

CORRELATION OF ANTI C1Q ANTIBODIES WITH DISEASE ACTIVITY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Objective: To study the correlation of anti C1q antibodies with disease activity in patients with systemic lupus erythematosus (SLE).

Study Design: Cross sectional, observational study.

Place and Duration of study: The Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi in collaboration with Military Hospital, Rawalpindi, Pakistan Institute of Medical Sciences, Islamabad and Benazir Bhutto Hospital, Rawalpindi, from Jan 2012 to Dec 2013.

Material and Methods: Patients with a clinical diagnosis of SLE were included in the study on fulfilling revised American College of Rheumatology (ACR) criteria (1997). Main outcome measures were SLE disease activity index (SLEDAI) score and anti C1q antibody levels in serum. SLEDAI scores were calculated for each patient on the basis of physical examination, patient interviews and previous clinical records. Anti C1q antibody levels in the serum were determined by enzyme-linked immunosorbent assay (ELISA) and correlated with the SLEDAI scores by calculating Pearson's correlation coefficient 'r'. The cutoff value for anti C1q antibody positivity in the serum was determined by evaluating the serum levels of anti C1q antibodies in 25 healthy subjects and was 12 U/ml.

Results: Six male and forty nine female SLE patients with an age range of 16-47 years (mean 34.5 years) and 8-70 years (mean 31.7 years) respectively were studied. The correlation between anti C1q levels and SLEDAI scores in all patients was demonstrated by calculating the correlation coefficient and was not significant ($r=0.19$, $p=0.14$). However, there was an inverse correlation between anti C1q levels and SLEDAI scores in patients with severe disease and this was statistically significant ($r=-0.448$, $p=0.037$). The difference in anti C1q antibody positivity between patients with and without nephritis was not significant. The anti C1q antibody levels correlated poorly with anti double stranded deoxyribonucleic acid (dsDNA) antibody positivity. A significantly higher percentage of patients with evidence of complement consumption was found to be positive for anti C1q antibodies ($p=0.01$). This significance was only seen in patients with reduced C3 levels ($p=0.04$) and not reduced C4 levels ($p=0.23$) or both ($p=0.23$). Anti C1q antibody levels had significant inverse correlation with serum C3 levels. ($p=0.007$).

Conclusion: A significant inverse correlation was found between SLEDAI scores and serum anti C1q antibody levels in patients with severe SLE. The anti C1q antibody positivity is significantly higher in patients with reduced C3 levels.

Keywords: Anti C1q antibodies, SLE, SLEDAI.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a relapsing and remitting multi organ auto immune inflammatory disorder characterized by the presence of multiple auto antibodies in

patient serum and disease spectra ranging from mild symptoms to life threatening multi organ failure. Owing to its complex pathogenesis, variable presentation and unpredictable outcome, it remains one of the major challenges to the physician and pathologist alike¹.

A major issue in therapeutic monitoring and disease prognosis of SLE is the frequent relapse or "flare" of disease activity after a quiescent

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period. The inability to reliably predict an oncoming flare has significantly contributed to disease associated morbidity².

The disease activity in SLE is often measured by various indexing scores like SLE disease activity index (SLEDAI) which provides a model of disease activity by a combination of clinical and laboratory features. SLEDAI has been demonstrated to have a positive correlation with disease activity in SLE. An SLE patient is assessed for the presence of 24 clinical and laboratory parameters to arrive at a SLEDAI score³. This is however cumbersome and often impossible in the

been ascribed to the differences in patient populations. By extension, any potential SLE disease activity marker like anti C1q antibody must also be tested for its utility in the local Pakistani population under study.

The exact disease burden in Pakistan is not known. It has been suggested that Lupus is under reported in Pakistan⁶. A prevalence study in India found a point prevalence of 3 per 100,000 which is a much lower figure than reported in the literature for the West⁷. A similar study for Iran found a disease prevalence of 40 per 100,000⁸. With a comparable disease burden Pakistan

Table-I: Anti C1q antibody positivity in SLE patients at the time of evaluation.

n=55		
Anti C1q antibodies	Positive(> 12 U/ml)	16
Anti C1q antibodies	Negative (< 12 U/ml)	39

Disease activity*	SLEDAI Score	No of pts	Anti C1q Antibody (Mean ± SD)
Mild	(SLEDAI = 1-10)	6	5.14 ± 1.72
Moderate	(SLEDAI = 11-20)	22	9.65 ± 8.21
High	(SLEDAI = 20 and higher)	27	19.08 ± 13.70

Table-II: Complement consumption in patients with anti C1q antibody positivity.

	Anti C1q antibody positive	Anti C1q antibody negative
Low C3	12	17
Low C4	12	22
Normal C3 and C4	3	23

face of the immense patient burden in outpatient settings in addition to the patients being lost to follow up.

Efforts are afoot to identify auto antibodies with significant predictive value for organ-specific damage and disease activity. Antibodies against complement in SLE have also been evaluated in this regard and anti C1q antibody has been demonstrated to have potential diagnostic utility as a marker of lupus nephritis and “possible activity indicator”⁴. Anti C1q antibodies are present in the serum of 30-60% of all patients with SLE¹ and their titers have been shown to correlate well with SLEDAI scores in various studies while no such correlation was found in one study⁵. This difference in results has

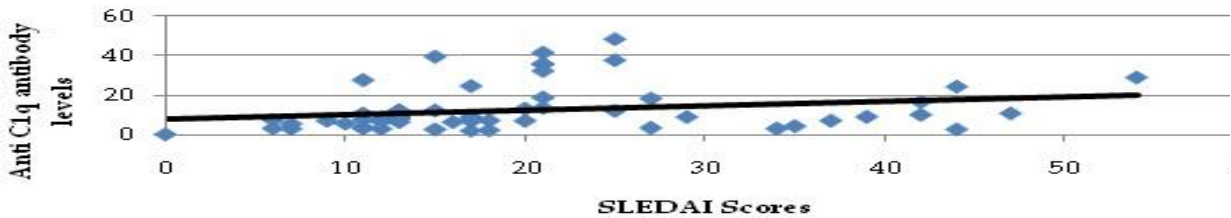
might have up to 72000 SLE patients. Two recent studies which correlated SLEDAI with the levels of anti-C1q antibodies found a positive correlation with r-values of 0.520 and 0.37 respectively^{9,10}. In the absence of a population wide tertiary health care services, a positive correlation between anti C1q antibodies and SLE disease activity might provide an inexpensive biomarker that correlates well with SLE disease activity and allows for a quick and cost effective disease activity assessment thus reducing costs and improving patient management.

MATERIAL AND METHODS

It was a cross sectional observational study carried out in the Department of Immunology, Armed Forces Institute of Patholog (AFIP), from

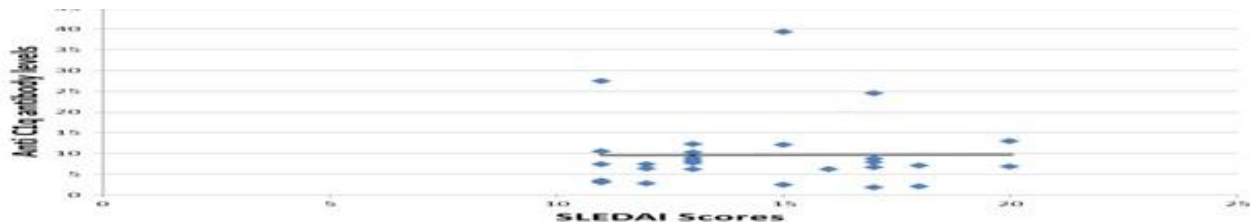
January 2012 to December 2013. A total of 55 patients were included in the study out of those screened for SLE by non-random consecutive sampling. The sample size was calculated by PASS software by utilizing $r=0.37$ as found in a recent study on the subject¹⁰. A sample size of 55 achieved 81% power to detect a difference of -0.37 between the null hypothesis correlation of 0.0000 and the alternative hypothesis correlation of 0.37 using a two-sided hypothesis test with a significance level of 0.05. Patients referred from the Military Hospital, Fauji Foundation Hospital and Benazir Bhutto Hospital, Rawalpindi for laboratory investigations with a clinical diagnosis of SLE were selected for evaluation on fulfilling

sample was obtained from each patient in plain blood collection tubes. Anti nuclear antibodies were detected in patient serum by indirect immunofluorescence using rat liver & HEp-2 cells as substrate whereas anti double stranded deoxyribonucleic acid (dsDNA) antibodies were detected in patient serum by indirect immunofluorescence with *Crithidia lucilae* as the substrate. Complement C3 & C4 levels were determined by radial immune diffusion & confirmed by chemiluminescence method as required. The presence of precipitating antibodies to extractable nuclear antigens including Ro(SSA), La(SSB), RNP and Sm was demonstrated by line enzyme linked



$r = 0.19$ (pearsons correlation coefficient), $r^2 = 0.03$, $p = 0.14$

Figure-I: Correlation of anti C1q antibody levels with SLEDAI scores in all patients (n=55).



$r = 0.005$ (pearsons correlation coefficient), $r^2 < 0.001$, $p = 0.98$

Figure-II: Correlation of anti C1q antibody levels with SLEDAI scores in patients with mild disease (n=6).

American College of Rheumatology (ACR) classification criteria after informed consent. Previous clinical diagnosis of SLE was confirmed by patient interviews, clinical examination and laboratory investigations.

Clinical and pathologic features were documented on a specially designed form. The study was approved by the ethical review committee at AFIP. All the necessary laboratory investigations were carried out at the Department of Immunology, AFIP. Two milliliter whole blood

immunosorbant assay (ELISA) method. The anti C1q antibody levels in patient serum were determined by ELISA and reported as IU/ml. We determined the cutoff level for anti C1q antibody positivity (mean \pm 3SD) by evaluating the presence of anti C1q antibodies in the serum of 30 known healthy subjects with no history of chronic disease or recent hospital admission over the last 3 months. This was found to be 12 IU/ml.

The patients were also assessed for the SLEDAI at the time of presentation⁷. Statistical

Package for Social Sciences version 20 was used for data entry & analysis. Frequency and percentages were calculated for gender & presence of anti C1q antibodies. Mean \pm standard deviation (SD) was calculated for SLEDAI score and age. The level of anti C1q antibodies in SLE patients (in units/ml) was correlated with SLEDAI score. Correlation was determined by calculation of Pearson’s correlation coefficient ‘r’ with the value of ‘r’ ranging from +1 through 0 to -1 with a positive value indicating a positive correlation and vice versa. Chi-square test was

The patients were stratified on the basis of SLEDAI scores (score ranging from 0 to 105) into mild, moderate and severe disease (mild: upto 10; moderate: 11-20 and severe: >20) and their percentage was 11%, 49 % and 40% respectively. The mean time duration between first onset of symptoms and diagnosis of SLE was about 7 months while mean duration of disease was 3 years. In female patients 50% of all patients presented before 30 years of age while only 25% of male patients presented before 30 years of age. The late presentation in males was found to be



$r = 0.3$ (pearsons correlation coefficient), $r^2 = 0.09$, $p = 0.57$

Figure-III: Correlation of anti C1q antibody levels with SLEDAI scores in patients with moderate disease (n=22).



$r = -0.407$ (pearsons correlation coefficient), $r^2 = 0.16$, $p = 0.007$

Figure-IV: Correlation of anti C1q antibody levels with SLEDAI scores in patients with severe disease (n=27).

applied for the comparison of qualitative variables. A p -value of less than 0.05 was considered to be significant.

RESULTS

A total of 55 patients out of those referred were included in the study over a period of six months from Jan 2012 to Dec 2012. There were 6 males & 49 females with a ratio of about 1 to 8. The mean age for males and females was 34.5 and 31.7 with a range of 16-47 years and 8-70 years respectively. Details of the anti C1q antibody positivity in the SLE patients included in this study, at the time of evaluation, are shown in table-I.

associated with moderate disease activity at the time of diagnosis in all cases whereas in females the late presentation was associated with more severe disease. Only 29% of SLE patients were found to be positive for anti C1q antibodies.

We calculated the Pearson’s correlation coefficient ‘r’ to determine the strength and significance of correlation between the anti-C1q antibody levels and SLEDAI scores in all patients and this was found to be insignificant ($r=0.19$, $p=0.14$) as shown in fig-I. We also evaluated the strength of correlation between anti-C1q antibody levels and SLEDAI scores in different SLE disease activity groups. In patients with mild

and moderate disease (n=6 & 22 respectively), no significant correlation was observed ($r=0.3$, $p=0.57$ & $r=0.005$, $p=0.98$ respectively) as shown in fig-II and III. However, in patients with severe disease (n=27), a moderately strong inverse correlation was found between anti-C1q antibody levels and SLEDAI scores as shown in fig-IV.

We calculated anti C1q antibody positivity in lupus patients with and without positive anti dsDNA antibodies. Anti-dsDNA positivity was slightly higher among the anti-C1q positives than in the anti-C1q negatives (68.7% vs 31%), but this was statistically insignificant. (Chi-square test of independence, $p=0.3$).

A total of 18/55 (32.7%) SLE patients had lupus nephritis and the remaining 37/55 (67%) patients were grouped as non-lupus nephritis SLE patients. We calculated anti C1q antibody positivity in lupus patients with and without lupus nephritis and the difference was not significant $p=1.0$.

A total of 52.7% of all SLE patients (29/55) had evidence of complement consumption (table-II). There was an increased incidence of complement consumption in patients with anti C1q antibody positivity. We calculated anti C1q antibody positivity in lupus patients with and without reduced C3 and C4 levels. The anti C1q antibody positivity was higher in the patient group with reduced C3 levels $p=0.04$, but a similar association was not seen with reduced C4 levels $p=0.23$.

DISCUSSION

The reported prevalence of autoantibodies against C1q (anti-C1q) in patients with SLE in various studies ranges from 20 to 66%. In the past decade, though there were increasing studies suggesting it is relatively specific in lupus nephritis (LN), however its overall diagnostic value in LN was only recently evaluated in a meta-analysis comprising 25 studies including 2,502 patients with SLE and 1,317 with LN which showed that anti-C1q antibodies had a fair sensitivity and specificity in the diagnosis of LN, suggesting that the presence of anti-

C1q antibodies may be a valuable additional tool for predicting LN and assessing renal activity⁸. However, we did not find any such association between anti C1q antibody positivity and lupus nephritis in our study, similar to the results of a recent Indian study on the subject as well⁹.

These discrepancies may be due to differences in the patient populations studied, as well as in the specificity and sensitivity of the anti-C1q ELISA and the variations in the commercially available kits used. Our study showed an overall prevalence of 29% for anti-C1q antibodies among the SLE patients studied¹⁰.

The prevalence of anti-C1q antibodies among the LN and non-LN patients in this study was 27.7 & 29.7% respectively, which was less than the reported prevalence in earlier studies (41.5-55.5%)¹¹. It has been suggested that the circulating anti-C1q antibodies may bind to the C1q deposits in the kidneys of LN patients and this consumption of serum anti-C1q antibodies by binding to C1q-containing immune complexes could be responsible for the lack of significant difference among LN and non-LN patients in some cases, as also in our study¹².

Our findings suggested that anti-C1q antibodies are not useful as possible biomarkers for LN in SLE in our patients.

Complement components such as C3 and C4 were usually low in our SLE patients. Anti-C1q antibodies were associated with reduced levels of both C3 and C4 together, as well as individually indicating their role in immune complex clearance via the classical pathway. Our study did not show a correlation between anti-C1q-positive patients and their SLEDAI scores. This was similar to the recent reports where anti-C1q antibodies were not associated with SLEDAI scores for disease activity or with the presence of dsDNA antibodies in them^{13,14}.

CONCLUSION

Anti C1q antibody levels in serum did not correlate with SLE disease activity in all patients ($r=0.19$). However, there was an inverse

correlation between anti C1q antibody levels and disease activity in SLE patients with severe disease ($r=-0.407$, $p=0.007$). The anti C1q antibody positively is significantly higher in patients with reduced C₃ levels.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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