The Egyptian Journal of Hospital Medicine (July 2022) Vol. 88, Page 2475-2479

Utility of Serum Anti-C1q Autoantibodies as a Biomarker of Lupus Nephritis in Children

Hany Elsayed[1], Ahmed Ibrahim Bayoumi Imam*[1], Hasan EL-Banna Khedr[1], Naglaa Ali Khalifa[2]
Departments of [1]Pediatrics and [2]Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt
***Corresponding Author:** Ahmed Ibrahim Bayoumi, **E-Mail:** medo938382@gmail.com

ABSTRACT

Background: Deficiencies in anti-C1q antibodies were substantially linked to the development of lupus nephritis. **Objective**: To investigate the diagnostic value of serum anti-C1q auto antibodies used as a reliable marker for diagnosis of lupus nephritis in children.

Patients and Methods: at Zagazig University Children's Hospital seventy-two child included in the study were classified into 3 groups: Group (A): 18 children diagnosed with lupus nephritis flare at time of study. Group (B): 18 children diagnosed with lupus nephritis quiescence stage at time of study, and Group (C): 36 healthy children. Serum anti-C1q autoantibodies was assessed in all participants.

Results: Anti C1q at cutoff point 35 in Group (A) 15 (83.3%) had anti C1q value more than or equal 35.1 (5.56%) had anti C1q value between 9 – 35 and 2 (11.1%) had anti C1q value less than 9 while in Group (B) 14 (77.8%) had anti C1q value between 9 – 35 and 4 (22.3%) had anti C1q value less than 9 and in group (C) all children had anti C1q value less than 9. Lupus nephritis had a superior positive prognostic marker in the form of anti-C1Q, which had a sensitivities of 100 and specificities of 81.82. Lupus nephritis activity was associated with anti-C1q antibodies, suggesting that they could be beneficial in forming predictions regarding the disease and in assessing its activity. **Conclusion**: Anti-C1q antibodies can be considered a reliable, sensitive, and specific biomarker for the diagnosis of nephritis flares in pediatric and Egyptian SLE patients, in addition to and possibly replacing other proven disease activity indices.

Keywords: Anti-C1q autoantibodies, Children, Lupus nephritis.

INTRODUCTION

One-fifth of all cases of systemic lupus erythematous (SLE) have symptoms that appear before the age of 18 due to a multisystem autoimmune illness called childhood onset (cSLE) [(1)].

Patients with SLE are more likely to develop a condition known as Lupus Nephritis (LN), which can lead to an increased risk of death and morbidity. An accurate assessment of renal flaring remains a major difficulty due to the complexity of SLE and LN serology and clinical presentation [(2)].

Several autoantibodies (Abs) have been linked to systemic lupus erythematous, including antibodies that target the classical route complement fragment 1 (C1q) [(3)]. C1q, the initial component of the classical complement pathway, plays a critical function in the clearance of immune complexes and apoptotic cell debris from tissues [(4)]. A small percentage of people with SLE have anti-C1q Abs, ranging from one-third to one-half of the population [(5)]. It was also found that lupus nephritis is related with C1q deficiency [(6)]. As a genetic risk factor for systemic lupus erythematous, inherited C1q deficiency has been found to be the most common one. Patients with LN who have anti-C1q antibodies have a secondary C1q deficit [(7)].

Anti-C1q antibody appears to be more closely associated to renal disease activity than other autoantibodies, such as anti-double-stranded DNA antibody, in terms of predicting a renal proliferative flare [(8)].

More research is needed on the diagnostic utility of anti-C1 autoantibody in SLE and LN because few studies show the positive correlation between LN and anti-C1q Ab positivity in childhood onset systemic lupus erythematous [(9)].

It was the goal of this study to investigate the diagnostic value of serum anti-C1q autoantibodies used as a reliable marker for diagnosis of lupus nephritis in children.

PATIENTS AND METHODS

At Children's Hospital, Zagazig University and Clinical Pathology Department, Faculty of Medicine, Zagazig University, seventy-two child included in the study were classified into 3 groups: Group (A): 18 children diagnosed with lupus nephritis flare at time of study. Group (B): 18 children diagnosed with lupus nephritis quiescence stage at time of study, and Group (C): 36 healthy children.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee (ZU-IRB#6071). 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:

- There were at least four patients diagnosed with childhood-onset systemic lupus (SLE) who satisfied the new American College of Rheumatology (ACR) criteria for SLE.
- Patients with SLE with renal activity.
- Normal children under 18 years of age not diagnosed

Received: 19/12/2021
Accepted: 16/02/2022

previously with any chronic illness.

Exclusion criteria:

- Patients were diagnosed as childhood onset systemic lupus and their age was over 18 year or expected to exceed 18 years of age during the study.

All patients were subjected to:

1. A thorough review of the patient's medical history.
2. Complete general and physical examination with multi system examination for signs of lupus nephritis, e.g., photosensitivity, malar rash, discoid rash, serositis, renal disorder, oral ulcers, hematologic disorders, neurological disorder, and immunologic disorder.

Serum e.g., anti C1q antibodies:

Principle [10]: This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antigen specific to C1q-Ab.

Other laboratory tests were performed including anti DNAs, ESR, serum creatinine, CBC, and urine tests for lupus.

Statistical analysis

In order to analyze the data acquired, statistical Package for the Social Sciences (SPSS) version 20 was used to execute it. In order to convey the findings, tables and graphs were employed. The quantitative data were presented in the form of rang, mean, and standard deviation (SD). The qualitative data were presented as frequency and percentage. The student's t test (T) was used to assess the data while dealing with quantitative independent variables. Pearson Chi-Square test was used to assess qualitatively independent data. P value of 0.05 or less was considered significant.

RESULTS

As regarding demographic data of the studied groups, no statistically significant difference existed between groups in terms of age or the time from the onset of the condition. It was found that the groups differed significantly in terms of gender, the percentage of females was higher especially among flare up group (**Table 1**).

Table (1): Characters of the studied groups

Age	**Group (A) (n=18)**		**Group (B) (n=18)**		**Group (C) (n=36)**		**P Value**
Min.-Max.	9-18		8-17		8-18		0.959
Mean± S.D	12.94±3.134		12.67±3.125		12.92±3.459		
Sex	**Group (A) (n=18)**		**Group (B) (n=18)**		**Group (C) (n=36)**		**P Value**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Male	1	5.6	2	11.1	17	47.2	0.001*
Female	17	94.4	16	88.9	19	52.8	
Total	18	100	18	100	36	100	
Duration	**Group (A) (n=18)**			**Group (B) (n=18)**			**P Value**
Min.-Max.	3 months – 63 months			6 months – 72 months			0.922
Mean± S.D	28.83±18.484			29.44±18.449			

In comparison between three groups as regard to C3 and C4, there was statistically significant differences between each 2 groups. Lowest values were recorded among Group A **(Table 2).**

Table (2): Comparison between three groups as regard to C3 and C4

C3	**Group (A) (n=18)**	**Group (B) (n=18)**	**Group (C) (n=36)**	**P Value**
Min.-Max.	0.6-1.1	0.7-1.3	0.9-1.8	<0.001
Mean± S.D	0.77±0.141	1.01±0.178	1.36±0.290	
C4				
Min.-Max.	0-0.2	0-0.3	0.1-0.4	<0.001
Mean± S.D	0.06±0.078	0.13±0.119	0.23±0.113	

As regard serum anti C1q, there was significant difference among the 3 groups **(Table 3).**

Table (3): Comparison between three groups as regard to serum Anti C1q

Anti C1q	Group (A) (n=18)		Group (B) (n=18)		Group (C) (n=36)		P Value
	No.	%	No.	%	No.	%	
≥35	15	83.3	0	0	0	0	<0.001*
9-35	1	5.56	14	77.8	0	0	
<9	2	11.1	4	22.3	36	100	
Min.-Max.	0.21-100.56		0.18-34.58		0.13-8.98		<0.001*
Mean± S.D	46.87±34.822		12.36±14.248		1.53±6.112		

As regard serum anti anti-dsDNA antibodies, there was significant difference among the 3 groups (**Table 4).**

Table (4): Comparison between three groups as regard to anti-dsDNA antibodies

Anti-dsDNA IgG	Group (A) (n=18)		Group (B) (n=18)		Group (c) (n=36)		P Value
	No.	%	No.	%	No.	%	
Negative	**1**	**5.6**	**13**	**72.2**	**36**	**100**	**<0.001**
Positive	**17**	**94.4**	**5**	**27.8**	**0**	**0**	

SLED**AI** score in Group (A) was significantly higher than in Group (B) **(Table 5).**

Table (5): Comparison between groups of patients as regard to patient's SLEDAI score

SLEDAI score	Group (A) (n=18)	Group (B) (n=18)	P Value
Min.-Max.	**10-35**	**0-4**	**<0.001**
Mean± S.D	**12.4±3.305**	**2.23±1.02**	

There was positive highly significant correlation between anti C1q and each of SLEDAI score and anti-dsDNA IgG while there was negative highly significant correlation between Anti C1q and each of C3 and C4 **(Table 6).**

Table (6): Correlation between anti C1q and other parameters

	Anti C1q	
	r	P
C3	**-0.844**	**<0.001**
C4	**-0.783**	**<0.001**
SLEDAI score	**0.711**	**<0.001**
Anti-dsDNA IgG	**0.600**	**<0.001**

Lupus nephritis has a superior positive prognostic marker in the form of anti-C1q, which has a sensitivity of 100 and specificity of 81.82 **(Table 7 and Figure 1).**

Table (7): ROC curve analysis of anti C1q, C3, C4 and anti dsDNA to prediction of lupus nephritis

	Sensitivity	Specificity	PPV	NPV	Standard error	Significance level
Anti C1q	100	81.82	77.8	100	0.29	<0.001
C3	88.46	71.74	63.9	91.7	0.046	<0.001
C4	92.31	73.91	66.7	94.4	0.042	<0.001
Anti dsDNA	95	67.31	52.8	97.2	0.041	<0.001

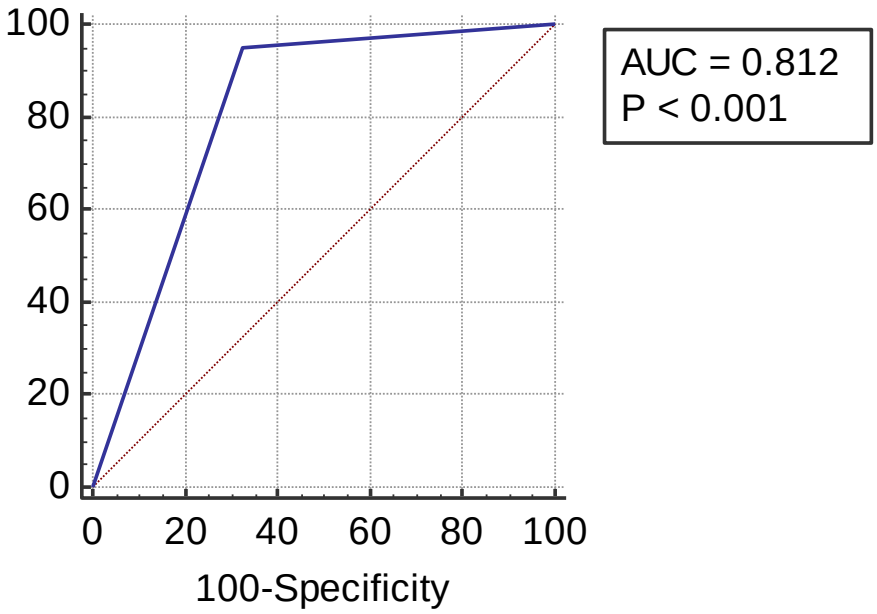

Figure (1): ROC curve analysis of Anti C1q to prediction of lupus nephritis

DISCUSSION

LN flare-ups can be detected by elevated levels of anti-dsDNA antibodies (anti-ds-DNA) and hypocomplementemia, however it's difficult to tell which biomarker is the most useful in diagnosing SLE activity [(11)].

The classical complement pathway's first component, anti-C1q antibodies, have been shown in a number of studies to be immunological indicators of SLE, particularly in cases where the kidneys are involved. An active kidney illness may be linked to the existence of anti-C1q. Additionally, the presence of anti-C1q, either alone or in combination with other markers of disease activity, could aid in the diagnosis of a renal flare [(12)].

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 [(13)].

Lupus nephritis has been linked to the formation of anti-C1q antibodies in a recent paper. Anti-C1q may also serve as a noninvasive biomarker of renal failure in SLE patients, according to some researchers [(13)].

Age and disease duration were not statistically different amongst the study groups in terms of demographic data. Among individuals who experienced flare-ups, the statistically significant difference in gender was most significant.

When it comes to the incidence of SLE, women are nine times more likely than men to develop the disease. Pregnancy and menopause are the most dangerous times for women, whereas middle age and old age are the most dangerous times for males [(14)].

The predominance of females in our study could be attributable to the fact that SLE exacerbations are frequently observed in the premenstrual, early pregnancy, and puerperium phases of women's lives. SLE flare-ups may be linked to an increase in plasma estrogen concentrations, according to one study. It appears that estrogen plays a significant influence in the generation of immune-related cytokines such as Th2 cytokines [(14)].

In our investigation, we found statistically significant differences between groups A, and groups B and C when it came to C3 and C4. Furthermore, there was a significant difference between group B and group C. Group A had the lowest results.

In the study of **Birmingham *et al.*** [(15)] C3 and C4 levels were evaluated twice a month for 35 months in individuals with lupus nephritis who had 70 renal flares during that time, assessing the connection between C3 and C4 levels and renal flares.

According to the results of our investigation, there was a significant difference in mean anti C1q levels between each group when compared to the other 2 groups (46.87±34.822 SD), (12.36±14.248 SD), (1.53±6.112 SD); in groups A, B, and C respectively. Anti C1q levels were significantly higher among the flaring group. This indicates the possible use of this antibody as a marker of disease activity.

Also anti C1q at cutoff point 35 in Group (A) 15 (83.3%) had anti C1q value more than or equal 35.1, (5.56%) had anti C1q value between 9 – 35 and 2 (11.1%) had anti C1q value less than 9 while in Group (B) 14 (77.8%) had anti C1q value between 9 – 35 and 4 (22.3%) had anti C1q value less than 9 and in group (C) all children had anti C1q value less than 9. There was a statistically significant difference between groups.

In a study done by **Glassock *et al.*** [(10)], in an Egyptian cohort of SLE patients, researchers looked to see if anti-nucleosome and anti-C1q antibodies were associated with nephritis, and they found that anti-C1q antibody had a statistically significant association with

vasculitis. Photosensitivity, vasculitis, and nephropathy, and anti-C1q antibodies were found in patients with a high ECLAM score, a high ESR, and low serum albumin. There is a strong association between LN and serum anti-C1q antibody levels in Egyptian people with SLE.

In our study, SLEDAI score in Group (A) ranged between 10-35 with mean ± S.D 12.4±3.305 while in Group (B) it ranged between 0-4 with mean ± S.D 2.23±1.02. There was statistically significant differences between groups, which reflects disease activity in group C

In comparison between the three groups as regard to anti-ds-DNA antibodies, antibodies in Group (A) show that 1 (5.6%) was negative and 17 (94.4%) were positive while in Group (B) 13 (72.2%) were negative and 5 (27.8%) were positive and in Group (C) all patients were negative.

Regarding correlation between Anti C1q and other parameter, correlation between Anti C1q and other parameter show that there was positive highly significant correlation between anti C1q and each of SLEDAI score (r=0.711) and anti-dsDNA IgG (r=0.600) while there was negative highly significant correlation between Anti C1q and each of C3 (r=-0.844) and C4 (r=-0.783), also regarding ROC curve analysis of anti C1q, C3, C4 and anti dsDNA to prediction of lupus nephritis, lupus nephritis was diagnosed with anti-C1Q antibodies, which had higher sensitivity and specificity than other predictors, with an AUC of 0.909. A connection between anti-C1q antibodies and the activity of lupus nephritis was found, suggesting that these antibodies could be useful in generating predictions about the disease and assessing its activity, which is consistent with the findings of **Glassock *et al.* [10], Gargiulo *et al.* [13] and Jesus *et al.* [16]**.

CONCLUSION

Anti-C1q antibodies can be considered a reliable, sensitive, and specific biomarker for the diagnosis of nephritis flares in pediatric and Egyptian SLE patients, in addition to and possibly replacing other proven disease activity indices.

Conflict of interest: The authors declare no conflict of interest.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

REFERENCES

1. **Kamphuis S, Silverman E (2010):** Prevalence and burden of pediatric-onset systemic lupus erythematosus. Nat Rev Rheumatol., 6 (9): 538–46.
2. **Goilav B, Putterman C, Rubinstein T (2015):** Biomarkers for kidney involvement in pediatric lupus. Biomarkers in Medicine, 9: 529–543.
3. **Pickering M, Botto M (2010)**: Are anti-C1q antibodies different from other SLE autoantibodies? Nat Rev Rheumatol., 6:490-493
4. **Cozzani E, Drosera M, Gasparini G *et al.* (2014):** Serology of lupus erythematosus: correlation between immunopathological features and clinical aspects. Autoimmune Diseases, 14: 1-13.
5. **Kallenberg C (2008):** Anti-C1q autoantibodies. Autoimmun Rev., 7: 612–615.
6. **Mohan C, Putterman C (2015):** Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. Nature Reviews Nephrology, 11(6): 329-41.
7. **Schejbel L, Skattum L, Hagelberg S *et al.* (2011):** Molecular basis of hereditary C1q deficiency—revisited: identification of several novel disease-causing mutations. Genes & Immunity, 12(8): 626–634.
8. **Chi S, Yu Y, Shi J *et al.* (2015):** Antibodies against C1q are a valuable serological marker for identification of systemic lupus erythematosus patients with active lupus nephritis. Disease Markers, 15: 1-11.
9. **Gilliam B, Ombrello A, Burlingame R *et al.* (2012):** Measurement of autoantibodies in pediatriconset systemic lupus erythematosus and their relationship with disease-associated manifestations. Semin Arthritis Rheum., 41: 840–848.
10. **Glassock R, Hebert L, Moroni G *et al.* (2019):** Infection-related and renal-limited glomerulonephritis. Treatment of Primary Glomerulonephritis. https://oxfordmedicine.com/view/10.1093/med/9780198784081.001.0001/med-9780198784081-chapter-9
11. **González L, Ugarte-Gil M, Alarcón G (2021):** Systemic lupus erythematosus: The search for the ideal biomarker. Lupus, 30(2) 181–203.
12. **Donia A, Amin A, Mohamed S (2017):** Study for the relationship between lupus nephritis and Anti-C1q antibodies. The Egyptian Journal of Hospital Medicine, 69(8): 2960-2974.
13. **Gargiulo M, Gómez G, Khoury M *et al.* (2015):** Association between the presence of anti-C1q antibodies and active nephritis in patients with systemic lupus erythematosus. Medicina (Buenos Aires), 75(1): 23-28.
14. **Quintero O, Amador-Patarroyo M, Montoya-Ortiz G *et al.* (2012):** Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. Journal of Autoimmunity, 38(2-3): 109-119.
15. **Birmingham D, Irshaid F, Nagaraja H *et al.* (2010):** The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. Lupus, 19(11): 1272-1280.
16. **Jesus A, Silva C, Carneiro-Sampaio M *et al.* (2009):** Anti-C1q antibodies in juvenile-onset systemic lupus erythematosus. Annals of the New York Academy of Sciences, 1173(1): 235-39.