclophosphamide had the highest mean reduction (69.43). Patient taking AZA despite they had mean reduction of (67.00) it was not significant, as illustrated in Table VI.

## DISCUSSION

This study is the first observational prospective cohort study about the relationship between anti-nucleosome antibody and LN in Iraq. Data analysis was showed that mean age of all patients is 32.6 ±9.3 years which are agreed with the age range of most populations (third decade of age). It is older than the age in Shabana et al. study that found SLE patients with a mean age of 25 ± 9.3 years (13) but close with Souza et al. study that found SLE patients with a mean age of 35.1 ± 11.9 years (14). Most of the patients were females (90.7%) which agrees with Stefanidou et al. study (15) and Tan et al study (16). Also this study was showed that mean of body mass index of all patients was around 26 kg/mm2 which indicate most patients were overweight as in Rizk et al. study (17).

Anti-dsDNA mean level was 59.6+30.4 IU/ml in SLE group while in LN group was 76.1+46.4 IU/ml which shows active disease in both groups. At baseline, Anti-dsDNA level is higher in LN patients and also after three months. In both SLE and LN patients, the reduction of anti-dsDNA to normal level was significant (p value <0.001) after three months which agree Linnik et al. study (18).

Both SLE and LN patients had significantly normalized their C3 level (p value <0.001) after three months, however; their normalization was similar in two groups while C4 level in SLE patients had more significant normalization as compared to LN patients at same periods as in Birmingham et al. study (19) and Hussain et al. study (20).

White blood cells of patients had significantly reduced in both groups (but all means of both groups within normal limits). The reduction was more in SLE group than LN group which indicate LN is higher inflammatory condition than SLE without renal involvement these findings agreed Lertchaisataporn et al. study (21), While ESR was high in both groups (indicate active disease) and significantly decreased to same degree in both groups after three months as in Stojan et al. study (22) and Delcea et al. study (23).

Other laboratory tests which measured in this study was serum albumin which had lower mean level in LN group than SLE group and this agrees Jonathan et al. study (24). Also this study approved by Manshu et al. study (25) that found decreased serum albumin levels may reflect the activity and severity of renal damage to SLE patients.

In this study found that anti-nucleosome antibody can establish a diagnosis of lupus nephritis due to its level was high. It has been demonstrated that SLE patients and LN patients had high anti-nucleosome antibodies level with elevated disease activity score, this agree with Valentina et al. study (26) that found anti-nucleosome antibodies demonstrated association with SLE and lupus nephritis activity, suggesting their potential usefulness in making predictions about lupus nephritis and assessment of disease activity.

This study found that serum of anti-nucleosome antibody is lower than 32.1U/ml may indicate inactive disease for LN patients while if lower than 76.1U/ml this may indicate inactive SLE disease with very high probability (100% sensitivity and 99.6% specificity) in comparing with Soha et al. study (27) that found level of 37.5 U/L was postulated as a cutoff point of 100% sensitivity, and 66.7% specificity in SLE patients.

This study found that anti-nucleosome antibodies have high sensitivity (100%) and high specificity (99.6%) in SLE patients in comparing with Su et al. study who reported that the sensitivity and specificity of anti-nuA in SLE was 61.8% and 97.6% respectively (28) and with Braun et al. study that stated anti-nuA had a sensitivity of 64.1% and specificity of 99.2% for SLE diagnosis (29). The results of this study are consistent with Tikly et al. study who achieved cross sectional study on 86 SLE patients. He is found that sensitivity and specificity of anti-nuA were 45.3% and 94.3%, respectively (30).

Among LN patients, anti-nuA is more likely to be detected in this study (mean 141.7+ 87.4) and may act as biomarker in the diagnosis of lupus nephritis as in Valentina et al. study (26), Koutouzov et al. study (31), Mortensen et al. study (32) and Putova et al. study (33).

This study confirmed that anti-nuA may be useful parameter for diagnosing lupus nephritis especially in those patients with negative anti-dsDNA antibodies (43% of them have positive anti-nuA) which agreed with Min et al. study (34) and Ghillani-Dalbin et al.study (35). Also, patients with LN had high Anti-nuA titer (the mean 141.7+87.4) than anti-dsDNA (the mean 76.1+46.4) as comparing with Valentina et al. study (41), Cervera et al. study (36) and Gutierrez- Adrianzen et al. study (37).

About treatment, this novel study was searched correlation between anti-nuA and therapy of LN. It was found that patients using MMF had the lowest mean reduction in serum anti-nuA (48.81) while patients taking IV cyclophosphamide had the highest mean reduction (69.43). Patients taking AZA despite them had mean reduction of (67.00) it was not significant due to only four patients take AZA. These results confirm with Grootscholten et al. study (38) that found patients with high titers of the antinucleosome antibodies had significantly higher SLE disease activity scores. A rapid decline in the level of antinucleosome antibodies occurred after treatment (38).

## CONCLUSION

The anti-nucleosome antibody is a valuable serologic marker because of high sensitivity and specificity in patients with SLE. The measuring of Anti-nuA is an excellent test of predicting SLE disease activity with suggestion of the elevation of anti-nuA level may suggest active LN.Measurement of Anti-nuA could be a capable parameter to evaluate the effect of medications on disease activity.

## RECOMMENDATIONS

The measuring of Anti-nuA titer in LN patients has beneficial ability to diagnose active disease so may be useful adding to the biomarkers and other laboratory tests that can help in the diagnosis and monitoring of LN.Long-term studies will identify the effectiveness of treatment (maintenance therapy) according to the titer of these antibodies, improve quality of life and increased survival which can be achieved for the many patients affected by LN each year.

**CONFLICT OF INTEREST** :none

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