Serological and Immunological Determination of Auto-Antibodies Against Myositis-Associated Antigens in Systemic Lupus Erythematosus Patients Using A Novel Immunoblot Assay

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Abstract

Jo-1, anti-Mi-2, anti-Ro52, anti-PM/Scl-100 and -75, anti-Ku, anti-SRP, anti-antii-PL-7, anti-PL-12, anti-EJ, and antiOJ are associated with myositis and are also found in systemic lupus erythematosus. Materials & methods: We studied serum of 170 patients with systemic lupus erythematosus (97 females & 63 males) had definite or probable myositis according to physicians, the clinical status of patients was determined from their medical records and 20 adults’ healthy controls. Sera were screened for anti- ANA IgG antibodies and Anti-dsDNAIgG antibodies by using ELISA kits obtained from AESKU Diagnostics. also11 autoantibodies were detected by using IgGimmunoblot kit were used (EUROLINE Myositis Profile3, Euroimmun AG, Germany). Results: The analysis revealed that Jo-1 were more frequencies were present in 76 patients with SE followed by Mi2 were present in 59 patients., Ku, SRP, PM-Scl75, PM-Scl100, Ro-52, PL-7, PL-12, EJ, and OJ were present in 46, 38, 34, 28, 28, 22, 19, 11 and 5 patients respectively. The immunoblot results showed the highest intensity of anti-Mi-2, anti-Ku, anti-PM/Scl-100, anti-SRP, anti-PL-12, anti-OJ in females more than males with rheumatoid arthritis diseases, while the anti-EJ, anti-PL-7, anti-Jo-1, and anti-PM-Scl75 showed lowest intensity was +/17, ++/48, ++/33, and ++/29 respectively in females. Conclusion: The level of these autoantibodies associated with myositis were detected in 170 patients with SLE. The present results indicate that it is clinically useful to determine the serum levels of myositis-associated autoantibodies in patients with SLE even when their underlying diseases are not myositis.

Key words: auto-antibodies, myositis, Systemic Lupus Erythematosus, Immunoblot

Introduction

The systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoreactive B and T cells, responsible for the aberrant production of a broad and heterogeneous group of autoantibodies. Shereret al. in 2004 documented that more than a hundred sixteen different antibodies found in SLE patients1. The Specific detection of autoantibodies in systemic autoimmune diseases same as the myositis , either systemic or organ specific, can have both diagnostic and prognostic importance. Some autoantibodies have a clear pathogenic role such as anti-Jo-1 in polymyositis2. Among the myositis-specific autoantibodies reported to date, are directed against cytoplasmic antigens, such as tRNAsynthetase (Jo-1 or PL-1, PL-7, PL-12, EJ, OJ, JS, and KS), signal-recognition particle (SRP), Mas, KJ, and Wa. Also antibodies to nuclear antigens include anti-Mi-2, anti-PMS (PMS1, and PMS2), PM-Scl, Ku, RNP (U1-RNP and U2-RNP, U4/U6-RNP, and U5-RNP), Ro 52 kDa and, more rarely, Ro 60 kDa3. Auto-antibodies in myositis are very different in antigen specificity and characteristics. thus, different in-house laboratory methods for their detection have been used to date. Recently, a single multi-analytic line blot assay has been developed representing a promising methodological approach for testing4.

Immunoblotting with positively sera of antibodies has been previously reported. So in this study, used
the diagnostic performance of a novel, commercially available immunoblot technician for the standardized detection of several autoantibodies was evaluated at reference laboratories using serum panels from clinically patients with SLE and various controls.

**Materials and Method**

**Samples**

A Total of 170 serum samples has been collected from SLE patients whom suffering from myositis as (97 females and 73 males) and 20 healthy controls, which were pay a visit and residence in hospitals from date of 15/9/2017 to 15/6/2019 and diagnosed by the treating physicians and confirm the diagnosis by specific tests for each one. Their ages are ranging from 9-61 years.

**Methods**

Laboratory investigation for the detection of anti-dsDNA and anti-ANA IgG antibodies

Anti-dsDNA IgG antibodies and anti-ANA IgG antibodies were determined by using commercial ELISA kits obtained from AESKU Diagnostics. All test items were operated strictly in accordance with the manufacturer’s instructions.

Measurement of myositis-specific autoantibodies and myositis-associated autoantibodies

The recruited patients were evaluated for a myositis-associated autoantibody antibodies or myositis-specific autoantibodies. For the detection, IgG immunoblot kit were used (EUROLINE Myositis Profile3, Euroimmun AG, Germany), this provides a qualitative in-vitro assay for human antibodies to 11 different myositis antigens includes: anti-Ro52, anti-PM/Scl-100 and -75, anti-Ku, anti-Mi-2, anti-SRP, anti-Jo-1(histidyl-), anti-PL-7 (threonyl-), anti-PL-12 (alanyl-), anti-EJ(glycol-), and antiOJ(isoleucyl-tRNAsynthetase). The manufacturer’s instructions were followed while carrying out the assay. After the membrane strips were dried and automated evaluations of these strips for the analysis of different bands and examined with EUROLineScan system provided by the manufacturer, the reading was taken by keeping the strips on a flatbed scanner (Canon) which enables the EUROLineScan to recognize the position of the strips, identify the bands and measure its intensity. The results were defined as positive when the signal intensity was more than 11. The intensity of autoantibodies is graded according to the signal intensity into weakly 11–25 (+), moderately 26–50 (++), and strong more 50 (+++).

**Findings**

Among 170 onset SLE patients, 119 (70%) were ANA seropositive apportioned as 80 (67.22 %) at females and 39 (32.78%) at males. One hundred and sixty SLE patients (94.17%) showed a positivity for anti-dsDNA apportioned as 91 (56.88%) and 69 (43.12%) at females and males respectively (Table 1).

Table (1): Percentage seropositive of ANA, anti-dsDNA and ANCA associated with SLE patients (n=170)

<table>
<thead>
<tr>
<th>Genders</th>
<th>SLE patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td>NO. (%)</td>
<td></td>
</tr>
<tr>
<td>ANA (n=119)</td>
<td>80 (67.22)</td>
</tr>
<tr>
<td>Anti-dsDNA (n=160)</td>
<td>91 (56.88)</td>
</tr>
</tbody>
</table>

ANA: Antinuclear antibody, Anti-dsDNA: Anti-double-stranded DNA

Univariate analysis (Table 2) showed that SLE patients with positive antibodies for ANA and anti-dsDNA according to age groups and gender. The results of ANA showed the highest percentage of ANAis in the females within the age group (31-40) years followed by the patients within the age group (41-50) years (24.37%, 17.65%) respectively, while the lowest percentage was shown in males within age group (10-20) years and females patients within the age group (> 50) years. Anti-dsDNA analysis showed the highest percentage of anti-dsDNA is in females within the age group (41-50) years followed by the patients within the age group (21-30) years (21.25%, 17.5%) respectively. Whilst the anti-dsDNA result has lowest percentage in females (2.5%); see (Table 2). The results of the statistical analysis showed significant differences P ≤ 0.05.
Table (2): Percentage seropositive of ANA, anti-dsDNA and ANCA in relation to age groups and genders associated SLE

<table>
<thead>
<tr>
<th>Genders</th>
<th>SLE patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANA (n=119)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>10 – 20</td>
<td>7(5.88)</td>
</tr>
<tr>
<td>21 -30</td>
<td>18(15.13)</td>
</tr>
<tr>
<td>31 - 40</td>
<td>29(24.37)</td>
</tr>
<tr>
<td>41 – 50</td>
<td>21(17.65)</td>
</tr>
<tr>
<td>&gt; 51</td>
<td>5(4.2)</td>
</tr>
<tr>
<td>Total</td>
<td>80(67.23)</td>
</tr>
</tbody>
</table>

ANA: Antinuclear antibody, Anti- dsDNA: Anti–double-stranded DNA,

Table 3 shows percentage of seropositive of myositis antigens in patients with systemic lupus erythematosus relation to age groups and genders. According to data in this study, the results of SLE patients showed the highest percentage of the diseases under study is in the females within the age group (31- 40) years followed by the patients within the age group (21 -30) years (17.65%, 15.30%) respectively, while the lowest percentage was showed within age group (10-20) years and males patients within the age group (> 50) years. See (Table 3). The results of the statistical analysis showed significant differences $P \leq 0.05$.

Table (3) Percentage seropositive of myositis antigens in relation to age groups and genders associated SLE

<table>
<thead>
<tr>
<th>Genders Age groups (years)</th>
<th>SLE patients(n=170)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>10 – 20</td>
<td>9(5.29)</td>
</tr>
<tr>
<td>21 -30</td>
<td>26(15.30)</td>
</tr>
<tr>
<td>31 - 40</td>
<td>30(17.65)</td>
</tr>
<tr>
<td>41 – 50</td>
<td>21(12.35)</td>
</tr>
<tr>
<td>&lt;51</td>
<td>11(6.47)</td>
</tr>
<tr>
<td>Total</td>
<td>97(57.06)</td>
</tr>
</tbody>
</table>
In present study, the Jo-1 and Mi2 were observed more frequently in patients with SLE were present as 76, 59 respectively. Ku, SRP, PM-Scl75, PM-Scl100, PL-7 and PL-12 were present in patients as 46, 38, 34, 28, 22, 19 respectively while the lowest frequently were OJ which present in 5 patient. According to genders, the results showed that Jo-1 and Mi2 were recorded more frequently in females patients as 41, 38 respectively while the lowest frequently were OJ recorded in 1 female patient. Moreover, we did not observe patients with positivity to EJ in females with SLE (Table 4).

Table (4) Frequency of myositis-specific and myositis associated autoantibodies in patients with SLE

<table>
<thead>
<tr>
<th>Markers Study groups</th>
<th>Mi2</th>
<th>Ku</th>
<th>PM-Scl100</th>
<th>PM-Scl75</th>
<th>Jo-1</th>
<th>SRP</th>
<th>PL-7</th>
<th>PL-12</th>
<th>EJ</th>
<th>OJ</th>
<th>Ro-52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>38/170</td>
<td>28/170</td>
<td>12/170</td>
<td>13/170</td>
<td>41/170</td>
<td>18/170</td>
<td>16/170</td>
<td>11/170</td>
<td>-1/170</td>
<td>1/170</td>
<td>21/170</td>
</tr>
<tr>
<td>Total</td>
<td>59/170</td>
<td>46/170</td>
<td>28/170</td>
<td>34/170</td>
<td>76/170</td>
<td>38/170</td>
<td>22/170</td>
<td>19/170</td>
<td>11/170</td>
<td>5/170</td>
<td>28/170</td>
</tr>
</tbody>
</table>

The overall immunoblot results with the classical serological markers of anti-PM/Scl-100 and -75, anti-Ku, anti-Mi-2, anti-SRP, anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, and anti-OJ showed the highest intensity of anti-Mi-2, anti-Ku, anti-SRP, anti-PL-12, anti-PM/Scl-100, and anti-OJ in females more than males in patients with SLE, while the Ro-52, PL-7, Jo-1, anti-PM/Scl -75 and anti-EJ showed lowest intensity in females than males were +++/53, ++/50, ++/47, ++/41 and ++/21 respectively in SLE patients (Table 5).

Table (5) Maker of IgG antibodies associated with SLE patient’s group under study

<table>
<thead>
<tr>
<th>Markers Study groups</th>
<th>Mi2</th>
<th>Ku</th>
<th>PM-Scl100</th>
<th>PM-Scl75</th>
<th>Jo-1</th>
<th>SRP</th>
<th>PL-7</th>
<th>PL-12</th>
<th>EJ</th>
<th>OJ</th>
<th>Ro-52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>+++/67</td>
<td>++/39</td>
<td>++/38</td>
<td>++/33</td>
<td>++/42</td>
<td>++/48</td>
<td>+++/61</td>
<td>+/17</td>
<td>+++/41</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Controls</td>
<td>-</td>
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</tr>
</tbody>
</table>

SLE = Systemic Lupus Erythematosus

(No.) indicate mean of intensity

- (less than 10), + (10-25), ++ (26-50), and +++ (≥ 51) indicate class

Discussion

To our knowledge, this is the first report that evaluates the presence of various myositis autoantibodies in patients with Systemic Lupus Erythematosus in Mosul city. Anti-nuclear antibodies (ANA), a heterogeneous group of autoantibodies against nuclear antigens, are
often tested as a rapid screening tool in patients with suspected systemic lupus erythematosus (SLE) or other connective tissue diseases\textsuperscript{4}. Multiple studies show ANA positivity to be highly prevalent in various patient populations. In this study, the prevalence of positive ANA in SLE patients was 70\%, which was lower than previous reports (90-100\%)\textsuperscript{6}. A different genetic background could explain these differences. SLE is a multifactorial disease in which genetic and environmental factors interplay, determining disease development\textsuperscript{7}. The genetic background could explain not only the disease susceptibility but also the autoantibodies production. On other hand, some of patients received much medication for treating their underlying diseases. Many drugs are reported to produce ANA and have been reviewed extensively such as Hydralazine, procainamide, chlorpromazine and quinidine\textsuperscript{8}.

In the present study, we registered a frequency of anti-dsDNA greater than 90\% that is similar to the data reported in the literature for SLE populations\textsuperscript{9}. Antibodies to dsDNA are of great importance in SLE. It is known that anti-dsDNA antibodies are present in 70–90\% of SLE patients and in less than 0.5\% of the controls. The majority of SLE patients are found to have anti-dsDNA antibodies at some time during their illness. The presence of anti-dsDNA has been found to precede the onset of lupus symptoms by up to 5 years and have been correlated with SLE activity\textsuperscript{10}. In discrete study, Chung and colleagues\textsuperscript{14} published that many previously identified SLE-associated genes are more strongly associated with the production of anti-dsDNA than with disease susceptibility\textsuperscript{11}. The authors demonstrated the association between polymorphisms (SNPs) located in the MHC, STAT4, IRF5, and ITGAM regions and the positivity for anti-dsDNA antibodies. Conversely, only SNPs in the MHC and IRF5 regions have been identified in negative patients\textsuperscript{11}.

We used in this study the Euroline blot immunoassay technique which is the diagnostic method, also seemed to detect a higher proportion of myositis autoantibodies among SLE patients compared to other studies. Our study showed that the systemic lupus erythematosus patients have a variety of autoantibodies which acceptable with previous studies\textsuperscript{12,13} have reported that were detected in patients with SLE\textsuperscript{12,13}. Also, in agreement with the data from the literature, we found a predominance of Anti-Ro52, Anti-Ku in SLE patients, compared to other of myositis autoantibodies\textsuperscript{14}.

Previous studies reported that Anti-EJ antibody and anti-Jo-1 antibody were also detected frequently in SLE and another non-myositis CTD and as for other myositis-associated autoantibodies, anti-PM-Scl75 antibody was detected in 7 patients with SLE while anti-Ku antibody was demonstrated in 3 patients with PM/DM, 4 patients with SLE and 3 patients with MCTD\textsuperscript{15}. However, our study revealed that anti Jo-1 is more common in females, with a approximately female-to-male ratio of 2:3 which confirmed with Cavagna et al. who documented in an international retrospective multicenter study that anti Jo-1 is more common in females\textsuperscript{16}.

The role of anti-Ku in the pathophysiology of autoimmune diseases is not entirely understood. However, the present study showed the high incidence of Anti-Ku autoantibodies with SLE patients were 27.06\% different with previous studies which revealed that Anti-Ku autoantibodies have been identified in 7-18\% of the patients with overlap syndromes such as SLE\textsuperscript{17}. Suggested that may be due to limitation in a cohort number of patients in this study.

The most important finding of previous studies has been reported the presence of anti-Ro52 antibodies in different systemic autoimmune rheumatic diseases\textsuperscript{18,19}. Our findings are similar to those previously reported\textsuperscript{19} that showed the presence of anti-Ro52 in SLE patients were 16.47\% and showed the high incidence in males more than females. However, In previous studies patients with SLE had higher frequency of anti-Ro52 in a prevalence between 36 and 64\%\textsuperscript{20}. Ro52 is a member of the tripartite motif (TRIM) family of single-protein E3 ligases and is known to be a target for autoantibody production in systemic autoimmune rheumatic diseases. Ro52 function is still unknown\textsuperscript{21}.

In summary, These results indicate that anti-Ro52, anti-PM/Scl-100 and -75, anti-Ku, anti-Mi-2, anti-SRP, anti-Jo-1 (histidyl-), anti-PL-7 (threonyl-), anti-PL-12 (alanyl-), anti-EJ (glycol-), and antiOJ (isoleucyl-tRNA synthetase) autoantibodies are closely associated with myositis as well as Systemic Lupus Erythematosus were detected in our series of Iraqi patients. Also, The present results indicate that it is clinically useful to determine the serum levels of myositis-associated autoantibodies in patients with SLE even when their underlying diseases are not myositis. This study is the first report in Mosul/ Iraq that evaluates myositis autoantibodies among SLE patients, so further studies in a larger number of Iraq patients may be
Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: This research was carried out with the patients.

References


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