Significance of Anti-C1q Antibodies in Patients with Systemic Lupus Erythematosus as A Marker of Disease Activity and Lupus Nephritis

1Amal F Soliman, 1Sami EL-Said Egailla, 2Eman R Abdel Gawad, 2Amira I Mansour, 3Tohamy H ElKhouly, 1Marwa YA Mahgoub

Departments of 1Rheumatology & Rehabilitation, 2Clinical & Chemical Pathology and 3Radiology; Faculty of Medicine, Benha University, Benha, Egypt.

Anti-C1q autoantibodies have been proposed as a useful marker in SLE. This study aimed at measuring serum levels of anti-C1q antibodies in patients with SLE and investigates correlations of this level with the histopathological classes of renal biopsies and disease activity. The anti-C1q antibody level was measured in 30 females SLE patients and 20 controls. The activity of SLE disease was calculated according to the SLE disease activity index. A renal biopsy from patients with clinical manifestations of renal disease was obtained. There was a significant increase in the level of anti-C1q antibodies in SLE patients than controls and in patients with active LN than inactive LN (P<0.05). There were significant positive correlations between anti-C1q antibody level and SLEDAI & rSLEDAI scores and activity index score of renal biopsies. Anti-C1q antibodies showed higher diagnostic sensitivity and specificity than anti-dsDNA antibodies. In conclusion, anti-C1q antibodies are useful and sensitive non-invasive biomarker with high specificity in combination with anti-dsDNA antibodies for the diagnosis of renal disease activity.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune condition with diverse clinical and immunological features. It is characterized serologically by autoantibodies targeting self-proteins, notably antinuclear antibodies (ANAs), which are present in virtually all affected individuals. The pathogenesis of SLE remains incompletely understood, but most likely involves the interaction of genetic, hormonal and environmental factors. It is substantially more common in females of childbearing age, where the reported female: male ratio is 8–15:1 (Murphy & Isenberg, 2013).

Lupus nephritis (LN), one of the major serious manifestations of SLE, usually arises within five years of diagnosis; but, renal failure rarely occurs before the ACR classification criteria is met. Early diagnosis and rapid treatment of LN are crucial to improving survival in SLE patients (GargiuloMde et al., 2015).

A main pathogenic hypothesis of LN is that in SLE there were defects in renal clearance of immune complexes, and consumption of C3, C4, C1q the early components of classical complement pathway, which are strongly associated with the development of active SLE (Walport, 2002).

Anti-C1q antibodies have been suggested to be associated with LN. (Pradhan et al., 2012) This association is suggested from the correlation between anti-C1q antibodies and renal involvement, the prediction of anti-C1q antibody titers for flares of nephritis and the accumulation of anti-C1q antibodies in LN kidneys. A Low C1q level may be due to the presence of anti-C1q antibodies with the formation of C1q/antiC1q immune complexes and subsequent development of glomerulonephritis (Meyer et al., 2009).

A kidney biopsy is considered the golden standard diagnostic tool for the assessment of renal involvement in SLE (Parikh et al., 2015). Repeated biopsies may be necessary to
associate the clinical symptoms with histological findings and to confirm the effectiveness of therapeutic management. However, a renal biopsy may be contraindicated in an active lupus flare, and, more importantly, it is not free of complications. Serial evaluations are sometimes needed but cannot be performed routinely due to its invasive nature (Cross & Jayne, 2005). Hence, it is vital to identify a biomarker that would be able to detect kidney involvement and help in monitoring LN activity (Illei et al., 2004).

Consequently, the present study aimed at (i) estimating the level of anti-C1q antibodies in patients with SLE (ii) attempt to correlate this level with the histopathological classes of renal biopsies and (iii) to evaluate its reliability as a biomarker for LN and disease activity.

Subjects and Methods

Thirty female patients fulfilling, at least, four of the updated ACR revised criteria for the classification of SLE (Hochberg, 1997) were enrolled in this study. These patients were selected from the inpatients and the out-patient clinics of the Rheumatology, Rehabilitation and Physical Medicine Department of Benha University Hospitals. A total of 20 apparently healthy volunteers, age and sex matched to SLE patients, were carefully chosen to serve as the control group.

All subjects gave a written informed consent. The study was approved by Benha Medical Ethics Committee.

All patients were subjected to the following:

-Full history taking with stress on symptoms of renal disease.
-Thorough clinical examination.

Assessment of disease activity

SLE Disease Activity Index (SLEDAI) and renal SLEDAI (rSLEDAI) score were used for assessment of disease activity and activity of renal disease respectively. The SLEDAI depends on twenty-four features that are attributed to lupus with a weighted score given to anyone that is present. The most serious manifestations (such as renal, neurologic and vasculitis) are weighted more than others (such as cutaneous manifestations). The maximum possible score is 105. (Lam & Petri, 2005) Lupus nephritis was assessed clinically with the renal SLEDAI which consists of the 4 kidney related parameters: hematuria, pyuria, proteinuria and urinary casts. Each item in renal SLEDAI was assigned 4 points. Thus the scores for the renal SLEDAI can range from 0 to a maximum of 16. A patient had SLEDAI ≥10 was defined as active SLE (Bombardier et al., 1992).

SLE patients enrolled in the present study had a negative urine bacterial culture and/or a negative CRP testing in the absence of antibiotic treatment to differentiate the active urinary sediment that is the result of a LN flare from that which is the result of urinary tract infection.

Patients were excluded from the study if they had diabetes or other inflammatory diseases.

Laboratory Investigations

The following investigations were done for all participants:

-Complete blood picture by automated hematology system (Sysmex XE 5000). (England et al., 1984)
-Erythrocyte Sedimentation Rate (ESR) by Westergren method. (Westergren, 1921)
-Detection of anti-dsDNA antibodies using the ELISA: (Abcam, Anti-dsDNA IgG ELISA Kit (ab178618)) (Koh et al., 1995). ELISA was performed according to the manufacture’s instructions.
--Determination of C3 and C4 levels by Radial Immune Diffusion. (Fahey & Mckelvey, 1965)
-Serum creatinine concentration by the standard colorimetric method. (Heinegård & Tiderström, 1973)
-Complete urine analysis for the urinary casts, hematuria and pyuria.
-Twenty-four-hour urine examination for protein by the turbidimetric method (Shahangian et al., 1984).

Measurement of Anti Complement-1q Antibody (anti C1q antibodies) by ELISA

BÜHLMANN anti-C1q Autoantibodies ELISA that was performed according to the manufacture’s instructions. To define our cutoff levels of abnormal results, we calculated an optimized cutoff value, that is
SLE patients were divided into two groups:

- **Group I**: Included SLE patients, who had never shown any past or present clinical and/or laboratory evidence of major renal manifestation attributable to SLE.

- **Group II**: Included SLE patients with renal disease who had past or present renal affection based on the ACR criteria: proteinuria \( \geq 500 \text{ mg/day} \) and/or red cell casts (Hochberg, 1997). A renal biopsy was done for these patients:

  A renal biopsy from each patient was obtained on the same day of blood sampling under computed tomography "CT" guidance using a true cut needle biopsy. Specimens were fixed in 10% formalin solution, embedded in paraffin, sliced into 4 \( \mu \text{m} \) sections, and then stained with hematoxylin – eosin, for examination under light microscopy using the criteria of the World Health Organization (WHO) classification system for grading of LN (Churg et al., 1995).

- The predominant histopathological class was reported for each sample.

- Activity and chronicity indices (AIS and CIS respectively) were used for biopsy assessment according to the standards of the National Institute of Health (NIH) for LN (Austin et al., 1984).

**Statistical Analysis**

The results of this study were tabulated and statistically analyzed by descriptive and analytic parametric statistics on an IBM personal computer using the SPSS version 6. Quantitative data were analyzed using mean and standard deviation, while frequency and percentage were used with qualitative data. Student t test was used to compare means of different groups. Pearson correlation test was used to analyze the relationship between two variables and ANOVA was used for normally distributed variables. A value of \( P <0.05 \) was considered to indicate statistical significance.

To assess the diagnostic value of anti-C1q antibodies, we calculated the following indices:

- **Sensitivity**: True positive / (true positive + false negative) \( \times 100 \)

- **Specificity**: True negative / (true negative + false positive) \( \times 100 \)

- **Positive Predictive Value (PPV)**: True positive / (true positive + false positive) \( \times 100 \)

- **Negative Predictive Value (NPV)**: True negative / (true negative + false negative) \( \times 100 \).

**Results**

This study included 30 patients with SLE. They were all females with a mean age of 27.7±8.2 years and disease duration of 4.2 ± 3 years. Of the 30 SLE patients, 24 patients (80%) showed renal disease. Of these, 18 patients (75%) had active LN disease. Thus the prevalence of active LN among SLE patients accounted for 60% (18/30).

After proper examination, SLE patients were stratified into one of 2 groups:

**Group I**

Included 6 SLE patients (20%) without renal disease. Their mean age was 29.7± 8.3 years with a mean disease duration of 2 ± 1.8 years.

**Group II**

Included 24 SLE patients (80%) with renal disease based on ACR criteria that were confirmed by the results of the renal biopsy. Their mean age was 28.7± 8.7 years and disease duration of 4.8 ± 3.1 years. The SLE disease duration was significantly longer in group 2 than in group 1 (\( P<0.05 \)).

Group II was subdivided into 2 subgroups:

- **Group II A**: included 18 patients (75%) with active LN (rSLEDAI score \( \geq 4 \)).

- **Group II B**: included 6 patients (25%) with inactive renal disease (rSLEDAI score = 0).

The mean anti-ds DNA and anti-C1q antibodies in SLE patients was significantly higher than the control group (\( P<0.05 \)) (Table 1).
Significance of Anti-C1q Antibodies in Patients with SLE as A Marker of Disease Activity and LN

Table 1. Comparison between SLE patients and controls as regards to anti-C1q and Anti-dsDNA antibodies levels:

<table>
<thead>
<tr>
<th></th>
<th>SLE (n=30)</th>
<th>Mean±SD</th>
<th>Control (n=20)</th>
<th>Mean±SD</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA u/ml</td>
<td>97±23</td>
<td></td>
<td>27.6±16.3</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Anti-C1q (U/L)</td>
<td>73.4±60.5</td>
<td></td>
<td>13.7±6.1</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05= significant

Several parameters including ESR, serum creatinine and proteins in urine (24 hours) were higher in group IIA (SLE patients with active LN) than in group IIB (SLE patients with inactive renal disease) (P<0.05), whereas C3 and C4 levels were lower in Group IIA than in group IIB (P<0.05). Also, anti-dsDNA and anti-C1q antibodies levels were higher in group IIA than in group IIB (P<0.05) (Table 2).

Table 2. Comparison of laboratory parameters between the studied SLE patient groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (n=6)</th>
<th>Group IIA (n=18)</th>
<th>Group IIB (n=6)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR mm / 1st hr.</td>
<td>42.3±6.8</td>
<td>71.9±18.01</td>
<td>50±15.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S. Creatinine mg/dl</td>
<td>0.97±0.19</td>
<td>1.4±0.5</td>
<td>0.8±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S. Urea mg/dl</td>
<td>11.7±6.8</td>
<td>23.9±7.3</td>
<td>16.3±3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Protein/24 hour urine collection (mg/24 hr.)</td>
<td>133±0.1</td>
<td>2500±1.0</td>
<td>600±0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C3 mg/dl</td>
<td>130.7±15.00</td>
<td>87.8±7.9</td>
<td>100.7±15.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C4 mg/dl</td>
<td>31±9.1</td>
<td>15.8±4.7</td>
<td>25±11.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-dsDNA u/ml</td>
<td>22.7±8.5</td>
<td>109±25.5</td>
<td>97.4±35.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-C1q antibodies (U/L)</td>
<td>26.1±7.01</td>
<td>126.4±6.1</td>
<td>27.7±6.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P>0.05 is not significant (NS).

According to SLE disease activity, study subjects were divided into two groups; Active SLE (n=20) and inactive SLE (n=10). Active SLE patients had higher mean levels of anti-ds DNA and anti-C1q antibodies than inactive SLE patients (P<0.05), whereas C3 and C4 mean levels were lower in active SLE patients than inactive SLE patients (P<0.05) (Table 3).

Table 3. Comparison between laboratory parameters in SLE patients, according to SLEDAI, regardless renal affection of SLE patients.

<table>
<thead>
<tr>
<th></th>
<th>Active SLE (n=20)</th>
<th>Inactive SLE (n=10)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-C1q antibody (u/ml)</td>
<td>133.26±54.22</td>
<td>27.3±13.22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-dsDNA antibody (u/ml)</td>
<td>140±20</td>
<td>43±15</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>60.4±0.13</td>
<td>130.6±0.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C4 mg/dl</td>
<td>15.8±4.7</td>
<td>31±9.1</td>
<td>NS</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>12.56±3.11</td>
<td>4.01±1.0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P>0.05 is not significant (NS).
Based on the WHO classification system for grading of LN, the 24 SLE patients with renal disease were histopathologically graded into 3 grades, III, IV and V (Table 4). The mean anti-C1q antibodies was higher in class IV followed by class III then class V ($P<0.001$).

Table 4. Comparison between the mean levels of anti-C1q antibody levels in different classes of lupus nephritis (according to the WHO morphological classification of lupus nephritis).

<table>
<thead>
<tr>
<th>Class</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>Mean±SD</td>
<td>N (%)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Anti C1q Abs (U/ml)</td>
<td>10(41.7%)</td>
<td>50.7±9.1</td>
<td>8(33.3%)</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*$P<0.05$= significant.

A positive correlation was observed between anti-C1q antibodies and ESR, ANA, anti-dsDNA, SLEDAI score, renal SELDAI score and activity index of renal biopsy, but not with the chronicity index of renal biopsy (Table 5). In addition, anti-C1q antibodies were negatively correlated with C3 and C4.

Table 5. Correlation coefficients between serum anti-C1q antibodies and disease activity markers, activity and chronicity index score of renal biopsy in SLE:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Anti-C1q antibody (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR mm / 1st hour</td>
<td>0.31*</td>
</tr>
<tr>
<td>HB% g/dl</td>
<td>-0.11</td>
</tr>
<tr>
<td>WBCs $10^9$/L</td>
<td>-0.12</td>
</tr>
<tr>
<td>Platelets $10^9$/L</td>
<td>-0.16</td>
</tr>
<tr>
<td>Serum creatinine mg/dl</td>
<td>0.12</td>
</tr>
<tr>
<td>Serum urea mg/dl</td>
<td>0.12</td>
</tr>
<tr>
<td>24 hours proteinuria</td>
<td>0.35*</td>
</tr>
<tr>
<td>$C_3$ mg/dl</td>
<td>-0.40*</td>
</tr>
<tr>
<td>$C_4$ mg/dl</td>
<td>-0.39*</td>
</tr>
<tr>
<td>ANA U/ml</td>
<td>0.31*</td>
</tr>
<tr>
<td>Anti-dsDNA U/ml</td>
<td>0.51*</td>
</tr>
<tr>
<td>SLEDAI score</td>
<td>0.46*</td>
</tr>
<tr>
<td>Activity index of renal biopsy</td>
<td>0.42*</td>
</tr>
<tr>
<td>Chronicity index of renal biopsy</td>
<td>0.11</td>
</tr>
<tr>
<td>Renal SELDAI score</td>
<td>0.63*</td>
</tr>
</tbody>
</table>

$r \geq 0.28$ (for all 30 SLE patients); *$P<0.05$= significant.
Significance of Anti-C1q Antibodies in Patients with SLE as A Marker of Disease Activity and LN

Anti-C1q antibodies showed a higher diagnostic sensitivity of 94.4% and specificity of 83.3% in active LN while, anti-dsDNA antibodies showed sensitivity 88.9% and specificity 66.7%. A combination of both the anti-ds DNA and anti-C1q antibodies yielded decreased sensitivity to 88.9% but increased specificity to 100%. Also, they could predict 100% of patients with active LN and exclude 75% of patients without the activity of LN proven by biopsy, (Table 6).

Table 6. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of anti-dsDNA and anti-C1q antibodies in detection of active LN (n=18).

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve anti–ds DNA antibodies</td>
<td>88.9%</td>
<td>66.7%</td>
<td>88.9%</td>
<td>66.7%</td>
</tr>
<tr>
<td>+ve anti-C1q antibodies</td>
<td>94.4%</td>
<td>83.3%</td>
<td>94.4%</td>
<td>83.3%</td>
</tr>
<tr>
<td>+ve anti-C1q and anti-dsDNA antibodies</td>
<td>88.9%</td>
<td>100%</td>
<td>100%</td>
<td>75%</td>
</tr>
</tbody>
</table>

Figure 1. A renal biopsy of an SLE patient of group IIA. This slide shows the focal segmental nature of proliferative LN (WHO Class III LN) (hematoxylin and eosin stain, original magnification x200).

Discussion

In this study, there was a significant increase in the mean level of anti-C1q and anti-dsDNA antibodies in SLE patients than in normal controls (P<0.05). As regards to the clinical presentation, serum anti-C1q antibody levels were significantly higher in patients with renal affection. Additionally, we observed significant associations and higher levels of anti-C1q antibodies, as well as anti-dsDNA antibodies in patients with active LN comparing it in patients with inactive LN that suggest an association of such antibodies and LN. Moroni and coworkers (2001), also detected higher titers of anti-C1q antibodies and anti-dsDNA antibodies in active SLE patients and an association with nephritis. Moreover, Matrat and his associates (2011),
confirmed that the presence of anti-C1q and anti-dsDNA antibodies were associated with a high risk of renal flare, whereas the absence of both antibodies excluded such an event.

Our results are also consistent with the results of a study done by Chi and coworkers (2015) who stated that anti-C1q was more frequent in patients with active SLE (78.4%) than those with inactive SLE (22.7%), also, they found anti-C1q antibodies were more often to be detected in the sera of LN patients (85.7%) than in sera of those without a renal flare (33.3%).

In our study there were positive correlations between anti-C1q antibodies and ESR, ANA, anti-dsDNA, SLEDAI score, renal SELDAI score and activity index of renal biopsy. Also, there were negative correlations between anti-C1q antibodies and C3 and C4, but insignificant correlation with CBC parameters, creatinine, urea and chronicity index of renal biopsy. Similar to our findings, three other studies found significantly increasing difference in titers of anti-C1q antibodies in patients with active disease compared with those with inactive SLE. (Horak et al., 2006, Wu et al., 2011 and Katsumata et al., 2011)

These results are also in agreement with Chi and coworkers (2015), who found that the titer of serum anti-C1q antibodies was positively correlated with anti-dsDNA antibodies \( r = 0.796 \) and SLEDAI \( r = 0.584 \) but inversely correlated with serum levels of complements C3 \( r = -0.563 \) and C4 \( r = -0.532 \). However, Fang and his colleagues (2009), demonstrated significant correlations with creatinine and leucopenia. This discrepancy in results may be due to differences in patients population and anti-C1q antibodies assays.

In addition, we found a significantly higher level of anti-C1q antibodies present in class IV followed by class III then class V. Different literature mentioned that more active classes of biopsy-proven LN are classes III and IV (proliferative LN), while other classes, namely classes I, II, V, and VI (nonproliferative LN) are considered less active that needs limited immunosuppressive therapy (Chi et al., 2015). In agreement with our study, Fang and his colleagues (2009), found a strong positive association between anti-C1q antibodies and the detection of proliferative LN.

These data also are in concordance with the studies done by Eggleton et al., (2014), GargiuloMde et al., (2015), Orbai et al., (2015); Tan et al., (2015).

Our data are corresponded to that reported by Olivier and coworkers (2009), who suggested that anti-C1q antibodies may be a good serological marker for the subsequent development of active proliferative glomerulonephritis in SLE patients and patients without anti-C1q antibodies are at a very low risk for severe proliferative glomerulonephritis, they also reported that patients with anti-C1q antibodies have an approximately 50% risk for LN within the next decade and, therefore, require close monitoring of renal disease activity.

In the current study, we found that the sensitivity and specificity of anti-dsDNA antibodies were 88.9% and 66.7%, respectively, for anti-C1q antibodies were 94.4 % and 83.3% respectively, and were 88.9% and 100% for both antibodies in combination. Detection of both anti-C1q and anti-dsDNA antibodies could predict 100% of active LN and could exclude 75% of those without LN proven by biopsy. Trendelenburg and his associates (2006), reported that for the detection of an active glomerulonephritis in SLE patients, the anti-C1q assay showed a particularly high sensitivity (97.2%) while specificity was 70.3%. Also, they found that the positive predictive value (PPV) was 68.4% and the negative predictive value (NPP) was 97.8. Also, they mentioned that
anti-C1q antibodies had a very high prevalence in biopsy-proven active LN, thus a negative test result almost excludes active nephritis.

Matrat et al., (2011) mentioned that the sensitivity of anti-C1q antibodies and/or anti-dsDNA antibodies in predicting renal flares reached 85%. The specificity of anti-C1q antibodies were 84%, of anti-dsDNA antibodies 77% and of both antibodies together 97%. Positive and negative predictive values were as follows: 56% and 70% for anti-C1q antibodies, 53% and 72% for anti-dsDNA antibodies respectively. The combination of both antibodies had the highest PPV (69%), whereas the absence of both antibodies was associated with the highest NPV (74%). Zivković et al., (2014) and Orbai et al., (2015), reported that anti-C1q antibodies demonstrated an association with SLE and LN activity, suggesting their potential usefulness in making predictions about LN and assessment of disease activity.

In conclusion, this study documented the presence of an association of anti-C1q antibodies with disease activity in SLE patients. Anti-C1q antibodies were found to be useful and sensitive non-invasive biomarker that have a high specificity in combination with anti-dsDNA antibodies for the diagnosis of renal disease activity. Thus, they could serve as good noninvasive markers for primary screening SLE patients to determine whether they developed LN.

References
13. Hochberg MC (1997). Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification


32. Wu FQ, Zhao Q, Cui XD, Zhang W (2011). C1q and anti-C1q antibody levels are correlated with