Antinucleosome antibodies as new marker in early diagnosis of systemic lupus erythematosus (SLE) in comparison with other autoantibodies

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Abstract. Systemic lupus erythematosus (SLE) is an autoimmune disorder of the multisystem, a multifactorial disease with generalized persistent inflammation that can involve multiple tissues and organs. Nucleosomes are essential chromatin molecules, and are thought to be the main antigens in SLE pathogenesis. A case-control study was performed to show the relationship between antinucleosome antibodies and systemic lupus erythematosus in Najaf province. This study was carried out in the Department of Microbiology in the Faculty of Medicine- University of Kufa, for the period of (January 2018 to December 2018), subjects were included in present study, divided into three groups: SLE patient (30), RA patient (30), and Healthy controls(20). Anti-NCS antibodies, ANA and anti-dsDNA antibodies were done for all individuals those included in this study. The findings of the current study showed that, in sera of SLE patients the mean level of antibodies against the following parameters NCS, ANA and ds-DNA were high in comparison with the mean levels of these parameters in sera of the normal control group (p<0.001). As well as, the levels of antibodies against NCS significantly higher in sera of the SLE group than in the rheumatoid arthritis RA (diseased control group) (p<0.001). On the other hand, the mean levels of antibodies against NCS were statically insignificant (p value 0.356) among the healthy individuals and the diseased control group. Anti-NCS antibodies have an important role in SLE diagnosis in comparison to other autoantibodies, and may have a role to predict the disease outcome.

Keywords. SLE, Antinucleosome , autoantibodies, rheumatoid arthritis.

1. Background
Systemic lupus erythematosus is an inflammatory illness that affect multisystems. The skin, joints, lungs, brain, and other organs may be affected. One of SLE’s immunological characteristics is immunity
to self-chromatin, which manifests as autoantibodies going to against three of its most important subunits, included dsDNA, histones and nucleosomes [1].

Patients with SLE may have various clinical symptoms that vary from person to person can be mild to severe. So the most frequent symptoms like painful and swelling of the joints, high temperature, pain in the chest, hair loss, ulcers in the mouth, swollen in the lymph nodes, tired feeling, and a most common red rash on the face [2]. The most common symptoms in new cases or chronic active SLE flares are hematological cytopenias and weight changes [3].

The disease may be initiated by interactions between genetic and environmental factors, especially exposure to UV light, infection with Epstein–Barr virus and hormonal factors, resulting in immune dysregulation at cytokine, T cell, B cell and macrophage levels. Although it is unknown the precise etiological mechanism, genetic, hormonal and environmental factors have been reported as well as immune disorders. Associations have also been identified between lupus onset and age, gender, geography and race [4].

Diagnosis SLE is mainly clinical but remains not easy due to the heterogeneity of SLE. The disease has various diagnostic markers; however, a mixture of clinical and laboratory parameters distinguishes it. Diagnosing systemic lupus erythematosus accurately is critical because treatment can minimize morbidity and mortality [4].

The nucleosome, is a chromatin structure, which consists of a central octamer containing two copies of each histone, H2A, H2B, H3 and H4 wrapped up with a helical DNA complex [5]. It has been shown that nucleosomes are more highly immunogenic (i.e. ability to induce immune response) than native histones or DNA and cause a positive cell response from the T-helper. In various connective tissue diseases anti-NCS antibodies have been detected like SLE, scleroderma and mixed connective tissue disease, however, these antibodies have high specificity and sensitivity in the diagnosis of SLE [6].

Anti-dsDNA Ab is one of a wide range of autoantibodies (Ab) found in systemic lupus erythematosus (SLE), it has been used for more than 60 years to diagnose and evaluate disease activity. Anti-dsDNA Ab is often coupled with anti-nucleosome Ab, as well as clinical practice combining are useful for diagnosis, prognosis and for monitoring treatment response as biomarkers, especially when drugs which target B cells are used. So, the combination of antibodies against dsDNA Ab plus anti-nucleosome Ab is related to flare of the disease and may propose lupus nephritis. However, because anti-nucleosome Ab has a higher specificity and sensitivity than anti-dsDNA Ab, therefore, anti-nucleosome Ab is a practical marker for diagnosis SLE patients and for the diagnosis of certain forms of lupus caused by the drug [7].

Antibodies against nucleosome Ab is detected in SLE-prone mice before spreading to anti-dsDNA Ab and anti-histone Ab by intramolecular epitope. The anti-dsDNA Ab and anti-nucleosome Ab are found in highest levels in untreated young patients in the diagnosis time, as well as in patients with lupus nephritis and those with high disease activity [7]. So, this study conducted to compare using of anti-nucleosome antibodies, ANA and antibodies against dsDNA in the diagnosis (SLE) Systemic Lupus Erythematosus.

2. Methods

2.1. Study Design: Case-Control Study.
This study was conducted on 80 subjects, their age ranged from 21–48 years, they were divided into three groups: SLE patient (30) were classified according to revised classification criteria for SLE by the American College of Rheumatology (ACR), RA (30) patient according to the ACR criteria, and Healthy controls (20). This study was carried out in the Department of Microbiology in the College of Medicine-Kufa University, during the period from April 2018 to March 2019. The following parameters were carried out on all participants, the (ESR) erythrocyte sedimentation rate, complete blood count (CBC). Anti-nucleosome antibodies, anti-dsDNA, and ANA were performed by Enzyme-Linked Immunosorbent Assay (ELISA) in serums of all individuals included in this study.
The current study before its commencement was approved by the ethical committee of the Faculty of Medicine, University of Kufa. As well as the informed consent was obtained from all individuals.

The concentration of anti-nucleosome antibody was measured in the sera of SLE patients and compared it with the concentration in other study groups (healthy and disease control) using a commercially available enzyme linked immunosorbent assay (ELISA) kit. Using a commercially available kits and according to the manufacture's instruction, the following kit (BINDAZYME MK200&MK017) were using to determine antibodies against nuclear antibodies (ANAs) and dsDNA antibodies by ELISA technique, also according to the manufacture's instruction, was measured IgG antibody against nucleosome (anti-NCS) for patients with SLE and control by enzyme-linked immunosorbent assay (ELISA) technique with the following kit (Anti-Nucleosome ORG 528, Orgentec Diagnostic, Germany).

2.2. Statistical Analyses

Statistical analysis was done using SPSS (Social Science Statistical Package) version 20 in which we use mean and standard deviation as descriptive statistics and LSD (the least significant difference) analysis of variance (ANOVA) for comparison between groups. The P value was considered significant if below 0.05.

3. Results

To investigate the relationship between anti-nucleosome antibody and systemic lupus erythematosus, eighty Iraqi individuals were enrolled in this study, they are divided into three groups; patients diagnosed with systemic lupus erythematosus (SLE), patients diagnosed with rheumatoid arthritis (RA) and healthy control group. Table (1) revealed that, statistically significant differences in regard to mean of different parameters (ANA, Ds-DNA and Anti-NCS) in compare between patients with SLE and healthy control group. The mean of ANA, Ds-DNA and Anti-NCS were (43.82, 37.7 and 108.36) respectively; whereas the mean of these parameters among healthy group were (12.7, 24.25 and 18.43) respectively. As well as this table showed that high mean level of antibodies against NCS among SLE patients in comparison to healthy control.

| Study groups | ANA (Mean±SD*) | Ds-DNA (Mean±SD*) | Anti-NCS (Mean±SD*) |
|--------------|----------------|-------------------|---------------------|
| SLE (N=30)   | 43.82±7.244    | 37.70±15.788      | 102.36 ±24.857      |
| Healthy controls (N=20) | 12.70±3.450 | 24.25 ± 7.254 | 18.43 ±6.133 |
| P- value     | <0.001         | 0.001             | <0.001              |

Table (2) revealed that, the mean levels of antibodies against nucleosome antibodies were higher in SLE patients than in RA (disease control group). There is a significant differences (P value <0.001) between SLE patients and RA in regard to mean of the Anti-NCS (102.36± 24.857, 20.05± 5.850) respectively.

| Study groups | Anti-NCS (Mean±SD*) |
|--------------|---------------------|
| SLE (N=30)   | 102.36 ±24.857      |
| Healthy controls (N=20) | 18.43 ±6.133 |
| P- value     | <0.001              |
SLE (N=30) 102.36± 24.857
RA ( N=35) 20.05± 5.850
P- value <0.001

To prove the importance of Anti-NCS in the SLE diagnosis in compared to other autoimmune diseases like RA. We compared the mean of anti nucleosome between healthy control group and RA patients, as shown in table(3) regarding the level of Anti-NCS between both groups the results show statistically insignificant differences between both groups (p value=0.356).

Table 3. Evaluation the mean levels of anti-nucleosome between Healthy controls and (RA) diseased control group

| Study groups   | Anti-nucleosome (Mean±SD*) |
|---------------|---------------------------|
| Healthy controls ( N=20) | 18.43± 6.134          |
| RA ( N=30)     | 20.05±5.850               |
| P- value       | 0.356                     |

4. Discussion
SLE is a disease described as loss of tolerance to self-chromatin and the development of self-antibodies to ds-DNA, histones, and nucleosomes [8]. Double stranded DNA (dsDNA) autoantibodies considered to be serological markers for SLE. It may stimulate antibodies against nucleosome (NCS) or anti-DNA response. Nevertheless, DNA is not immunogenic per se but necessary bound to proteins (histones/viral proteins). Nucleosome (DNA histone complexes) are therefore considered to be most important autoantigenic substances. There has been much research on anti-nucleosome antibodies and their clinical relationship with the severity of the disease since the last decade [9]. So the aim of this study was to measure the level of anti-NCS antibodies in patients with SLE and to compare them with other autoimmune diseases such as RA in addition to assess their association to anti-dsDNA and anti-ANA.

The findings of the current study showed statically significant differences in the levels of Anti-NCS between SLE and healthy control, as well as significant differences in ANA (p = 0.001), anti-dsDNA (p = 0.001) were found between the two groups. Furthermore, in patients with SLE the anti-NCS mean tended to be higher compared to other parameters (ANA and anti-dsDNA).

These results correspond to other studies, such as Manson et al. [10] Dina et al [11] Amoura et al [12] Tikly et al. [13] they were reported the levels of anti-NCS antibodies was significantly higher than the level in the control group, and anti-NCS antibodies have been suggested to be important marker and complement antibodies against dsDNA in the SLE diagnosis. The findings of the present study are inconsistent with Pradhan et al. [14]. Anti-NCS was reported to be valuable serological parameter with higher specificity and sensitivity in comparison with anti-dsDNA, anti-DNP, anti-Sm and anti-cmDNA antibodies those used in SLE diagnosis.

The present study findings are compatible with Manson et al. [21] who confirmed that mean levels of anti-nucleosome were higher in patients with LN in compared to levels in the healthy controls (0.32 versus 0.01, P < 0.001).

Further analysis of mean of Anti-NCS Proved that there were significant differences between SLE and RA, These results confirm the role of Anti-NCS in diagnosis of SLE and not in other autoimmune diseases. This finding has been supported by prior studies such as Saigal et al. [15], Yin Su et al [16]. Gupta et al. [17], no significant association of anti-NCS antibodies with arthritis was found. The results of this study also correspond to Cairns et al. [18] who reported that positive results for serum anti-NCS
antibodies were 61 of 95 (64%) of SLE patients, whereas negative results were in the 95 healthy controls and 2 out of 48 (4%) control.

In the recent study, statistical analysis of the mean of anti-NCS between disease control (rheumatoid arthritis patients) and healthy control showed statistically insignificant difference between both groups was found.

The current study findings agree with Min et al. [19] Bose et al. [20] in this context Duzgun et al. [21] who reported that seropositive for ANuA were 72 (54.9%), which was significantly higher than RA patients where seropositive only 3 of 74 (4%) whereas none of the healthy individuals were seropositive.

However the present study in consistent with Cairns et al. [18] who reported that 61 out of 95 (64%) patients with SLE were positive for ANuA, and in rheumatoid arthritis (RA) disease controls group only 2 out of 48 (4%)while none of 95 healthy controls were seropositive.

5. Conclusion
The present study confirms that in SLE patients the antibodies to the antinucleosome are common. We have also revealed that mean antinucleosome antibodies are high in SLE groups in compared to healthy subjects or in rheumatoid arthritis patients. So, it might be a helpful addition to the laboratory tests that can aid with SLE diagnostics.

6. Recommendations
There are various measures that exist for SLE diagnosis. The ELISA anti-nucleosome antibody used in this research is an uncomplicated laboratory procedure that can be conducted over a relatively short period of time on large numbers of samples. However, if anti-dsDNA antibodies are negative, the anti-nucleosome antibody test may be particularly helpful in the SLE diagnosis.

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