Autoantibody Profile in Systemic Lupus Erythematosus Patients

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Abstract. Systemic lupus erythematosus (SLE) is an autoimmune disease, in which the etiology is not well-understood; however, interactions between environmental and genetic factors in predisposed individuals have been recognized. As a consequence, immunological alternations occur and immune cells are involved, especially T and B lymphocytes that are activated to produce different immune components. Among these components are autoantibodies that react with self-antigens aside from non-self-antigens due to the proposed theory of molecular mimicry. Accordingly, the current study was designed to examine the profile of different autoantibodies in SLE patients by using the indirect membrane based enzyme immunoassay for the quantitative measurement of IgG class antibodies. Subjects: Sixty-four SLE patients (32 arthritis and 32 nephritis patients) and 32 healthy subjects (control) were enrolled in the study, and their sera were tested for anti-nucleosome, anti-histone, anti-smD1, anti-PCNA, anti-PO, anti-SS-A/Ro-60, anti-SS-A/Ro-52, anti-SS-B/La, anti-CENP, anti-SCI-70, anti-U1snRNP, anti-AMA-M2, anti-Jo-1, anti-PM-SCI, anti-Mi2 and anti-Ku autoantibodies in order to evaluate the autoimmunity status in SLE patients. Results: The sera of control subjects were negative for these antibodies; therefore, the comparisons were limited to the two groups of SLE patients; arthritis and nephritis. The highest percentage of seropositive arthritis patients was observed for anti-SS-A/Ro-60, anti-CENP and anti-U1snRNP antibodies (100.0%), while the lowest percentage was recorded for anti-Jo-1 antibody (15.6%). For nephritis patients, anti-U1snRNP antibody (87.5%) was also observed to have the highest percentage, and anti-Jo-1 antibody (3.1%) also recoded the lowest percentage. However, four autoantibodies (anti-PCNA, anti-SS-A/Ro-60, anti-SS-B/La and anti-CENP antibodies) showed different profiles in arthritis and nephritis SLE patients. They showed a significant increased percentage in arthritis patients compared to nephritis patients (anti-PCNA: 87.5 vs. 50.0%, p = 0.003; anti-SS-A/Ro-60: 100.0 vs. 81.2%, p = 0.02; anti-SS-B/La: 75.0 vs. 43.8%, p = 0.02; anti-CENP: 100.0 vs. 43.8%, p = 0.001). Conclusion: These findings suggest the diagnostic potential of autoantibodies as early markers for SLE development.

Keywords: Autoantibodies, Systemic lupus erythematosus, arthritis, nephritis.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that impact different tissues, organs and systems of the human body. The blood vessels, heart, lungs, liver, kidneys, joints, nervous system and skin are the considerable targets for the disease, in which the immune system abnormally attacks self-targets through generating different autoantibodies specific for self-components causing inflammation and damage [1]. The presence of different
autoantibodies; such as anti-double-strand deoxyribonucleic acid (anti-dsDNA), anti-ribonucleic acid (anti-RNA), anti-ribonucleoprotein (anti-RNP), anti-smith antigen (anti-Sm) and anti-nucleosome antibodies in SLE patients, represent an important feature of the disease. Therefore, SLE is considered as a B lymphocyte-mediated disease [2]. Several clinical features of SLE are reported in patients, and these are related to the complexity and risk factors involved in disease etiology; such as the genetic, hormonal, and environmental factors, besides the the assortment of autoantibodies that circulate in the peripheral blood [3]. Thus, the hallmarks of SLE are presented by circulating autoantibodies, deposition of immune complexes, and activation of complement proteins; however, it is also evident that an imbalance of cytokines is a further important further [4]. In addition, autoantibodies are usually present before the onset of symptoms [5]. Different autoantibodies have been detected in sera of SLE patients and 98% of them bind to cell nucleus antigens like anti-nuclear antibody (ANA) [6 – 9]. Globally, the typical model of SLE patients is distinguished by the presence of anti-dsDNA antibody [10, 11], anti-nucleosome antibody (ANuA) [12, 13], anti-histones antibody (AHA) [14, 15], anti-Smith antigen (anti-Sm), anti-Sjögren syndrome-A/Ro antibody (anti-SSA/Ro) [8, 11, 16, 17], anti-Sjögren's syndrome antigen-B (anti-SS-B/La) [11, 17, 18], anti-systemic sclerosis-70 (Scl-70) antibody [19], anti-U1 small nuclear ribonucleoprotein antigen (anti-U1snRNP) [20, 21], anti-histidyl-tRNA synthetase antibody (Jo-1) [8, 22], anti-RNP, anti-Ku and Ki antibodies [23], anti-ribosomal P protein (RPP) antibody [24] and anti-polymyositis-systemic scleroderma antibody (PM-Scl) [25]. However, in Iraqi SLE patients, such profile of autoantibodies has not been determined; therefore, the present study was designed to examine such profile in sera of SLE patients with some emphasis on nephritis and arthritis as clinical complications of the disease.

2. Subjects and Methods

2.1. Subjects

In this study, 64 SLE female patients were enrolled, with a mean age ± standard error (SE) of 32.5 ± 1.1 years. They attended a consulting clinic at the Rheumatology Department of Baghdad Teaching Hospital during the period January – March 2016 for diagnosis and treatment. The diagnosis was made by the consultant medical staff at the clinic according to the 1997 revised criteria for SLE of the American College of Rheumatology (ACR), which are based on clinical examination and laboratory evaluation [26]. As suggested by the consultants, the patients were distributed into two clinical groups (nephritis and arthritis); each of 32 patients with a mean age ± SE of 30.7 ± 2.0 and 29.7 ± 2.1 years, respectively. In addition, 32 apparently healthy women were also enrolled in the study (control group); matched patients for age (33.1 ± 1.4 years).

2.2. Study parameters and methods

The studied groups were screened for the following anti-ANA, anti-dsDNA, ANuA, AHA, anti-SmD1, anti-PCNA, anti-PO(RPP), anti-SS-A/Ro60, anti-SS-A/Ro52, anti-SS-B/La, anti-CENP, anti-Scl-70, anti-U1snRNP, anti-AMA-M2, anti-Jo-1, anti-PM-Scl, anti-Mi2 and anti-Ku IgG autoantibodies by using a commercial kit (IMTEC-ANA-LIA MAXX, Human company, Germany) that is based on indirect membrane based enzyme immunoassay for the quantitative measurement of IgG class antibodies, and instructions of manufacturer were followed.

2.3. Statistical analysis

The autoantibody profile data were given as numbers and percentage frequencies, and significant differences between these frequencies were assessed by the two-tailed Fisher's exact probability. The epidemiological statistical software WINPEPI (version 11.65) was used to carry out such assessments.

3. Results

The sera of SLE patients and controls were tested for 17 autoantibodies. They were anti-dsDNA, anti-nucleosome, anti-histone, anti-smD1, anti-PCNA, anti-PO, anti-SS-A/Ro-60,
anti-SS-A/Ro-52, anti-SS-B/La, anti-CENP, anti-SCI-70, anti-U1snRNP, anti-AMA-M2, anti-Jo-1, anti-PM-SCI, anti-Mi2 and anti-Ku IgG antibodies. The sera of control subjects were negative for these antibodies; therefore, the comparisons were limited to the two groups of SLE patients; arthritis and nephritis. The highest percentage of seropositive arthritis patients was observed for anti-SS-A/Ro-60, anti-CENP and anti-U1snRNP antibodies (100.0%), while the lowest percentage was recorded for anti-Jo-1 antibody (15.6%). For nephritis patients, anti-U1snRNP antibody (87.5%) was also observed to have the highest percentage, and anti-Jo-1 antibody (3.1%) also recorded the lowest percentage. However, four autoantibodies (anti-PCNA, anti-SS-A/Ro-60, anti-SS-B/La and anti-CENP antibodies) showed different profiles in arthritis and nephritis SLE patients. They showed a significant increased percentage in arthritis patients compared to nephritis patients (anti-PCNA: 87.5 vs. 50.0%, \( p = 0.003 \); anti-SS-A/Ro-60: 100.0 vs. 81.2%, \( p = 0.02 \); anti-SS-B/La: 75.0 vs. 43.8%, \( p = 0.02 \); anti-CENP: 100.0 vs. 43.8%, \( p = 0.001 \)) (Table 1).

Table 1: Autoantibody profile in arthritis and nephritis systemic lupus erythematosus patients.

| Autoantibodies | Seropositive Systemic Lupus Erythematosus Patients | Arthritis (No. = 32) | Nephritis (No. = 32) | p-value |
|----------------|-------------------------------------------------|----------------------|---------------------|---------|
| Anti-dsDNA     | 29                                              | 25                   | 78.1                | NS      |
| Anti-nucleosome| 28                                              | 21                   | 65.6                | NS      |
| Anti-histone   | 28                                              | 21                   | 65.6                | NS      |
| Anti-SmD1      | 30                                              | 26                   | 81.2                | NS      |
| Anti-PCNA      | 28                                              | 16                   | 50                  | 0.003   |
| Anti-PO (RPP)  | 14                                              | 10                   | 31.2                | NS      |
| Anti-SS-A/Ro 60| 32                                              | 26                   | 81.2                | 0.02    |
| Anti-SS-A/Ro 52| 19                                              | 14                   | 43.8                | NS      |
| Anti-SS-B/La   | 24                                              | 14                   | 43.8                | 0.02    |
| Anti-CENP      | 32                                              | 14                   | 43.8                | 0.001   |
| Anti-SCI-70    | 7                                               | 2                    | 6.2                 | NS      |
| Anti-U1snRNP   | 32                                              | 28                   | 87.5                | NS      |
| Anti-AMA M2    | 10                                              | 6                    | 18.8                | NS      |
| Anti-Jo-1      | 5                                               | 1                    | 3.1                 | NS      |
| Anti-PM-Scl    | 11                                              | 5                    | 15.6                | NS      |
| Anti-Mi-2      | 27                                              | 22                   | 68.8                | NS      |
| Anti-Ku        | 29                                              | 26                   | 81.2                | NS      |

\( p \): Two-tailed Fisher’s exact probability; NS: Not significant (\( p > 0.05 \))

4. Discussion

In agreement with the general theme of these findings, it has been reported that there are more than 20 autoantibodies that have a role in etiology of SLE [27]. Anti-dsDNA antibody is the first autoantibody that has been certified as a clinical diagnostic marker for SLE [28, 29], and the present results support such diagnostic importance irrespective of the clinical subtypes of SLE; arthritis and nephritis, as both groups showed an increased percentage of positive cases (90.9 and 78.1%), and without significant difference between them. Anti-dsDNA antibodies have also been detected in certain SLE patients prior to the onset of nephritis as measured by severe proteinuria. However, a retrospective study concluded that anti-dsDNA antibodies are not predictive of renal flare in SLE patients, but they have a strong sensitivity (80%) for severe SELENA-SLEDAI flare in patients [30]. It has also been suggested that these antibodies may participate in initiating SLE nephritis, and SLE has been proposed to be a prototype of immune complex nephritis in human [31].
Mannik *et al.* detected a number of autoantibodies in the sera of nephritis SLE patients; anti-nucleosome, anti-smD1, anti-SS-A/Ro and anti-SS-B/La; however, they did not prove that these autoantibodies may have a role in the development of SLE, but it has a role in causing inflammation [32]. These autoantibodies may arise after apoptosis of inflamed kidney cells. Whereas, Gronhagen and Nyberg and Fu *et al.* referred to the role of anti-histone, anti-nucleosome and anti-SS-A/Ro in cutaneous lupus, and these auto-antibodies were associated with an increased risk of photosensitive rash development [31, 33]. However, there have been no recorded results about the role of these autoantibodies in arthritis SLE.

A recent study referred to the high positivity of anti-SmD1 antibody in Chinese SLE patients; 68% in non-treated SLE and 58.8% in treated SLE patients [34]. While, a previous study referred that this autoantibody is associated with clinical symptoms; such as malar rash, hypocomplementemia, seizures, proteinuria, renal disorder and arthritis. The seropositive prevalence of anti-SmD1 antibody was 55.6% in SLE patients, 44.8% in arthritis patients, and 39.7% in renal disorder [28, 35]. These findings are compatible with the present findings, which documented that 93.8% of arthritis SLE and 81.2% of nephritis group were positive for these autoantibodies. Anti-SmD1 antibody is directed against many epitopes that found in the core of several proteins. These epitopes induce T-cell response, and Epstein Barr virus (EBV) is one of these epitopes that has a similar self-amino acids sequence, leading to a reaction between anti-SmD1 antibody and these epitopes, and then stimulating T-lymphocyte immune response [36].

Anti-Ribosomal P protein (RPP) antibody was observed in both arthritis and nephritis SLE patient groups with a prevalence of 43.8 and 31.2%, respectively. This finding is well-matched with Aguila *et al.*, who detected that 55.6% of SLE patients were seropositive for anti-RPP antibody [28]. However, other study mentioned that anti-RPP antibody was present in minority of Caucasian SLE patients, and its level was lower than the levels of anti-dsDNA, anti-ANA, and anti-SmD1 antibodies, but the authors considered this autoantibody as a marker for specific diseases such rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PA) and SLE [37]. Anti-RPP antibody was recorded with a frequency of 6-20% of SLE patients in different ethnic groups [38]; whereas it had a frequency of 36% in Chinese SLE patients [39]. The presence of anti-RPP antibody has also been associated with arthritis, nephritis, photosensitivity and malar rash [37]. The etiopathogenesis mechanism of this autoantibody could be illustrated by the binding affinity of this autoantibody to three subunits of antigen epitopes located on the surface of some immune cells; such activated T-cells, leading to production of tumor necrosis factor-α (TNF-α), IL-6 and other inflammatory cytokines, which finally cause the autoimmune diseases such SLE [40].

Present study findings also highlighted that 21.9 and 6.2 of arthritis and nephritis SLE patients were respectively seropositive for anti-Scl-70 antibody. In agreement with such theme, more than 20% of SLE patients and 9.6% of RA patients have been reported to be seropositive for scl-70 autoantibody [28, 41]. El-Awady *et al.* reported that 18% of Egyptian SLE patients were seropositive for anti-Scl antibody and they were suffering from a renal disorder [42]. In a previous study, the presented frequency of this autoantibody in the circulation of Kuwaiti SLE patients was 13% with the presence of anti-ds-DNA (35.5%), anti-ANA (96.8%), anti-SmD1 (13%), anti-RNP (13%), anti-SS-A/Ro (35.5%), and anti-SS-B/La (19.4%) autoantibodies [43]. The increased level of this autoantibody may risk the patient for pulmonary fibrosis, diffuse cutaneous disease and nephritis [44]. Anti-U1-snRNP antibody was observed in 100.0 and 87.5% of arthritis and nephritis SLE patients, respectively. In agreement with such observation, a strong association between this autoantibody and SLE development in both arthritis [45] and nephritis [46] has been reported. However, the studies showed some variation in the prevalence on anti-RNP in SLE patients, and it was within the range of 20 - 72% [47]. Anti-snRNP antibodies participate in both innate and adaptive immune response by binding with Sm antigen (which is shared with Sm antigen in the core structure) in the cytoplasm of cells, leading to a damage in connective tissues [45, 48].
The prevalence of anti-AMA M2 antibody was 31.2 and 18.8% in arthritis and nephritis SLE patients, respectively. Koszarny et al. also documented the positivity of SLE patient’s sera for such autoantibody but at a lower prevalence (13.3%) in Polish SLE patients especially those that had arthritis [49]. It has been suggested that production of this autoantibody in sera of patients with autoimmune diseases can result from the exposure to some chemical xenobiotics compounds such 2-nonynoic acid, in addition, to the cross reactivity due to molecular mimicry of some infectious agents (for instance E. coli) [50]. A previous study referred to the role of CpG motif that have the ability to induce the innate immunity response and up-regulation of B-cells expression of TLR-9 can also result in production of auto-AMA in the circulation [51].

Anti-Jo-1 antibody was presented with 15.6 and 3.1% among arthritis and nephritis SLE patients, respectively. In other study, the prevalence of this antibody was 11.1% in SLE and 14.3 in RA patients [28]. In an earlier study, Gomard-Mennesson et al. noticed a higher prevalence of anti-Jo-1 antibody (100%) in SLE patients especially those who were seronegative for anti-ANA antibody. They suggested that the assessment of anti-Jo-1 antibody is a useful marker in the diagnosis of anti-ANA sero-negative SLE patients [52]. Whereas, Cruellas et al. suggested that anti-Jo-1 and anti-SS-A/Ro 52 antibodies were strongly associated [53].

The anti-PM-Scl antibody was observed in 34.4 and 15.6% of arthritis and nephritis SLE patients, respectively. In contrast with such observation, a Japanese study documented that there was no role of anti-PM/Scl antibody in autoimmune diseases [54], while other studies referred to its prevalence in many cohort studies of autoimmune diseases in Germany [55]. A lower percentage of anti-PM/Scl antibody sero-positivity has also been reported in SLE (6.7%) and in RA (3.2%) patients (Aguila et al., 2014). A further study attributed its pathogenic role to the strong association with HLA-DRB1*0301 allele [25]. Anti-Mi-2 antibody was highly presented (84.4 and 68.8%) in arthritis and nephritis SLE patients, respectively. Such highly prevalence has been attributed to the major role of cross reactivity and to the expression of some regulatory participating genes [56]. Cruellas et al. reported that 8.1% of photosensitivity patients were seropositive to anti-Mi-2 antibody in their sera [53], while Aguila et al. reported that both SLE and RA patients were 100% seronegative for anti-Mi-2 antibody [28].

The final autoantibody was anti-Ku, which was observed to have a prevalence of 90.6 and 81.2% of arthritis and nephritis SLE patients, respectively. This finding agreed with the results of Allenbach and Benveniste, who suggested that anti-Ku antibody is associated with SLE in concurrence with the presence of anti-SS-A/Ro 52, anti-PM/Scl and anti-Mi-2 antibodies [57]. Several previous studies also referred to the presence of anti-Ku antibody in many autoimmune diseases including SLE (0.7 - 27%), rheumatoid arthritis (up to 16%), mixed connective tissue disease and undifferentiated connective tissue disease (up to 8.3%) and Sjogren syndrome (<1 - 20%) [28, 58, 59].

5. Conclusion

These present study suggests the diagnostic potential of autoantibodies as early markers for SLE development.

Conflicts of Interest: There are no conflicts of interest.

6. References

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