Impact of Autoantibodies to Complement Components on the Disease Activity in SLE

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Abstract

Introduction: Systemic Lupus Erythematosus (SLE) is a chronic multi-system autoimmune disease with varied clinical presentations. Complement components are the major players in disease pathogenesis. This retrospective cross-sectional study was aimed at assessing the role of autoantibodies to these complement components and their association disease activity in newly diagnosed SLE patients from India.

Method: Clinically diagnosed SLE patients (n=57) classified as per 2015 ACR/SLICC revised criteria were enrolled between November 2016 to April 2017. Patients’ sera were tested for C3 and C4 by nephelometry, while serum levels of factor H, factor P (properdin) as well as autoantibodies to C3, C4, factor H and factor P were detected by ELISA. GraphPad Prism Version 6.01 was used for statistical analysis. Mean, SD, SEM were calculated. Mann Whittney U-test, ANOVA, Chi-square test, Odd’s Ratio were calculated. Pearson’s correlation was used to study relativeness of the study parameters.

Results: Among the 57 SLE patients, low C3 were seen in 51% patients, low C4 in 49%, low factor H in 19% and low factor P in 49% patients. Positivity for autoantibodies against complement components, anti-C3 were seen in 42% patients, anti-C4 in 7%, anti-factor H in 19% and anti-factor P in 28% patients. Serum levels of C3 (p=0.0009), C4 (p=0.0031) and anti-C3 autoantibodies (p=0.0029) were significantly associated with ACR/SLICC 2015 scores.

Conclusion: Hypocomplementemia was found to be associated with higher disease damage score in newly diagnosed SLE patients. This study adds novel arguments for the importance of the anti-C3 autoantibodies as a marker of SLE.

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic multi-system autoimmune disease with varied clinical presentations.1 SLE pathogenesis involves loss of immune tolerance and abnormally functioning T-cells and B-cells. Complement system from the innate immunity plays a key role in this disease.2 Quantitative and functional deficiencies of the components of the classical pathway are associated with SLE due to inefficient clearance of dying cells and immune complexes and failure in the self-tolerance.3,4 Nevertheless, these cases remain rare. Most frequently complement is overactive in SLE due to the strong accumulation of immune complexes, leading to tissue inflammation and damage. This leads to disease progression rapidly and requires aggressive immunosuppressive therapy.2,5-6 The levels of complement components C3 and C4, hence have been attributed to as immune markers for disease activity in SLE.1-3 Hypocomplementemia is a vital aspect of disease activity in SLE. However, interplay between complement components and disease activity is mainly studied in SLE patients with renal involvement. The pathogenesis of SLE is assumed to arise from a combination of raised levels of autoantibodies and the reduced levels of components of the complement systems.3,4 Alteration of the function of the complement cascade in SLE can be caused also by autoantibodies against different complement components.

Majority of the autoantibodies which bind with high affinity to complement proteins are directed against the neoepitopes or to structurally modified components. Autoantibodies like anti-C3, anti-C4 bind to the complete proteins but also to their activation fragments.5-11 Autoantibodies to the C1q component, are the most common antibodies found in around one-third of SLE patients, bind also preferentially to neoepitopes, exposed after binding of C1q to apoptotic cells.12-17 Despite the large spectrum of anti-complement antibodies found in SLE patients, only anti-C1q antibodies are screened in the routine diagnostics. The clinical associations of the remaining types of antibodies are reported in limited number of cohorts and their clinical relevance needs further investigation, before introduction to routine screening.18-22

This study was aimed at assessing the role of autoantibodies to various complement components in association
Table 1: Levels of Complement components in SLE patients

<table>
<thead>
<tr>
<th>Complement components</th>
<th>Range</th>
<th>Mean ± SD in SLE patients (n=57)</th>
<th>Number of patients with hypo-complementemia</th>
<th>Mean ± SD in SLE patients with hypo-complementemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (mg%)</td>
<td>90 – 180</td>
<td>99.71 ± 58.41</td>
<td>29</td>
<td>57.24 ± 21.97</td>
</tr>
<tr>
<td>C4 (mg%)</td>
<td>15 – 40</td>
<td>20.74 ± 16.44</td>
<td>27</td>
<td>7.84 ± 3.41</td>
</tr>
<tr>
<td>factor H (ng/ml)</td>
<td>5 – 16</td>
<td>9.12 ± 4.49</td>
<td>11</td>
<td>3.67 ± 1.09</td>
</tr>
<tr>
<td>factor P (ng/ml)</td>
<td>130 – 309</td>
<td>144.5 ± 77.15</td>
<td>27</td>
<td>82.84 ± 26.49</td>
</tr>
</tbody>
</table>

Levels of factor H and factor P were estimated using commercially available ELISA kits (AssayPro, USA).

Estimation of autoantibodies to complement components

Autoantibodies to C3, C4, factor H and factor P were detected by indigenous ELISA as per protocols described by Vasilev et al (2015) with slight modifications. Coating of ELISA plates was performed at 1µg/ml for C3 and C4 and 5 µg/ml for factor H and factor P (Complement Technology, Tyler, TX, USA). In brief round-bottom ELISA plates (NUNC, Denmark) were coated overnight at 4°C with native human complement components in carbonate-bicarbonate buffer (0.05M; pH 9.6). Plates were given 5 wash with 0.01M PBS and blocked with 5% non-fat dry milk powder in PBS for 1 hour at 37°C. Plates were given 5 wash with 0.01M PBS. Serum samples were diluted 1:100 and Conjugate (Sigma A-3150 goat anti-human IgG alkaline phosphatase) was diluted 1:2000 in 0.01M PBS. p- Nitrophenyl phosphate (PNPP) (Sigma N2765) at 2mg/ml in carbonate-bicarbonate buffer (pH 9.8) was used as substrate. Reaction was stopped by adding 4N NaOH. The ELISA plates were read at 405nm wavelength using Biotek ELx800 ELISA reader (Winooski, VT, USA).

The reference range for complement components and autoantibodies to complement components were determined by assessing levels in healthy controls (n=100).

Statistical analysis

GraphPad Prism Version 6.01 was used for statistical analysis. Arithmetic Mean, Standard Deviation (SD) and Standard Error of the Mean (SEM) were calculated. Parametric and non-parametric t-tests, ANOVA were used to analyse quantitative data. Chi-square test and Odd’s Ratio were calculated for estimating qualitative data. Pearson’s and Spearman’s correlations were used to study relativeness of the study parameters.

Results

Patient Demographics

Out of 57 SLE patients enrolled for the study, there were 50 females and 7 males (Female-Male ratio 7:1). Average age of these patients was 27.7 (± 10) years with mean disease duration 3.5 (± 3.3) months. Forty-five patients (79%)
had anemia (haemoglobin < 10 mg%). Five patients (9%) had leukopenia (WBC < 4000/µl) while 13 patients (23%) had thrombocytopenia (Platelets < 150,000/µl). Arthralgia (Joint disease) (n=47; 83%), followed by alopecia (n=27; 47%) and oral ulcers (n=25; 44%) were observed. Photosensitive rash were seen in 17 patients (30%), while serositis (pleuritis and pericarditis) was seen in 14 patients (25%). Renal involvement was seen in 9 patients (16%) only. Neuropsychiatric involvement was seen in 3 patients (5%).

Autoantibody profile in these patients showed ANA positivity in all (100%) except for 3 patients with ANA titres < 1:80. ANA titres > 1:160 were seen in 43 patients (75%) whereas lower ANA titres (< 1:160) were observed in 14 patients (25%). Anti-dsDNA antibodies were seen in 14 patients (25%). Renal involvement was seen in 9 patients (16%) only. Neuropsychiatric involvement was seen in 3 patients (5%).

Age and sex matched normal healthy controls (n=100) were tested for determining the normal range of complement components and autoantibodies to complement components. Following were the cut-off levels set for complement components and respective autoantibodies.

C3 : 90 – 180 mg%; C4 : 15 – 40 mg%; factor H : 5 – 16 ng/ml; factor P : 130 – 309 ng/ml; anti-C3 abs : 0 – 18 AU/ml; anti-C4 abs : 0 – 85 AU/ml; anti-factor H abs : 0 – 35 AU/ml; anti-factor P abs : 0 – 34 AU/ml.

Estimation of complement components

Mean levels of C3 among SLE patients (n=57) were 99.71 (± 58.41) mg%. Low C3 levels (<90 mg%) were seen in 29 patients (51%). Mean levels of C4 (n=57) were 20.74 (± 16.44) mg%. Low C4 levels (<15 mg%) were seen in 27 patients (49%). Mean levels of factor H were 9.12 (± 4.49) ng/ml. Low factor H levels (<5 ng/ml) were seen in 11 patients (19%). Mean levels of factor P were 144.5 (± 77.15) mg%. Low factor P levels (<130 mg/ml) were seen in 27 patients (49%) (Table 1).

Estimation of autoantibodies to complement components

Anti-C3 antibodies (> 18 AU/ml) were present in 24 patients (42%) with a mean of 37.15 (± 26.39) AU/ml. Anti-C4 antibody levels (> 85 AU/ml) were present in only 4 patients (7%) with a mean of 116.9 (± 39.33) AU/ml. Anti-factor H antibody levels (> 35 AU/ml) were present in 11 patients (19%) with a mean level of 59.30 (± 20.92) AU/ml. Anti-factor P antibody levels (>34 AU/ml) were seen in 16 patients (28%) with a mean of 50.00 (±16.44) AU/ml (Figure 1).

Association of Complement components and respective autoantibodies with ACR/SLICC 2015 Scores

ACR/SLICC 2015 scores were calculated based on clinical manifestations noted at the time of evaluation. Pearson’s correlation was calculated for ACR/SLICC 2015 scores and the complement components and respective autoantibodies. Complement components C3 (p=0.0009; r=-0.4294) and C4 (p=0.0031; r=-0.3854) were significantly associated with ACR/SLICC 2015 scores. Autoantibodies to complement components were not significantly associated with ACR/SLICC 2015 scores (Figure 2).

Hypocomplementemia has significant effect on the disease activity in SLE as is seen in patients with low C3 and C4 as against patients with normal C3 and C4 levels (p=0.0003 and p=0.0028 respectively) (Figure 3). Hypocomplementemia for other complement components and respective autoantibody positivity did not have a significant impact on the ACR/SLICC 2015 Scores.

Among the group with anti-C3 autoantibody positivity, serum C3 levels were found to be significantly associated with ACR/SLICC 2015 scores (p=0.0029). The same was not observed with other complement autoantibody positivity with respective complement levels and its association with ACR/SLICC 2015 scores (Figure 4).

Discussion

The complement system plays an important role in maintaining homeostasis and also preventing autoimmune diseases. The acquired hypocomplementemia seen in SLE is detrimental and contributes to disease pathogenesis. Complement proteins C3 and C4 are assessed in patients regularly to evaluate the disease status and prognosis. Our findings demonstrated 51% patients having
low C3 levels, whereas C4 levels were low in 47% patients. C3 and C4 levels also significantly correlated with ACR/SLICC 2015 scores indicating their role in disease severity. The autoantibodies to complement components did not show a significant association with SLE disease activity.

Hypocomplementemia (low C3 and low C4) was recorded among 29% SLE patients in the Argentine cohort by Gandino et al. Low C4 levels were seen among 14% SLE patients from Romania [28], while it was 54% in the Tunisian SLE patients. Brazilian SLE patients were reported with 75% low C4 levels. A study from Northern Indian early onset SLE patient cohort showed 20% hypocomplementemia. Our study demonstrated 47% lower levels of C4 and factor P, while low C3 levels were seen in 51% patients. 19% patients had low factor H levels. Hypocomplementemia was mostly associated with presentation of malar rash, followed by renal involvement and arthritis.

Autoantibodies to complement components also form a significant component of SLE disease pathogenesis. The functional role of autoantibodies to complement components in complement consumption is not completely understood. However the presence of complement autoantibodies has been associated with tissue destruction, impairment of complement regulation and functional modification of target complement proteins.

Anti-factor H antibodies have been predominantly studied with atypical hemolytic uremic syndrome (HUS), and other renal pathologies. Although our study cohort had very few patients with renal involvement, we encountered anti-factor H antibodies in 20% of our SLE patients, but the observed titres were low. Anti-C3/C3b antibodies have been reported with SLE and LN and have been significantly correlated with the decrease in complement C3 levels among patients. In our study group, 42% patients had raised titres of anti-C3 antibodies. Among the studied autoantibodies to complement components, only anti-C3 antibodies were seen to contribute significantly to C3 hypocomplementemia and resultanty contributing to increased disease damage index. Our study has also demonstrated 20% patients having significant anti-factor P antibody titres where as anti-C4 antibodies were rare (7%). Except anti-C3, the remaining tested autoantibodies did not contribute significantly to either complement deficiency or the disease pathology.

The significant association of anti-C3 autoantibodies with the disease process could be explained with their clear functional consequences. Interestingly, they have been reported to bind to neoepitopes, exposed after activation. This property allows them to act locally, at the site of deposition of the immune complexes and to concentrate their deleterious effects, causing thus tissue damage.

In conclusion, this study adds novel arguments for the importance of the anti-C3 autoantibodies as a marker of SLE. It also confirms that anti-C1q antibodies are a useful biomarker for this disease as well as the quantification of C3 and C4 levels. These two types of antibodies could bring useful information for the clinicians. The remaining tested antibodies: anti-C4, anti-FH and anti-factor P did not show significant correlations with the disease activity and their screening would not be informative for SLE.

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**Key Points**

- C3 and C4 complement levels are directly correlated with anti-C1q antibodies in active SLE patients.
- Anti-C3 and anti-C1q autoantibodies have role in disease pathogenesis of SLE.
- Anti-C4, anti-factor H and anti-factor P autoantibodies have no association with the disease activity and hence show no impact on screening in active SLE patients.

**References**


