

## Role of Anti-C1q Antibodies as Indicator of Renal Activity in Systemic Lupus Erythematosus

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### ABSTRACT

**Background:** Several investigations have found a correlation between serum anti-C1q autoantibodies and peripheral lymphocyte apoptosis among systemic lupus erythematosus (SLE) patients.

**Objective:** It was to assess correlation between anti-C1q, lupus nephritis and other markers of lupus activity.

**Patients and Methods:** This case-control study was conducted in Internal Medicine Department in cooperation with Clinical Pathology Department. This study was performed on 72 cases and were allocated into three equal groups: SLE with nephritis group, SLE without nephritis group, and control group. Measurements of anti-C1q titers were carried out with by (ELISA) kits. **Results:** Anti-C1q antibody levels varied significantly amongst the groups. Post hoc test showed that there was a statistical significance increase in anti-C1q among SLE with nephritis compared to SLE who don't have nephritis and control and among SLE without nephritis compared to control. Anti-C1q antibodies validity to diagnose LN among the studied group showed that anti-C1q at cut off >88.058 ng/ml had sensitivity 75%, specificity 75%, accuracy 75%, PPV of 75% and NPV of 75% in diagnosis of LN among cases groups.

**Conclusion:** Anti-C1q autoantibodies, like other standard markers like renal SLEDAI, correlate with renal flare-ups as well as renal disease activity.

**Keywords:** Systemic Lupus Erythematosus, Renal Activity, Anti-C1q antibodies.

### INTRODUCTION

A variety of immunological abnormalities that manifest in a variety of systemic manifestations are characters of systemic lupus erythematosus <sup>(1)</sup>.

The most up-to-date speculations on the cause of SLE center on the idea that abnormal apoptosis and necrosis release nuclear antigens into the immune system, immunological complexes containing nucleic acids that induce Type I interferon overexpression upon uptake by plasmacytoid dendritic cells <sup>(2)</sup>. The complement system participate in waste material collection, immunological tolerance, and the formation of an adaptive immune response. Expression of the adaptive immune response's humoral component occurs through antibodies; these antibodies have a dynamic connection with the body's complement system <sup>(3)</sup>.

The complement system's first component, C1q, is expected to perform a key role in clearing away immune complexes and other waste products of apoptotic cells. Autoimmune disease can be triggered by the immune system coming into prolonged link with C1q epitopes <sup>(4)</sup>.

The Systemic Lupus Activity Measurement (SLAM) as well as the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) are considered representative of the global scoring systems used to evaluate SLE activity, while the specific scales of organ/system evaluation used to evaluate SLE activity on a per-organ basis are typical of the other important kind of SLE activity assessment <sup>(5)</sup>.

Lupus nephritis affects up to 60% of those who have lupus that may progress to proteinuria and chronic kidney disease if untreated <sup>(6)</sup>.

In individuals with SLE, death of peripheral lymphocytes has been linked to higher serum levels of

anti-C1q autoantibodies, showing that these antibodies could have a harmful role, especially in the case of active disease <sup>(4)</sup>.

This study aim was to assess correlation between antiC1q, lupus nephritis and other markers of lupus activity.

### PATIENTS AND METHODS

At Internal Medicine Department in cooperation with Clinical Pathology Department, Zagazig University hospitals. We conducted this case-control study on total of 72 people who were randomly assigned to one of three groups for this case-control study:

**Group (I):** Twenty-four SLE with active lupus nephritis (LN) (24 female, no male, age range 18-40 year, mean 29.21±7.23 year). Proteinuria > 0.5 g/day and an increased serum creatinine level higher than 1.2 and 1.1 among males and females respectively, and estimated eGFR <60 mL/min/1.73 m<sup>2</sup> are both hallmarks of clinical nephritis.

**Group (II):** Twenty-four SLE patients with no lupus nephritis (22 female, 2 male, age range 18-42 year, mean 32.38±5.92 year). In addition to having normal kidney function (serum creatinine level less than 1.2 and 1.1 among males and females respectively) and an estimated GFR higher than 90 mL/min/1.73 m<sup>2</sup>, all of these patients also have low proteinuria (0.5 g/day), no urinary casts sediment, and no hematuria upon admission.

**Group (III):** Twenty-four healthy-looking participants who were of a similar age and sex distribution to the patients served as controls (22 female, 2 male, age range 22-42 year, mean 31.71±5.72 year).

Systemic lupus erythematosus was identified and diagnosed using criteria of Systemic Lupus

International Collaborating Clinics (SLICC) (7).

**Ethical consent:**

An approval of the study was obtained from Zagazig University Academic and Ethical Committee (IRB #6712-9-2-2021). Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

**Inclusion Criteria:** Male and female patients aged > 18, all lupus patients fulfill SLICC criteria, and those who were healthy and showed no signs of chronic disease by clinic or laboratory data.

**Exclusion criteria:** Individuals suffering from other systemic autoimmune diseases, individuals who have a urinary tract infection, patients with LN who are undergoing hemodialysis or underwent renal transplantation, patient with chronic kidney disease due to other causes, and patients refuse to be enrolled to the study.

Medical History taking, clinical examinations were performed on all study participants. Systemic lupus erythematosus disease activity was measured by a panel of expert clinicians using a recognized approach; the index was called the Lupus Disease Activity Score (SLEDAI) (5).

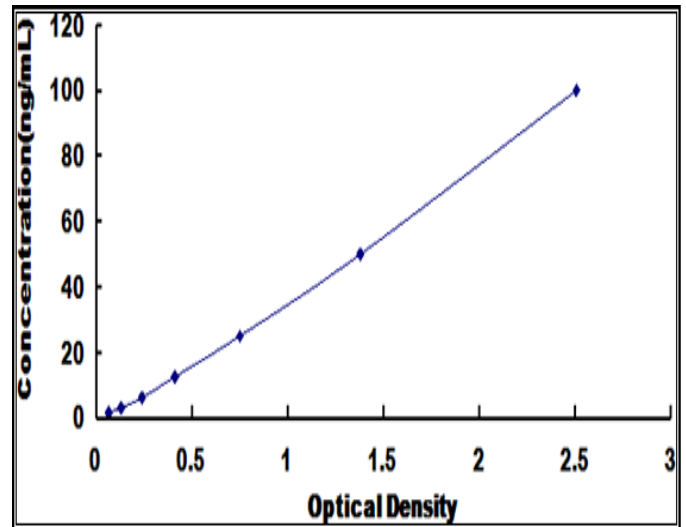
**Lab investigations:**

Include any investigations that verify inclusion and exclusion criteria:

- 1) **Complete blood count (CBC):** differential leucocytic count in peripheral blood smears stained with Leishman's solution.
- 2) **Liver function tests:** Albumin, AST, ALT, total protein, total bilirubin, and direct bilirubin.
- 3) **Kidney function tests:** Urea and Creatinine and urinary/albumin creatinine ratio
- 4) **Erythrocyte sedimentation Rate (ESR).**
- 5) **CRP.**
- 6) **Special laboratory investigation:** Anti-nuclear antibody (ANA), serum complements (C3& C4), anti-

double stranded deoxyribonucleic acid (anti-ds DNA) by immunofluorescence technique.

7) **Blood for the assay Levels of anti-C1q** utilizing kits of enzyme-linked immunosorbent assay (ELISA).



**Fig. (1):** Typical Standard Curve for Anti-C1q, Human ELISA.

**Statistical analysis**

In order to analyze the data acquired, Statistical Package of Social Sciences (SPSS) version 20 was used to execute it on a computer. In order to convey the findings, tables and graphs were employed. The quantitative data was presented in the form of the mean, median, standard deviation, and confidence intervals. P values of 0.05 or below were used to be statistically significant.

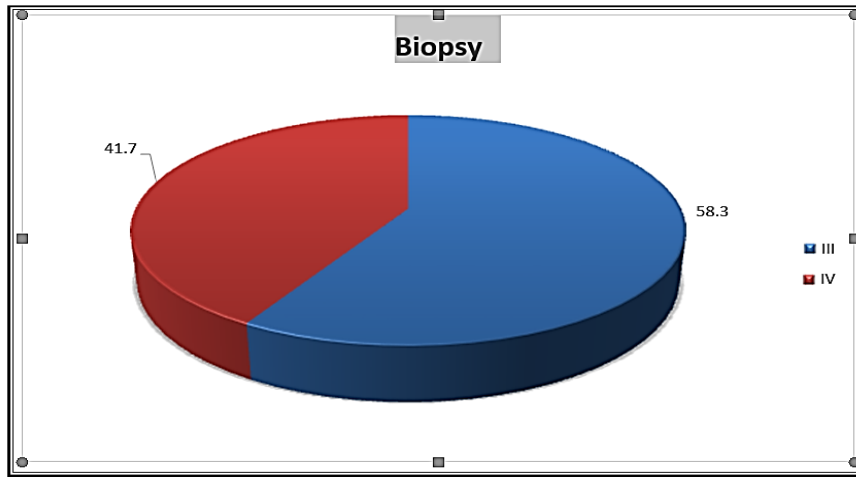
**RESULTS**

We included in our study seventy-two individuals' [four males (5.56%) and 68 females (94.44%)]. Study participants mean age was 31.1 ± 6.29 years. They were distributed to three groups according to presence of SLE with or without nephritis, we included sex as well as age matched individuals in group III as healthy control group with non-significant differences regarding sex or age between the 3 groups (Table 1).

**Table (1):** Demographics between studied groups

Variable		SLE with Nephritis (n=24)		SLE without Nephritis (n=24)		Control (n=24)		F	P
		No	%	No	%	No	%		
Age: (years)	Mean ± SD	29.21±7.23		32.38±5.92		31.71±5.72		1.67	0.20
	Range	18-40		18-42		22-42			
<b>Variable</b>		<b>No</b>	<b>%</b>	<b>No</b>	<b>%</b>	<b>No</b>	<b>%</b>	<b>χ2</b>	<b>P</b>
Sex:	Female	24	100	22	91.7	22	91.7	2.12	0.35
	Male	0	0	2	8.3	2	8.3		

ANA titers were not different LN patients (group I) in comparison versus those with SLE but without LN (group II). As regards C3 and C4 levels, they were statistically significant lower in patients with SLE and LN (Mdn=0.6 and 0.2 respectively) and patients with SLE but without LN (Mdn=0.86 and 0.5 respectively), (p=0.04). Regarding renal biopsy results in patients with lupus nephritis, the most frequent classes founded were class III (58.3%) followed by class IV (41.7%) as shown in figure (2).



**Fig. (2):** Biopsy results among Group I (Active LN).

The SLEDA score in SLE patients with LN varied between 20 and 28 and it was found to be higher in this group (Mdn=22) compared to lupus patients without nephritis [(Mdn=11),  $p < 0.001$ ].

Statistical analysis using the Kruskal Wallis H test revealed a significant difference in anti-C1q levels between the three studied groups (including control group III), it was statistically significantly higher among LN group compared to other groups and among SLE group compared to control group as shown in table (2).

**Table (2):** Anti C1q antibodies level among the studied groups:

Variable		SLE with nephritis (n=24)	SLE without nephritis (n=24)	Control (n=24)	KW	P	Post hoc
Anti C1q (ng/ml)	Mean ± SD	154.18±35.34	83.001±18.34	42.24±8.44	45.96	<0.001**	0.002* <sup>1</sup>
	Range	55.78-362.72	31.48-198.52	23.51-53.79			<0.001** <sup>2</sup>
	Median	134.02	79.34	44.26			<0.001** <sup>3</sup>
	IQR	76.57-240.53	57.65-90.35	37.36-47.75			

**Table (3):** Anti-C1q Antibody Levels, Age, and Laboratory Parameters in the Studied Case Groups:

Variable	Anti-C1q antibodies (n=48)	
	R	P
Age (years)	0.08	0.57 NS
C3: (mg/dl)	-0.04	0.79 NS
C4: (mg/dl)	-0.03	0.81 NS
ANA:	0.15	0.31 NS
Antids DNA:	0.16	0.29 NS
SLEDA score	<b>0.36</b>	<b>0.01*</b>
S.creatinine (mg/dl)	<b>0.45</b>	<b>0.001*</b>
Bl.Urea (mg/dl)	0.18	0.23 NS
ACR (mg/g)	<b>0.54</b>	<b>&lt;0.001**</b>
GFR (ml/min/1.73m)	<b>-0.42</b>	<b>0.003*</b>
24 h protein	<b>0.38</b>	<b>0.008*</b>
S.Albumin (g/dl)	-0.02	0.92 NS
Total Protein (g/dl)	0.08	0.60 NS
Hb (gm/dl)	-0.09	0.52 NS
TLC (x10 <sup>9</sup> /L)	0.15	0.30 NS
ESR. (mm/hr)	<b>0.34</b>	<b>0.04*</b>

The correlation between levels of anti C1q and other study parameters were tested using appropriate correlation analysis. Patients with SLRE had a positive correlation between anti C1q and SLEDA. (n= 48, r = 0.36, P=0.01), anti C1q and serum creatinine (n= 48, r = 0.45, P=0.001), anti C1q and urinary albumin to creatinine ratio (n= 48, r = 0.54, P <0.001) and ESR in first hour (n= 48, r = 0.34, P =0.04). While there was negative correlation between anti C1q and eGFR (n= 48, r = -0.42, P = 0.03). Other correlation analyses between UFCR and study parameters were reviewed in table (3). As regard the distribution of anti C1q according to class of LN in renal biopsy, it was found that Class IV LN in the SLE with nephritis group had a significantly higher Anti C1q level (Mdn=160.28) compared to Class III LN [(Mdn=105.75),  $p = 0.03$ ]. Anti C1q levels were substantially greater in patients with active SLE without nephritis compared to those who were inactive (as evidenced by SLEDA score) as shown in table (4).

**Table (4):** Anti C1q antibody titers and SLE disease activity in patients without nephritis.:

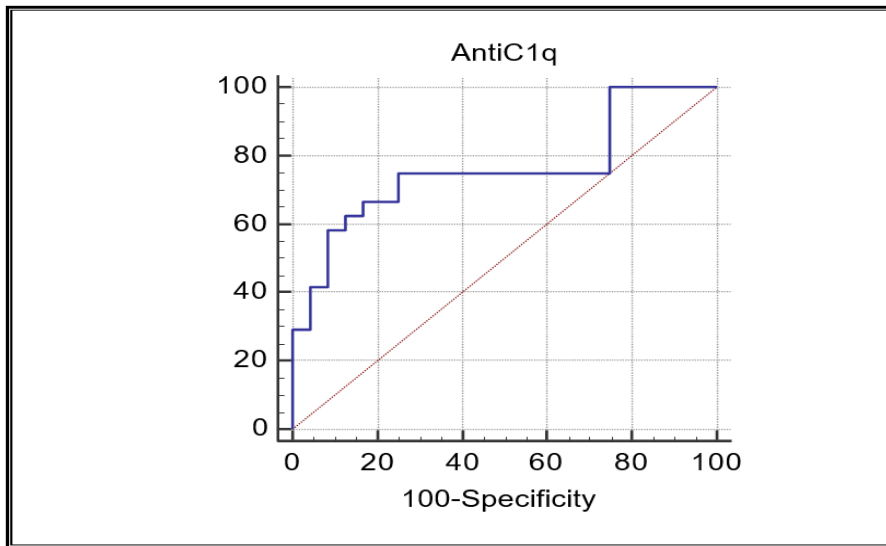
Variable	No	Anti C1q antibodies			MW	P	
		Mean	SD	Median (IQR)			
SLEDA	Non active	21	73.81	16.47	73.35 (49.03-85.82)	2.58	0.01*
	Active	3	147.36	33.58	107.06 (106.03-168.53)		

The receiver operating characteristic curve was used to determine whether or not anti C1q was able to predict nephritis in patients with SLE. It was found that anti C1q at cut off > 88.08 ng/ml had sensitivity and specificity of 75% for both and accuracy 75% in diagnosis of LN in patients with SLE (Table 5 & figure 3).

**Table (5):** Anti C1q validity to diagnose LN among the studied cases groups:

Cut off	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	Accuracy	P
>88.08 ng/ml	0.76 0.62-0.90	75%	75%	75%	75%	75%	0.002*

This table shows that Anti C1q at cut off >88.058 ng/ml had sensitivity 75%, specificity 75% and accuracy 75% in diagnosis of LN among cases groups.



**Fig. (3):** ROC curve showing the validity of anti-C1q in the diagnosis of LN in the patient populations examined.

**DISCUSSION**

Immune dysregulation and the inappropriate generation of autoantibodies are hallmarks of SLE. Abnormalities in the activation of the innate and adaptive immune systems are generally recognized, even if the precise pathophysiology in SLE remains to be explained (9).

Kader *et al.* (10) had a study included 120 individuals aged 0-19 years, with a mean SD for the SLE with nephritis group of 16.7± 3, for the SLE without nephritis group of 16.1 ± 2, and for the control group of 15.9 ± 3. Non-significant difference was found as regards age between the groups.

In contrast to our study's findings on gender distribution, Elsayed *et al.* (11) observed that the examined groups differed significantly in terms of gender, with a higher number of females in the SLE with nephritis group in particular.

The results of the present study showed significance observed increase in Anti-C1q among SLE who have nephritis compared to SLE with no nephritis and control and also among SLE without nephritis

compared to control.

Inconsistent with the current study, in another study researchers evaluated sixty-one SLE patients, forty of them of had biopsy-proven lupus nephritis, and found that Anti-C1q antibodies were found in 44% of SLE patients compared to 4% of healthy blood donors (detected by in-house ELISA). Sixty percent of those with lupus nephritis had anti-C1q antibodies, but just 14 percent of those with SLE but no nephropathy did. Active lupus nephritis patients were observed to have elevated anti-C1q antibody titers in comparison to patients with inactive nephritis (p =.89) (12).

The current findings verified Kader *et al.* (10) findings that Higher levels of anti-C1q antibodies were found in patients with active lupus nephritis compared to SLE patients without active nephritis or control subjects, with medians (ranges) of [27.5 (14-83), 9 (2.5-30), and 7 (2-13), respectively. Positive anti-C1q antibody titers were significantly higher in cases than in the other two groups.

Elsayed *et al.* (11) study was in line with our study regarding serum anti-C1q levels that there was

significant increase among SLE with nephritis compared to control group.

These conclusions have been disputed by several researchers, though, anti-C1q may be linked to systemic disease activity or only to severe renal disease activity. However, this is still up for debate. Even if anti-C1q antibodies are linked to a certain form of LN, no one can agree on whether they're helpful in the long-term monitoring of LN. It may be speculated that anti-C1q can be used as a noninvasive biomarker of renal failure in people with SLE<sup>(6)</sup>.

Anti-C1q antibodies were found to have a statistically significant negative link with GFR among the examined cases groups and a positive correlation with SLEDA score, serum creatinine, ACR, 24-hour urine protein, and ESR.

This finding is consistent with that of **Kader et al.**<sup>(10)</sup>, who discovered a favourable association between anti-C1q antibodies and renal SLEDAI, activity index, and 24-hour urine protein in patients with active lupus nephritis. They discovered a negative association between anti-C1q antibodies and C3 and C4, but no statistically significant correlation with proteinuria, a biological marker of SLE activity and renal impairment.

In line with our findings, a recent study by **Elsayed et al.**<sup>(11)</sup> discovered a positive, highly significant association between anti-C1q and the SLEDAI score and anti-dsDNA IgG, but a negative, highly significant correlation between anti-C1q and the C3 and C4 scores.

The present study revealed that Class IV LN had a significantly higher prevalence of Anti-C1q than Class III LN in the SLE with nephritis group ( $p < 0.05$ ).

**Donia et al.**<sup>(8)</sup> revealed a high significant difference in anti-C1q levels between the normal and lupus nephritis groups, which is consistent with our findings.

The present study revealed that the mean anti C1q levels were 73.81 and 147.35 ng/ml in non-active and active SLEDA among SLE with nephritis group respectively. There was a statistically significant increase in Anti-C1q among active cases compared to non-active cases among SLE without nephritis group.

In a prospective multi-center study of 38 patients with lupus nephritis, 97.2 percent of those with active proliferative lupus nephritis tested positive for anti-C1q, but only 35% of those with inactive lupus nephritis and 25% of those with active non-renal lupus did<sup>(13)</sup>.

Anti-C1q antibodies validity to diagnose LN among the studied group showed that Anti C1q at cut off  $> 88.058$  ng/ml had sensitivity 75%, specificity 75%, accuracy 75%, PPV of 75% and NPV of 75% in diagnosis of LN among cases groups.

In line with the current findings, **Kader et al.**<sup>(10)</sup> found that lupus nephritis patients who had anti-C1q antibodies were more likely to have the disease than those who did not. (cut off  $> 18$  ng/ml had sensitivity 97.5 percent, specificity 65.0 percent, accuracy 75.0

percent, PPV 74.0 percent, and NPV 75.0 percent). In the instance of highly sensitive lupus nephritis, C3 was deemed to be a better positive sign than a negative marker. With regards to lupus nephritis, C4 was found to be a more sensitive positive marker than a negative marker.

When predicting severe lupus nephritis, anti-C1q antibody achieved 100% sensitivity, 95.70% specificity, 50% positive predictive value, and 100% negative predictive value<sup>(14)</sup>.

A recent study was compatible with our results with higher sensitivity that **Elsayed et al.**<sup>(11)</sup> found that lupus nephritis had a superior positive prognostic marker in the form of anti-C1Q, which had an NPV of 100%, a PPV of 78%, a sensitivity of 100%, and a specificity of 81.82%. Anti-C1q antibodies were found to have a correlation with the severity of lupus nephritis, suggesting that they may be useful in diagnosing and monitoring the condition.

**Limitation of the study:** Our research has some limitations. First off, the outcome was predicated on just one institution. Secondly, lupus nephritis patients were few in number, and the follow-up period was brief.

## CONCLUSION

Our study's findings provide conclusive proof that anti-C1q plays a significant role in the pathogenesis of active SLE nephritis. As a result, it is now possible to advance treatment targets for SLE kidney damage caused by C1q. Similar to other common measures, such as renal SLEDAI, Anti-C1q autoantibodies have been linked to renal disease activity as well as renal flare-ups. In addition to other validated disease activity indices, patients with SLE who test positive for anti-C1q antibodies have a biomarker for nephritis flare.

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